Gerard Marshall Raj
Ramasamy Raveendran *Editors*

Introduction to Basics of Pharmacology and Toxicology

Volume 1: General and Molecular Pharmacology: Principles of Drug Action

Springer

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Dedicated to all my mentors including my parents (for their love, care, and affection), my darling "chinnu" @ Dr. Rekha (for her everlasting love and exceptional support), and finally to my kiddo Gershin (for lending his time).

Gerard Marshall Raj
Puducherry, India
April 2019

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Foreword

It gives me immense pleasure that some of my former bright postgraduates have made a venture to write a series of textbooks to meet the demands of information in higher studies in Pharmacology. My student Dr. Gerard Marshall Raj contacted me to write a "foreword" of the book which I could not decline. I have gone through some portions of the manuscript and also gave my suggestions for improvement. The book chapters on "General Pharmacology and Principles of Drug action" are clearly written. I felt that they should also have incorporated "clinical trials" in this section. The text is written in lucid language, easy to understand, and will be a good companion book in Pharmacology for higher studies.

All my best wishes to these young writers in the field of Pharmacology!

Dr. Suresh Chandra Pradhan

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of Allergy, Asthma & Applied Immunology
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Preface

It was in the mid-2016s, when I had been in the phase of transformation from a post-graduate student to a teaching faculty, this idea of writing a book especially for M.D. Pharmacology post-graduates came out from nowhere. Though it was a light-bulb thought, I had been ruminating on that since then.

And it was in the late 2017s and early 2018s, I had actually started working on this herculean task. The first thing I did was to browse through the various syllabi of Indian Universities imparting M.D. Pharmacology course. I could retrieve (online search) around 20 syllabi of Universities in India from the States of Bihar, Delhi, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Puducherry (UT), Punjab, Tamil Nadu, Uttar Pradesh, and West Bengal. Then, I sifted through the course contents and also the recommended reference books and textbooks to finalize the topics to be covered.

The ultimate goal of this book is to cater to a prospective M.D. Pharmacology post-graduate appearing for his/her formative or summative (final) assessment examinations. Hence, I had divided the book into exam paper-wise volumes.

Volume 1: General and Molecular Pharmacology: Principles of Drug Action
- *deals about General Pharmacology*

Volume 2: Essentials of Systemic Pharmacology: From Principles to Practice
- *deals about Systemic Pharmacology*

Volume 3: Experimental Pharmacology: Research Methodology and Biostatistics
- *deals about Experimental Pharmacology*

Volume 4: Pharmacology and Therapeutics
- *deals about Clinical Pharmacology*

For this project, I had included five other contributors from the field of Pharmacology, namely, Dr. Mageshwaran, Dr. Abialbon, Dr. Avinash, Dr. Neel, and Dr. Nishanthi. They are the major contributors of this four-volume book and also serve as the primary editors of the other three volumes. I know them both personally and professionally for the past 8 to 10 years. They are a bunch of young-yet-proficient and enthusiastic academicians whom I suppose could satisfy the demands of this project. They are currently working in different medical institutes and pharmaceutical industries around the globe.

The present book (*Volume 1: General and Molecular Pharmacology: Principles of Drug action*) is divided into the following five parts.

Part I on *Historical Aspects of Drug Discovery* comprises renowned contributions to the field of Pharmacology by personalities both from the Indian arena and from the world over—including the Nobel Laureates.

Part II on *General Pharmacological Principles* discusses in detail about the various facets of general pharmacology ranging from sources of drugs; routes of drug administration; basic pharmacokinetics including drug transporters and pharmacodynamics including adverse effects; and drug interactions to structure-activity relationships.

Special Topics in Pharmacology encompassing drug information; pharmacogenetics; chrono- and ethno-pharmacology; pharmaco-epidemiology and pharmacoeconomics; orphan drugs; fixed-dose combinations; translational and reverse pharmacology; gene and stem cell therapy; and principles of prescription writing are compiled under **Part III**.

Part IV is about *Toxicology* including two chapters on environmental toxicology and basic principles of management of drug poisoning.

Part V deals about *Molecular Biology in Pharmacology* with chapters on PCR, blotting techniques, antisense oligonucleotides, among others.

The M.D. Pharmacology post-graduates rely on multiple texts for their assessment examinations, which could be highly time-consuming especially during the preparation period. Moreover, by referring to different texts of varied patterns can at times be extremely distracting to a potential exam-going student. Hence, a review book of this sought can be in a way lessen the arduous task of referring to numerous books or materials with mixed patterns.

The book could also be of use to young pharmacologists working in different job portfolios ranging from teaching-research faculty in academia, pursuing medical advisory roles in pharmaceutical industry to drug safety physicians in pharmacovigilance sectors.

The following are the key features of our review book.

1. Point-wise listing of facts.
2. Inclusion of around 146 figures, tables, and boxes.

These features make the book more concise and precise. Thereby, they make the reading all the more easier and reproducible.

Wishing you all a very happy reading!

Puducherry, India
April 2019

Gerard Marshall Raj

Acknowledgments

I place my wholehearted gratitude to the publishers Springer-Nature for having given the due shape to this proposal. Foremost, I wish to express my special thanks to Dr. Gaurav Singh, the Acquisition Editor, who was in constant touch with me from the very beginning and was instrumental in giving valuable suggestions all along in the making of this project. I would also wish to extend my regards to Ms. RaagaiPriya Chandarasekaran, the Production Coordinator, and her team for their concerted efforts in the preparation of this book.

I am greatly indebted to my fellow editors of other volumes of this book, namely, Dr. Mageshwaran, Dr. Abialbon, Dr. Avinash, Dr. Neel, and Dr. Nishanthi—but for them, the whole proposal would not have seen the light of the day.

I also place my sincere regards and profound thankfulness to Professor (Dr.) Ramasamy Raveendran, Dean (Research)-cum-Head of the Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), for his unparalleled support as a senior editor of this book despite his busy schedules.

I would also like to acknowledge Professor (Dr.) Suresh Chandra Pradhan, Former Head of the Department of Pharmacology of JIPMER, for sparing time to read the entire chapter drafts and readily obliging to write a Foreword for this book.

Nothing is possible without the Divine Grace ("The LORD is my shepherd!"). Therefore, I thank the Almighty for good health and well-being that were necessary to complete this book.

Puducherry, India
April 2019

Gerard Marshall Raj

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Editors and Contributors

About the Editors

Dr. Gerard Marshall Raj completed his M.B.B.S. and M.D. in Pharmacology. Currently, he is working as an Assistant Professor in the Department of Pharmacology, Sri Venkateshwaraa Medical College Hospital & Research Centre, Puducherry, India. He has published two book chapters, one on the "Intellectual Property Rights in Drug Development and Biotechnology" in the book titled *Intellectual Property Issues in Biotechnology* (CABI; ISBN: 9781780646534) and the other on "Intellectual Property Issues Surrounding Antimicrobial Agents" in the book titled *Intellectual Property Issues in Microbiology* (Springer-Nature; ISBN: 9789811374654). He was awarded the "Prof. M. N. Ghosh Gold Medal" for outstanding performance in M.D. Pharmacology course and was also the topper in the M.D. Pharmacology University Examination in Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER). He has also published many research articles, reviews, commentaries, case reports, and letters to the editor in peer-reviewed and indexed journals. Pharmacogenomics, diabetes, antimicrobial resistance, drug patents, and intellectual property rights are some of his fields of research interests.

Dr. Ramasamy Raveendran Dean (Research)-cum-Professor (Senior Scale) and Head of Pharmacology, JIPMER, Puducherry, graduated from the University of Madras and obtained his M.D. degree in Pharmacology from JIPMER, Puducherry, and Diploma in Clinical Pharmacology from the St Bartholomew's Medical College, London, UK. He has over 34 years of experience in research and teaching. His main interests include Computer Assisted Learning (CAL) and toxicology. He has authored about 55 research papers, 7 CAL software packages, and 1 book. He has conducted more than 50 workshops on "research methodology," "biomedical communication," and "biostatistics" in various places in India. He was the chief editor of the *Indian Journal of Pharmacology* from 2001 to 2006, and currently he is the editor-in-chief of the *Journal of Pharmacology and Pharmacotherapeutics* from 2010. Presently, as the Dean (Research) of JIPMER, he is coordinating all research activities in the premier institute.

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Part I

Historical Aspects of Drug Discovery

The Great Pharmacologists and Their Revolutionary Discoveries

1

Gerard Marshall Raj

Abstract

Ever since the subject of pharmacology was liberated from the realms of the other major basic biological sciences including physiology, pathology, and chemistry, the drastic growth in the branch of pharmacology as a medical speciality was very much perceptible. The domain of pharmacology, as an independent speciality, in the last century-and-half, has transformed from the usage of crude plant extracts as drugs to the more synthetically engineered lab-based therapeutics like the application of monoclonal antibodies and gene and stem cell therapies. However, revisiting the conventional modes and sources of drugs are sometimes inevitable – so as to learn from both what had gone wrong or correct. Hence, perusing the history of a subject, which deals with the substances that are involved in the diagnosis, prevention, and treatment (sometimes, cure), is all the more important. The efforts put forth by some of the stalwarts in the field of biomedical sciences, who were involved in interdisciplinary research with special inclination towards pharmacology, are worth reviewing. In this chapter, the many doyens in the international arena of pharmacology from Jonathan Pereira (the *Founder of British Pharmacognosy*) to Louis C. Lasagna (the *Father of Clinical Pharmacology*) are discoursed with relevance to their contributions in pharmacology; many of whom were Nobel Laureates.

Keywords

History of pharmacology · Nobel Prize · Drug discovery · Materia medica

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1.1 Introduction

The field of pharmacology, as an independent medical subject, had its inception around the middle of the nineteenth century. Initially, pharmacology was considered as a sister concern to the basic sciences of physiology, pathology, and chemistry. The discovery of alkaloids and anesthetics as potential drugs with the likes of Friedrich Sertürner (purified morphine from opium, 1805), Horace Wells (failed demonstration of N_2O as an anesthetic, 1845), William Morton (successful demonstration of ether as an anesthetic, 1846), and James Simpson (use of chloroform in obstetric anesthesia, 1847) had progressively led to the development of this branch of medical science as a separate entity.

1.2 The Pioneers in Pharmacology

Some of the landmark discoveries made in the discipline of pharmacology and the major contributors of the same are discussed below.

1.2.1 Jonathan Pereira (1804, London, United Kingdom–1853, London, United Kingdom) (Fig. 1.1)

- Jonathan Pereira was the author of the first standard (English) book on pharmacology, namely, *The Elements of Materia Medica and Therapeutics*.
- He was keen on gathering knowledge about the use of drugs on a scientific basis. His *materia medica* consisted of three parts:

Fig. 1.1 Jonathan Pereira. (Jonathan Pereira. Wood engraving after J. Mayall. Credit: Wellcome Collection. CC BY; adapted)

  - Pharmacognosy, pharmacology, and pharmacopathia or the history of simple drugs
  - Pharmacy
  - Pharmacodynamics
- He was more inclined in the study of crude drugs, and it was he who used the term *pharmacognosy* to refer the same. He is also regarded as the *Founder of British Pharmacognosy*.

1.2.2 Rudolf Buchheim (1820, Bautzen, Germany–1879, Giessen, Germany) (Fig. 1.2)

- Rudolf Buchheim introduced animal experiments (*Experimental Pharmacology*) and worked on the mechanisms of drug effects, thereby a more natural scientific basis for the pharmaceutics (drugs) heralded.
- He was the first to classify drugs based on their mode of action – called the "natural system of drugs." He also introduced the domain of *bioassay* to pharmacology and created a methodology for determining the quantitative and qualitative aspects of chemical substances in living systems.
- He converted part of his home into a pharmacology lab (1847) and also self-financed for the scientific works to be done over there. He detected the hypnotic effects of chloral hydrate.

Fig. 1.2 Rudolf Buchheim. (Portrait of Rudolf Buchheim. Credit: Wellcome Collection. CC BY; adapted)

- He wrote the textbook on pharmacology by the name *Lehrbuch der Arzneimittellehre* (1853–1856) in which he emphasized his postulate that "pharmacology is an elucidating science which should provide all the information on drugs necessary for the precise understanding of their therapeutic values." He also translated *The Elements of Materia Medica and Therapeutics* by Pereira to German and also revised the same; he included a chapter titled *Art der Wirkung* meaning "the pharmacological action."
- He established the pharmacological institute which is now called as the *Rudolf Buchheim Institute of Pharmacology* located as a part of the Biomedical Research Center Seltersberg (BFS) in Justus Liebig University Giessen, Germany.
- Hence, he is regarded as the originator of pharmacology and was justifiably called as the first "real" pharmacologist.

1.2.3 Oswald Schmiedeberg (1838, Kurland, Latvia–1921, Baden-Baden, Germany) (Fig. 1.3)

- Schmiedeberg was a protégé and successor to Buchheim. He wrote his medical thesis for M.D. degree on the determination and fate of chloroform in the blood under the guidance of Buchheim (1866).
- He made important discoveries in the study of drug action, as follows:
  - Hypnotic effects of urea derivatives (urethane) and paraldehyde
  - Effects and interactions of muscarine, nicotine, and atropine on the frog heart muscle

Fig. 1.3 Oswald Schmiedeberg. (Portrait of O. Schmiedeberg. Credit: Wellcome Collection. CC BY; adapted)

  - Action of digitalis on the heart
  - Synthesis of hippuric acid by the kidneys
  - Detoxification of organic compounds by coupling to glucuronic acid (xenobiotic metabolism)
- He tutored around 120 pupils in the field of pharmacology, and around 40 pharmacological and clinical chairs were occupied by his students' world over including the likes of Abel and Meyer (the mentor of Otto Loewi).
- He published over 200 scientific books and articles. One of his well-known works is the book *Grundriss der Arzneimittellehre* meaning "fundamentals of pharmacology" (1883).
- Oswald Schmiedeberg is commonly referred to as the *Father of Modern Pharmacology*.

1.2.4 John Newport Langley (1852, Newbury, United Kingdom–1925, Cambridge, United Kingdom) (Fig. 1.4)

- Langley postulated the existence of a "receptive substance" which was "the site of action of chemical mediators liberated by nerve stimulation" – receptor theory (1905).

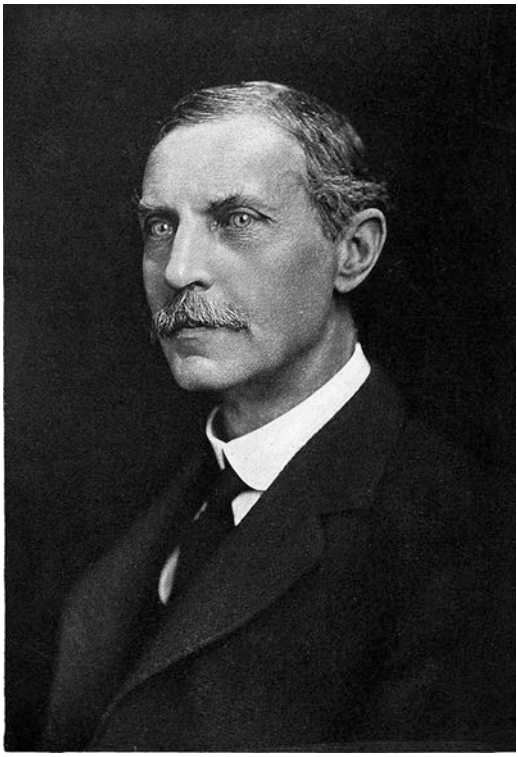

Fig. 1.4 John Newport Langley. (Portrait of John Newport Langley. Credit: Wellcome Collection. CC BY; adapted)

– He took cues from the responses exerted by the adrenomedullary extracts in different tissues; these extracts contained both epinephrine and norepinephrine.
– The responses were found to be similar to those provoked by sympathetic nerve stimulation.

1.2.5 John Jacob Abel (1857, Ohio, USA–1938, Maryland, USA) (Fig. 1.5)

- John J. Abel was appointed as the first professor (Chair) of Pharmacology in the USA at the University of Michigan (1891) under the recommendation of Schmiedeberg.
- The active principle from the adrenal medulla was isolated and refined by Abel and A. C. Crawford. Later, Abel named the active principle as *epinephrin* (without *"e"*) (1897).
- Abel also isolated and crystallized insulin (1927).
- He played a pivotal role in establishing the American Society for Pharmacology and Experimental Therapeutics (ASPET) (1908). He also founded the *Journal of Experimental Medicine* (1896), the *Journal of Biological Chemistry* (1905), and the *Journal of Pharmacology and Experimental Therapeutics* (1909).
- Abel is aptly called as the *Father of American Pharmacology*.

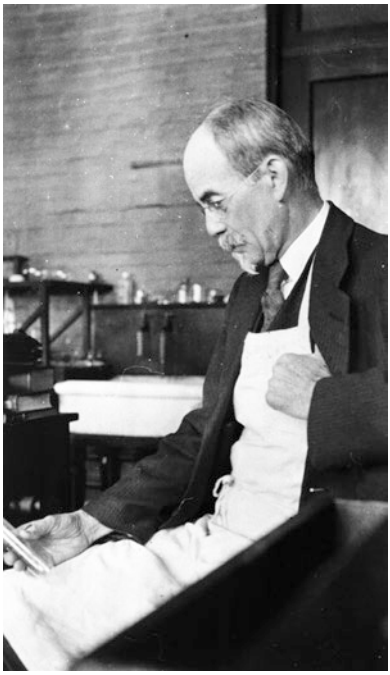

Fig. 1.5 John Jacob Abel. (From the National Library of Medicine, http://resource.nlm.nih.gov/101408190.Public Domain; reproduced)

1.2.6 Sir Henry Hallett Dale (1875, London, United Kingdom–1968, Cambridge, United Kingdom) (Fig. 1.6)

- Dale, along with George Barger, coined the term *sympathomimetic amine* to describe the actions of a cascade of amines that produced physiological effects strikingly similar to those induced by sympathetic nerve stimulation (1910).
- Dale delineated the cholinergic and also the muscarinic actions of acetylcholine (ACh) in 1914.
  - The exogenous administration of ACh displayed a remarkable resemblance to the responses of parasympathetic nerve stimulation. Both the muscarinic and nicotinic actions were elicited; the muscarinic actions were blocked by atropine, and the nicotinic actions were similar to that exerted by nicotine alkaloid.
- He suggested that the prompt degradation of ACh was due to the existence of esterases in blood and tissues (1914). He also formed the terms *cholinergic* and *adrenergic* to label the actions of motor and autonomic nerve fibers (1926).
- Dale also revealed that atropine antagonized the effects of ACh at muscarinic receptors and the action of norepinephrine and epinephrine at postganglionic sympathetic receptors was negated by ergot alkaloids.

Fig. 1.6 Sir Henry Hallett Dale. (Portrait of H. H. Dale, head and shoulders. Credit: Wellcome Collection. CC BY; reproduced)

1.2.7 Otto Loewi (1873, Frankfurt-on-the-Main, Germany–1961, New York, USA) (Fig. 1.7)

- Loewi positioned two frog hearts in a single bath, and the vagus nerve associated with one heart was stimulated resulting in slowing of its rate of contraction, and also the rate of the second heart was reduced. He deduced that an element released from the first heart was the reason for blockade of the second heart. Loewi called the unknown substance *vagusstoff*, which was later found to be ACh (Easter Monday, 1920).
- By utilizing the frog heart preparation, he also demonstrated that a substance was released with the stimulation of the sympathetic nerves; the substance he termed as *acceleransstoff*. He disclosed that the substance shared many of the properties of epinephrine.
- He proposed that sympathomimetic effects were transmitted by epinephrine, and parasympathomimetic effects were mediated by ACh.
- Loewi, along with Navratil, stated that the extracts of frog heart tissue promptly broke down ACh by a type of acetylcholinesterase (1926). They also established that eserine (physostigmine) not only blocked this enzyme but also strikingly enhanced the inhibitory response of ACh and vagusstoff on the frog heart.

Fig. 1.7 Otto Loewi. (Portrait of Otto Loewi in Boston, 1929. Credit: Wellcome Collection. CC BY; reproduced)

- Dale and Loewi were jointly awarded the Nobel Prize "for their discoveries relating to chemical transmission of nerve impulses (1936)."

1.2.8 Raymond P. Ahlquist (1914, Montana, USA–1983, Georgia, USA) (Fig. 1.8)

- Ahlquist categorized α- and β-adrenergic receptors by hypothesizing that the effect of norepinephrine on the postsynaptic sites was exerted by two different forms of adrenergic receptors (1948).

1.2.9 Daniel Bovet (1907, Neuchâtel, Switzerland–1992, Rome, Italy) (Fig. 1.9)

- Bovet and his associates presented the theoretical background for examining the pharmacological effects of cholinergic antagonists (succinylcholine) at the neuromuscular junction.
- They also synthesized pyrilamine (1944); this drug is used even today as a selective H_1 blocker in treating acute allergic conditions.
- Bovet along with the other affiliates of Fourneau's laboratory at the Pasteur Institute in Paris established that azo reduction of prontosil in tissues resulted in the formation of sulfanilamide; later the same group reaffirmed that sulfanilamide was the only active constituent of the red dye.

Fig. 1.8 Raymond P. Ahlquist. (From Little RC. Raymond P. Ahlquist (1914–1983). Clin Cardiol 1988;11:583–4. https://onlinelibrary.wiley.com/doi/pdf/10.1002/clc.4960110815; Copyright © 1988 Wiley Periodicals, Inc.; reproduced with permission)

Fig. 1.9 Daniel Bovet. (From https://upload.wikimedia.org/wikipedia/commons/b/b5/Daniel_Bovet_nobel.jpg. Public Domain; reproduced)

- Daniel Bovet was awarded the Nobel Prize in 1957 "for his discoveries relating to synthetic compounds that inhibit the action of certain body substances, and especially their action on the vascular system and the skeletal muscles."

1.2.10 Ulf von Euler (1905, Stockholm, Sweden–1983, Stockholm, Sweden) (Fig. 1.10)

- Euler discovered norepinephrine as the adrenergic neurotransmitter (1946).
- He made use of two bioassays (cat blood pressure and hen rectal caecum) with different sensitivities to epinephrine from norepinephrine to differentiate their activities.
- He was considered as the frontier of research in biogenic amines.

1.2.11 Julius Axelrod (1912, New York, USA–2004, Maryland, USA) (Fig. 1.11)

- Axelrod aided in the treatment developments for the relief of pain and depression following in-depth elucidation of neurotransmission and drug metabolism.
- He was also involved in the advancements made in the management of depression and anxiety; his investigations paved the way for the screening of drugs that block the uptake of both serotonin and catecholamines – the present-day selective serotonin reuptake inhibitors (SSRIs).

Fig. 1.10 Ulf von Euler. (Ulf Svante von Euler. Photograph. Credit: Wellcome Collection. CC BY; reproduced)

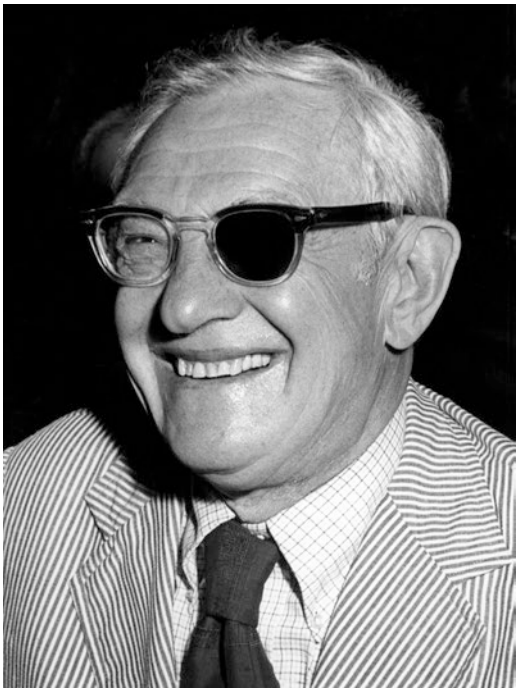

Fig. 1.11 Julius Axelrod. (From https://upload.wikimedia.org/wikipedia/commons/4/4e/Axelrod01.jpg. Public Domain; reproduced)

- He demonstrated that the antipyretic actions of acetanilide were due to its conversion to *N*-acetyl-*p*-aminophenol (acetaminophen) (1948).
- He named, isolated, and purified the enzyme, catechol-*O*-methyltransferase (1959).
- The presynaptic neuronal uptake, sequestration into the vesicles, and subsequent release of norepinephrine were elaborated by Axelrod.
- He also worked on the pineal gland and its crucial hormone melatonin; he became a stalwart in research related to pineal gland along with Richard Wurtman.

1.2.12 Sir Bernard Katz (1911, Leipzig, Germany–2003, London, United Kingdom) (Fig. 1.12)

- Katz expounded the steps involved in neurotransmission at the neuromuscular junction.
- The *vesicle hypothesis* (*quantal theory*) illustrating the neurotransmitter release was given by Katz and his colleagues.
- The 1970 Nobel Prize was awarded to Ulf von Euler, Julius Axelrod, and Sir Bernard Katz "for their discoveries concerning the humoral transmitters in the nerve terminals and the mechanism for their storage, release and inactivation."

Fig. 1.12 Sir Bernard Katz. (From Bernard Katz. 26 March 1911—1920 April 2003, Biographical Memoirs of Fellows of the Royal Society, https://doi.org/10.1098/rsbm.2007.0013. © 2007 The Royal Society; reproduced with permission)

1.2.13 Sir James W. Black (1924, Uddingston, Scotland–2010, London, United Kingdom) (Fig. 1.13)

- Black has been quoted as responsible for developing "among the most successful agents in the history of medicine," namely, propranolol and histamine.
- He synthesized pronethalol (nethalide), a naphthyl analog of isoproterenol; this relatively pure antagonist of β-adrenoceptor failed owing to its side effects.
- Later on, he discovered the supposedly first β-adrenoceptor blocker – propranolol – which had blocked the β-receptor activity 10 times more than that of pronethalol.
- Black postulated the existence of a new histamine receptor variant and developed the first antagonist for histamine-induced acid secretion – burinamide – which became the first H_2 receptor blocker.
- Ultimately, Black and his group were involved in the production of the world's first billion-dollar drug cimetidine [Tagamet] – the first clinically useful H_2 receptor blocker (1975).

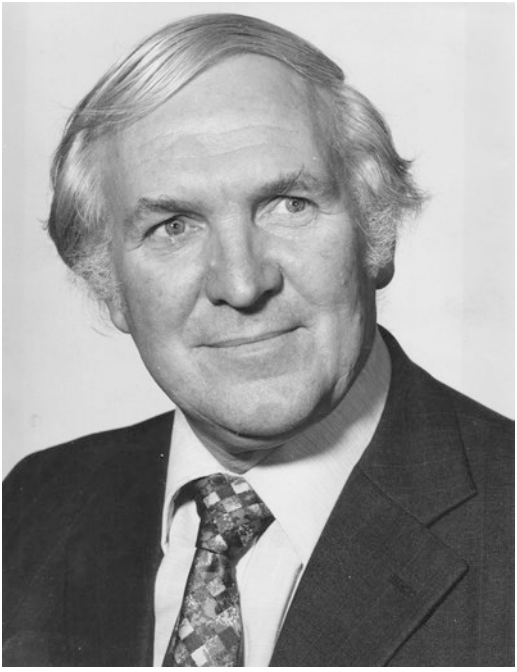

Fig. 1.13 Sir James W. Black. (From https://upload.wikimedia.org/wikipedia/commons/b/bd/Sir_James_W._Black.png. CC BY; reproduced)

1.2.14 Gertrude B. Elion (1918, New York, USA–1999, North Carolina, USA) (Fig. 1.14)

- Elion was the one who synthesized the purine analogs 6-mercaptopurine (6-MP) and thioguanine. These drugs transformed the treatment of leukemia.
  - She discovered these effective inhibitors of purine metabolism by substituting the oxygen atom with sulfur at the 6-position of guanine and hypoxanthine using *Lactobacillus casei* assay system.

1.2.15 George H. Hitchings (1905, Washington, USA–1998, North Carolina, USA) (Fig. 1.15)

- Hitchings along with Elion proposed that the antimetabolite-induced deficit in folic acid formation would result in abnormalities in the synthesis of purines and pyrimidines and consequently of DNA. Hitchings hypothesized that by utilizing the blockers of nucleic acid bases (analogs), the rapidly dividing cells like the neoplastic cells and bacteria can be halted from growing.
- Hitchings and his team went on to develop methotrexate (a structural analog of folate), pyrimethamine (antimalarial), and trimethoprim (antibacterial); the first antiviral acyclovir was also developed by them.

Fig. 1.14 Gertrude B. Elion. (From https://upload.wikimedia.org/wikipedia/commons/2/24/Nci-vol-8236 300_Gertrude_Elion.jpg. Public Domain; reproduced)

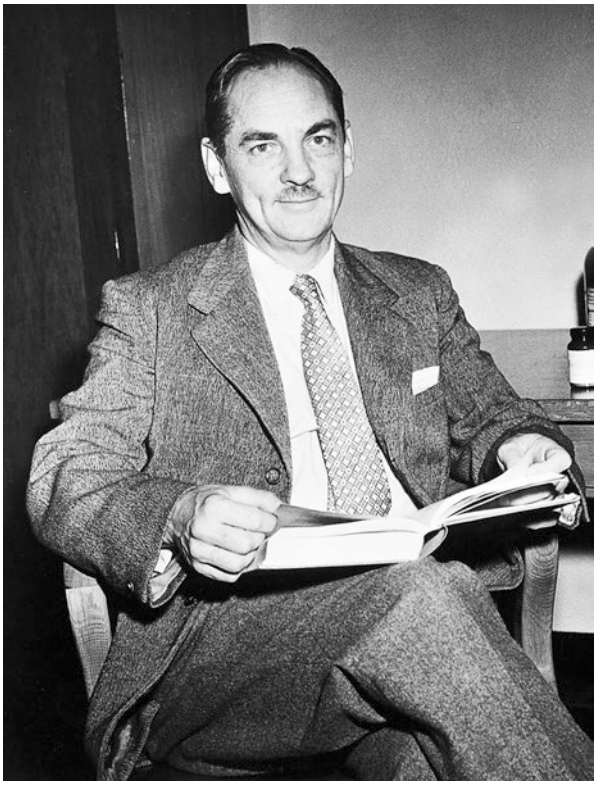

Fig. 1.15 George H. Hitchings. (From https://upload.wikimedia.org/wikipedia/commons/a/a1/George_H._Hitchings2.jpg. CC BY; reproduced)

- Together with Elion, Hitchings generated allopurinol for its putative antineoplastic effects, though later allopurinol was used for the management of gout and other forms of hyperuricemia.
- Black, Elion, and Hitchings received the Noble Prize (1988) "for their discoveries of important principles for drug treatment."

1.2.16 Paul Ehrlich (1854, Strzelin, Poland–1915, Bad Homburg vor der Höhe, Germany) (Fig. 1.16)

- Paul Ehrlich laid the foundation for modern chemotherapy.
- He formulated his own receptor theory of selective binding of nutritive substances and toxins; the binding of drugs to receptors was conceded by him comparatively later, and Ehrlich coined the term *chemoreceptors* (1907).
- It was Ehrlich's notion of the chemical substances selectively interacting with receptors present on the pathogenic microorganisms led to the early development of antibacterial agents.

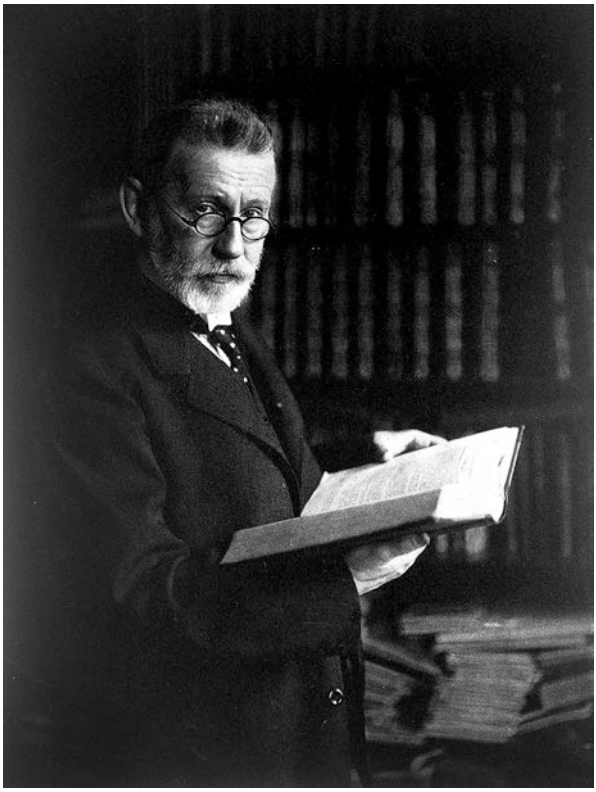

Fig. 1.16 Paul Ehrlich. (Photograph: portrait of P. Ehrlich in his. Credit: Wellcome Collection. CC BY; adapted)

- Ehrlich used methylene blue for treating patients with mild type of malaria. It was the first account of a synthetic drug being used effectively to treat a specific disease.
- He hypothesized that any infection can be controlled if the drug is specifically taken up by the microbe (*magic bullet*). Ehrlich after rigorous screening of more than 600 arsenical compounds identified that the 606th compound *(salvarsan or arsphenamine)* was active against the microbe *Treponema pallidum* which causes syphilis.
- Ehrlich was awarded the Nobel Prize in 1908 for "outlining the principles of selective toxicity and for showing preferential eradication of cells by chemicals." He shared the Prize along with Ilya Mechnikov.

1.2.17 Gerhard J. P. Domagk (1895, Lagow, Poland–1964, Burgberg, Germany) (Fig. 1.17)

- Domagk and his team at the I.G. Farben (a bygone German chemical and pharmaceutical conglomerate) worked on dyes which were derivatives of sulfonamide.

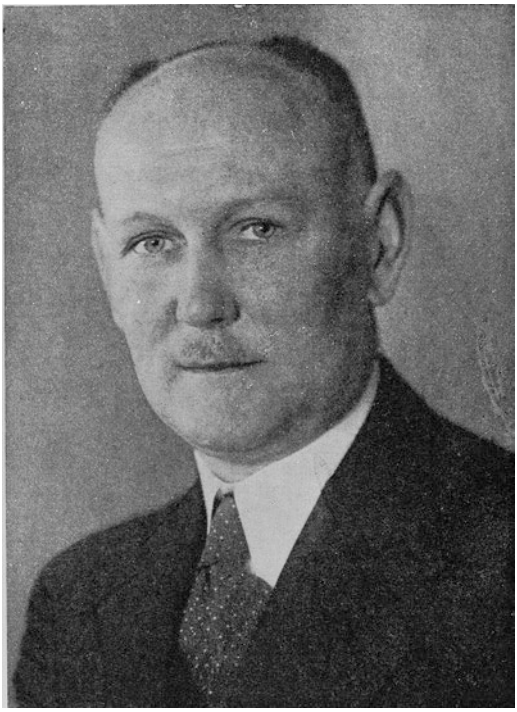

Fig. 1.17 Gerhard J. P. Domagk. (Portrait of Gerhard Domagk. Credit: Wellcome Collection. CC BY; adapted)

- The chemists at I.G. Farben produced a red dye and named it prontosil *rubrum* – it was found to be effective against *H. streptococci*. A 100% mouse protection rate with prontosil was pronounced by Domagk who was the then Research Director of I.G. Farben's Institute of Experimental Pathology (1935).
- Domagk successfully treated his daughter who had a needle prick injury-induced fatal septicemia (1935).
- The public acceptance of prontosil was possible when Franklin Jr., the son of the then President Franklin D. Roosevelt, was positively managed for tonsillitis (1936). *The New York Times* stated that "A new control for infections had been discovered."
- Prontosil was heralded as the first effective agent for treating systemic bacterial infections; however, the elixir (liquid) form of prontosil (containing the active moiety sulfanilamide) was attributed to the death of nearly 100 patients – the cause being acute kidney failure due to the solvent diethylene glycol (1937).
- The 1936 Nobel Prize was awarded to Domagk "for the discovery of the antibacterial effects of prontosil."

1.2.18 Sir Alexander Fleming (1881, Lochfield, Scotland–1955, London, United Kingdom) (Fig. 1.18)

- Fleming isolated an antibacterial material from the nasal passages of an acute rhinitis patient and termed it as *lysozyme*. The lysozyme was found to protect against certain nonpathogenic microbes from becoming virulent, and also it exerted a very different mode of action on these microorganisms.
- A spore from a mold got accidentally deposited in a staphylococci-impregnated Petri dish with agar in Fleming's lab. Returning back after several weeks of holidays, Fleming was perplexed to note that the area around the mold was completely clear without the growth of staphylococcal colonies (1928).
- Fleming coined the term *penicillin* to refer to the antibacterial substance. The susceptibility of streptococci, pneumococci, gonococci, meningococci, and diphtheria bacilli to penicillin was also deciphered by him; he also found that penicillin was more effective in overhauling gram-positive cocci than gram-negative bacilli.

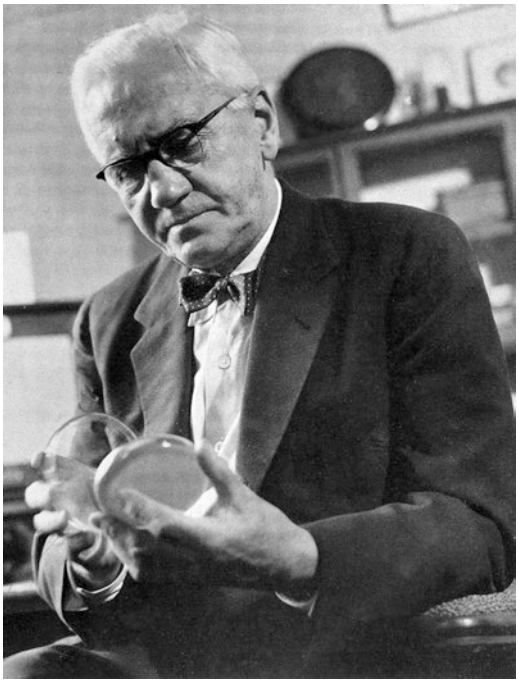

Fig. 1.18 Sir Alexander Fleming. (Sir Alexander Fleming. Credit: Wellcome Collection. CC BY; reproduced)

1.2.19 Ernst Boris Chain (1906, Berlin, Germany–1979, Mulrany, Ireland) (Fig. 1.19)

Fig. 1.19 Ernst Boris Chain. (From https://upload.wikimedia.org/wikipedia/commons/0/0a/Penicillin_Past%2C_Present_and_Future-_the_Development_and_Production_of_Penicillin%2C_England%2C_1944_D17806.jpg. Public Domain; reproduced)

1.2.20 Sir Howard Walter Florey (1898, Adelaide, Australia–1968, Oxford, United Kingdom) (Fig. 1.20)

- Chain and Florey confirmed the effectiveness of penicillin by pretreating a group of mice with penicillin and challenging the same with fatal doses of streptococci; they found that the group pretreated with penicillin survived 2 days longer than the other untreated and streptococci-infected mice group. Hence, they corroborated the antibacterial potential of penicillin against *Streptococcus pyogenes* and *Staphylococcus aureus* in animals (1940); the same was confirmed clinically (1941).
- Sir Alexander Fleming, Ernst Boris Chain, and Sir Howard Walter Florey were jointly awarded the Nobel Prize in 1945 "for the discovery of penicillin and its curative effect in various infectious diseases." Penicillin was rightly considered as the new "miracle drug" for terminally ill patients with infectious diseases.

Fig. 1.20 Sir Howard Walter Florey. (From https://upload.wikimedia.org/wikipedia/commons/d/d2/Howard_Walter_Florey_1945.jpg. Public Domain; reproduced)

1.2.21 Selman Waksman (1888, Nova Pryluka, Ukraine–1973, Massachusetts, USA) (Fig. 1.21)

Fig. 1.21 Selman Waksman. (From https://upload.wikimedia.org/wikipedia/commons/3/33/Selman_Waksman_NYWTS.jpg. Public Domain; reproduced)

- Waksman and his colleagues isolated actinomycin in 1940, streptomycin in 1944, and neomycin in 1949 from *actinomycetes*.
- Albert Schatz, a postgraduate assistant of Waksman, isolated one of the two streptomycin-producing strains of actinomycetes and subsequently extracted and tested the new antibiotic against various organisms, including the tubercle bacilli (1944). He obtained his Ph.D. degree based on this work from the Rutgers University (1945).
- It was Waksman who conceived the term *antibiotic* for categorizing the actions of streptomycin so as to resound the fact that the effect of one microbe (pathogen) is antagonized by a substance produced by another microorganism.
- Selman Waksman received the Nobel Prize "for his discovery of streptomycin, the first antibiotic effective against tuberculosis" in 1952. The Nobel Committee commended him as "one of the greatest benefactors to mankind" as his discovery of streptomycin had dramatically altered the prognosis for tuberculosis.

1.2.22 Sir Frederick Grant Banting (1891, Alliston, Canada–1941, Newfoundland, Canada) (Fig. 1.22)

Fig. 1.22 Sir Frederick Grant Banting. (Frederick Grant Banting. Photograph. Credit: Wellcome Collection. CC BY; reproduced)

1.2.23 Charles Herbert Best (1899, Maine, USA–1978, Toronto, Canada) (Fig. 1.23)

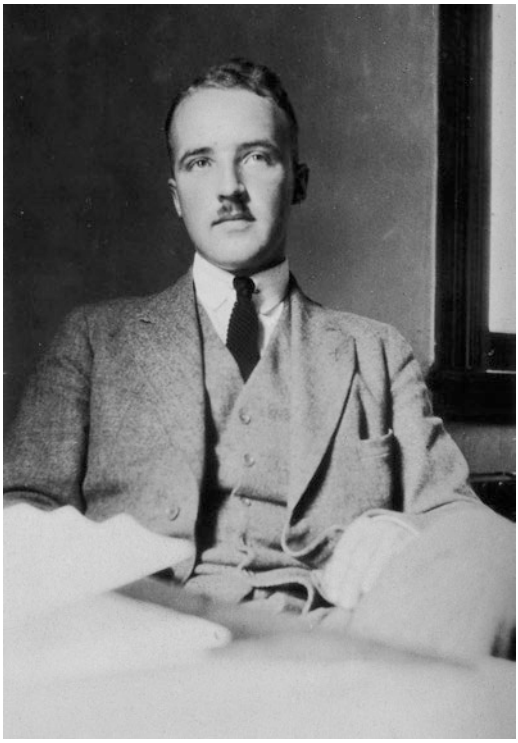

Fig. 1.23 Charles Herbert Best. (Charles Herbert Best. Photograph. Credit: Wellcome Collection. CC BY; reproduced)

1.2.24 John James Rickard Macleod (1876, Cluny, Scotland–1935, Aberdeen, Scotland) (Fig. 1.24)

Fig. 1.24 John James Rickard Macleod. (From https://upload.wikimedia.org/wikipedia/commons/7/70/John_James_Rickard_Macleod.jpg. CC BY; adapted)

1.2.25 James B. Collip (1892, Ontario, Canada–1965, Ontario, Canada) (Fig. 1.25)

Fig. 1.25 James Bertram Collip. (From https://upload.wikimedia.org/wikipedia/commons/c/c5/J._B._Collip_in_his_office_at_McGill_University_ca._1930.png. Public Domain; reproduced)

- The insulin secretion of the pancreas was first elucidated by Banting and Best (1922); they called the extract from the pancreas as *isletin*.
- Macleod, the Head of the Department of Physiology at the University of Toronto, guided Banting; Best, a 4th year student pursuing the honors physiology and biochemistry course, was nominated by Macleod to assist Banting.
- Collip not only successfully purified insulin but also devised effective methods for measuring blood sugar.
- The prolific and fruitful pursuit of Banting in the discovery and development of insulin is well portrayed by one of the National Historic Sites of Canada, namely, the *Banting House* also known as *the birth place of insulin*. In 1989, Queen Elizabeth kindled the *Flame of Hope* located at the center of Banting Square which is adjacent to the Historic Site. The Flame of Hope was lit to remind the fact that a cure to diabetes is yet to be known – till then the flame will not be extinguished.
- The 1923 Nobel Prize was jointly awarded to Banting and Macleod "for the discovery of insulin." Banting and Macleod later magnanimously shared their prize money with Best and Collip, respectively, with due recognition for their contribution.

1.2.26 Edward Calvin Kendall (1886, Connecticut, USA–1972, New Jersey, USA) (Fig. 1.26)

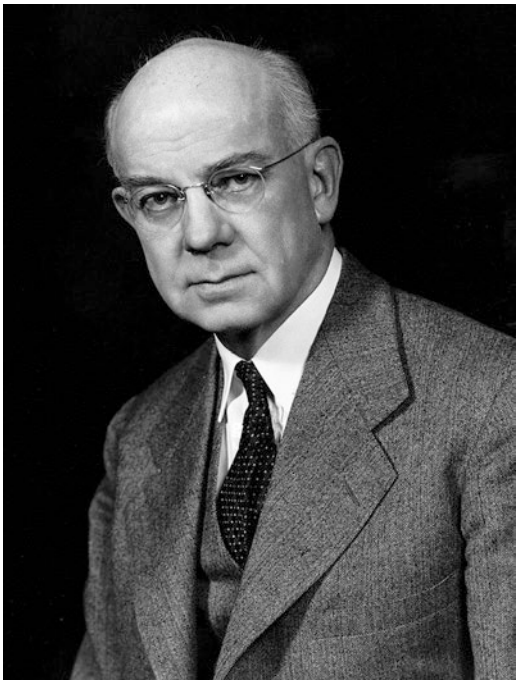

Fig. 1.26 Edward Calvin Kendall. (From https://upload.wikimedia.org/wikipedia/commons/4/41/Edward_Calvin_Kendall_1940s.jpg. Public Domain; reproduced)

1.2.27 Tadeus Reichstein (1897, Wloclawek, Poland–1996, Basel, Switzerland) (Fig. 1.27)

Fig. 1.27 Tadeus Reichstein. (From https://upload.wikimedia.org/wikipedia/commons/7/75/ETH-BIB-Reichstein%2C_Thadeus_%281897-1996%29-Portrait-Portr_00222.tif.Public Domain; reproduced)

1.2.28 Philip Showalter Hench (1896, Pennsylvania, USA–1965, Ocho Rios, Jamaica) (Fig. 1.28)

Fig. 1.28 Philip Showalter Hench. (From https://upload.wikimedia.org/wikipedia/en/a/a9/Philip_Showalter_Hench.jpg. Public Domain; reproduced)

- Edward Kendall was the first chemist to separate thyroxine (thyroid hormone) in crystalline form (1914). He also patented the thyroid hormone preparation and assigned the intellectual property rights to the University of Minnesota which was then affiliated with the Mayo Clinic.
- Philip Hench, the then Head of the Rheumatic Disease Service at the Mayo Clinic, postulated that a *substance X* was released by adrenal glands which aided in the recovery of symptoms related to arthritis (1929).
- Cortisone, called then as *compound E*, was isolated, identified, and synthesized by Kendall and his associates. They were also fruitful in extracting more than 20 chemically related compounds from adrenal glands (1937).
- Reichstein's laboratory in 1937 synthesized desoxycorticosterone – this facilitated in establishing the steroidal nature of the adrenal hormones, and also the dual distinct properties (mineral and carbohydrate metabolism) of these hormones were reaffirmed.
- Hench also deciphered the suppression of hypophyseal-pituitary-adrenal axis activity (negative feedback) by administration of supraphysiological doses of cortisone.
- Kendall, Reichstein, and Hench were the Nobel Laureates in 1950 "for their discoveries relating to the hormones of the adrenal cortex, their structure and biological effects."

1.2.29 Sune K. Bergström (1916, Stockholm, Sweden–2004, Stockholm, Sweden) (Fig. 1.29)

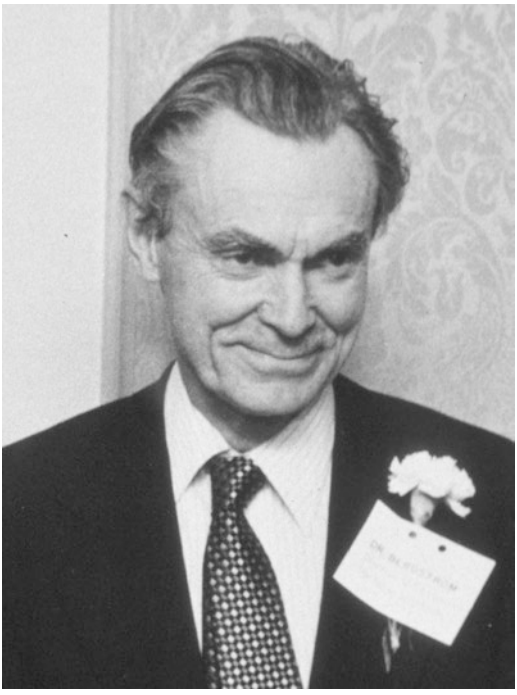

Fig. 1.29 Sune K. Bergström. (From https://upload.wikimedia.org/wikipedia/commons/f/fc/Sune_Bergstr%C3%B6m_3.jpg. Public Domain; reproduced)

1.2.30 Bengt Ingemar Samuelsson (b. 1934, Halmstad, Sweden) (Fig. 1.30)

Fig. 1.30 Bengt Ingemar Samuelsson. (From the National Library of Medicine, http://resource.nlm.nih.gov/101441459. Public Domain; reproduced)

1.2.31 Sir John Robert Vane (1927, Tardebigge, United Kingdom–2004, Farnborough, United Kingdom) (Fig. 1.31)

Fig. 1.31 Sir John Robert Vane. (Sir John Robert Vane. Photograph. Credit: Wellcome Collection. CC BY; reproduced)

- Bergström and his colleagues isolated and determined the chemical structure of prostaglandins (1962).
- Samuelsson mapped the main biosynthetic pathways of prostaglandin metabolism via the cyclooxygenase- and lipoxygenase-catalyzed reactions. In the process, he identified leukotrienes including those cysteine-containing leukotrienes (namely, LTB4, LTC4, LTD4, and LTE4) in various tissues and cells (1979).
- Bioassay methods rather than biochemical techniques as a mode of identification of products of arachidonic acid metabolism were documented by John Vane. He was also aware of the fact that bioassay could distinguish biologically relevant compounds from physiologically unimportant adducts.
- Vane proposed that the synthesis of prostaglandins could be inhibited by aspirin, and he experimentally reaffirmed the same with both aspirin and indomethacin.
- Vane also identified prostacyclin (PGI_2) which he initially labelled as *PGX* (1976).
- Bergström, Samuelsson, and Vane were awarded the Nobel Prize in 1982 "for their discoveries concerning prostaglandins and related biologically active substances."

1.2.32 Earl Wilbur Sutherland Jr. (1915, Kansas, USA–1974, Florida, USA) (Fig. 1.32)

- Sutherland started to study the effects of epinephrine and glucagon.
- Sutherland and Ted Rall demonstrated that the regulation of phosphorylase activity incorporated a balance between the contribution of a phosphate group to the enzyme (phosphorylation) and the repression of this process by a phosphatase (dephosphorylation).
- Sutherland and Rall discovered that glucose production by epinephrine was through cAMP signaling peptide (1962).
- Sutherland and his associates also identified the enzymes adenylyl cyclase and phosphodiesterase (1962).
- Sutherland's prophecy that the hormones act through generation of multiple "second messengers" was found to be true. He generalized that the raise in intracellular cAMP levels is obligatory for hormone action.
- The guiding words of Sutherland made a reluctant Alfred G. Gilman (discussed later) to choose the subject of pharmacology rather than biochemistry which was his initial personal interest.
- Earl Sutherland was the sole Nobel Laurette in 1971 "for his discoveries concerning the mechanisms of the action of hormones."

Fig. 1.32 Earl Wilbur Sutherland Jr. (From https://upload.wikimedia.org/wikipedia/commons/5/5c/Earl_Wilbur_Sutherland_Jr.jpg. Public Domain; reproduced)

1.2.33 Arvid Carlsson (1923, Uppsala, Sweden–2018, Gothenburg, Sweden) (Fig. 1.33)

Fig. 1.33 Arvid Carlsson. (From https://upload.wikimedia.org/wikipedia/commons/6/6e/Arvid_Carlsson_2011a.jpg. CC BY; reproduced)

1.2.34 Paul Greengard (1925, New York, USA–2019, New York, USA) (Fig. 1.34)

- Arvid Carlsson along with Brodie demonstrated almost a full depletion of serotonin from the neurons in the brain by using reserpine (1957); this marked the first instance of an association between certain intraneuronal biochemical changes in the brain with significant brain functions.
- Subsequently, Carlsson and Nils-Ake Hillarp found that reserpine could deplete catecholamines including epinephrine, norepinephrine, and dopamine and thereby, the sympathetic neurotransmission is blocked. They also observed that if a reserpine-treated animal is administered with DOPA, these effects are reversed (1957).
- Carlsson and Hillarp confirmed the much required method to localize dopamine, serotonin, and norepinephrine in the central nervous system (1959).
- Carlsson and his colleagues were instrumental in bringing L-DOPA in the management of Parkinson's disease (1959) and also for the generation of a novel class of drugs, namely, the SSRIs for the treatment of depression and anxiety (2001); the first SSRI to be used was zimelidine which was later withdrawn due to toxic effects.
- Paul Greengard and his team worked in continuation with the sustained efforts of Carlsson in deciphering the most intricate biological organ system, namely, the brain. They established that the enzyme adenylyl cyclase, the second messenger cAMP, and its dependent protein kinase A (PKA) activity were involved in the synaptic neurotransmission of the central nervous system like that of some of the peripheral organs (like liver) (1970).
- Greengard and his associates also identified the involvement of cGMP and Ca^{2+}-calmodulin-dependent kinases in the central neurotransmission (1978).
- The millennial Nobel Prize in the year 2000 was shared by Carlsson and Greengard with Eric R. Kandel (who is a physiologist, psychoanalyst, and neurobiologist) "for their discoveries concerning signal transduction in the nervous system."

Fig. 1.34 Paul Greengard. (From https://www.rockefeller.edu/our-scientists/heads-of-laboratories/1177-paul-greengard/. © Piotr Redlinski, The Rockefeller University; adapted with permission)

1.2.35 Martin Rodbell (1925, Maryland, USA–1998, North Carolina, USA) (Fig. 1.35)

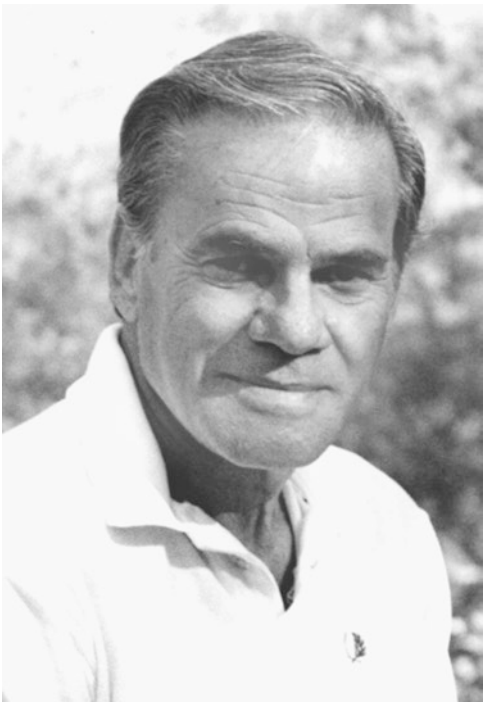

Fig. 1.35 Martin Rodbell. (From https://upload.wikimedia.org/wikipedia/commons/e/e7/Dr._Martin_Rodbell.jpg. Public Domain; reproduced)

1.2.36 Alfred Goodman Gilman (1941, Connecticut, USA–2015, Texas, USA) (Fig. 1.36)

- Martin Rodbell coined the term *signal transduction* (1969).
- Rodbell proposed that an intermediary was interposed between receptor (discriminator) and enzyme (effector/amplifier). He postulated a three-step mechanistic model to elaborate the steps involved in "cell signaling" incorporating a discriminator, transducer, and amplifier. This three-step hypothesis is considered to be a fundamental biological principle even today.
- As the transducer was bound to GTP and involved in the process of transmitting signals across the cell membrane, he theorized that the transducer be called as *G-proteins*. He also speculated the heterotrimeric (α, β, and γ subunits) structure of G-protein (1985).
- Later, Alfred G. Gilman categorically proved that G-proteins were obligatory for hormone action by utilizing a combination of genetic and biochemical techniques.

Fig. 1.36 Alfred Goodman Gilman. (From Bourne (2016). Copyright © 2016 by PNAS; reproduced with permission)

- Gilman and his co-workers isolated the α, β, and γ subunits of the G-protein. They also determined the conformational changes that occur in the G-protein complex during hormonal activation, namely, the dissociation of the α subunit from the β-γ subunit leading on to the activation of adenylyl cyclase by G_{α}-GTP (1983).
- Alfred G. Gilman was none other than the son of Alfred Gilman who initially co-authored (along with Louis Goodman) and then, edited the classical treatise on pharmacology, *The Pharmacological Basis of Therapeutics*, currently in its 13th edition. The textbook is reckoned as the "blue bible of pharmacology." The junior Gilman also contributed as an editor of the later editions of the book.
- Martin Rodbell and Alfred G. Gilman were jointly awarded the Nobel Prize in 1994 "for their discovery of G-proteins and the role of these proteins in signal transduction in cells."

1.2.37 Robert F. Furchgott (1916, South Carolina, USA–2009, Washington, USA) (Fig. 1.37)

Fig. 1.37 Robert F. Furchgott. (From SoRelle (1998). Copyright © 1998 by American Heart Association; reproduced with permission)

1.2.38 Ferid Murad (b. 1936, Indiana, USA) (Fig. 1.38)

Fig. 1.38 Ferid Murad. (From SoRelle (1998). Copyright © 1998 by American Heart Association; reproduced with permission)

1.2.39 Louis J. Ignarro (b. 1941, New York, USA) (Fig. 1.39)

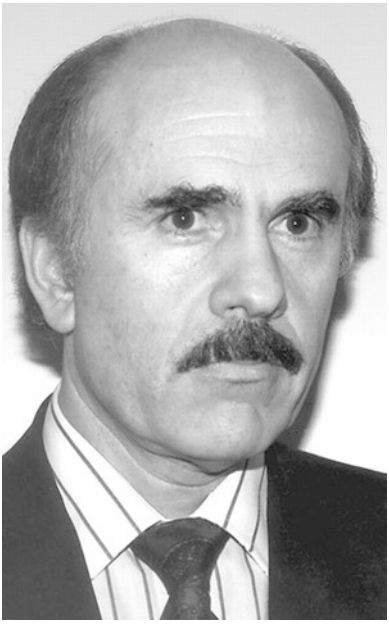

Fig. 1.39 Louis J. Ignarro. (From SoRelle (1998). Copyright © 1998 by American Heart Association; reproduced with permission)

1.2.40 Sir Salvador Moncada (b. 1944, Tegucigalpa, Honduras) (Fig. 1.40)

- Robert Furchgott, the then Chair of the Department of Pharmacology at The State University of New York Health Science Center, elucidated the possible role of endothelial cells in controlling vascular tone. He observed that the helical strip of rabbit aorta showed a contractile response to ACh (1953).
- In 1982, Furchgott proposed that the ACh after binding to the muscarinic receptors on the endothelial cell surface incite the release of an unknown product from the endothelium, and this product would then diffuse to adjacent smooth muscle cells to bring about relaxation. He also named this product as endothelium-derived relaxing factor (EDRF). Later, he also delineated the subsequent steps in the pathway as the released EDRF would stimulate the guanylyl cyclase of vascular smooth muscle causing the release of cGMP.
- Ferid Murad proposed that the nitric oxide (NO) formed from some unknown endogenous precursor could function as a triggering agent for cGMP synthesis in intact tissues (1978).

Fig. 1.40 Sir Salvador Moncada. (From https://www.thefamouspeople.com/profiles/salvador-moncada-5847.php. © FAMOUS PEOPLE; reproduced with permission)

- Louis Ignarro and his associates by using a bioassay setup had demonstrated that the vasorelaxation of isolated bovine coronary artery is due to the release of NO and accompanying raise in cGMP levels (1979).
- As the physiological characteristics of EDRF and the gaseous signaling molecule, NO, were found to be astoundingly similar, Furchgott proposed that the EDRF he identified was nothing but NO (1986). Ignarro independently proposed the same in the same year (1986).
- Salvador Moncada elucidated the synthetic pathway of NO with the involvement of the essential substrate L-arginine (amino acid) and the enzyme NO synthase (1994).
- The three pharmacologists Robert Furchgott, Ferid Murad, and Louis Ignarro were jointly awarded the Nobel Prize in 1998 "for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system."

1.2.41 Louis C. Lasagna (1923, New York, USA–2003, Massachusetts, USA) (Fig. 1.41)

- Louis Lasagna was the first to establish the Division of Clinical Pharmacology in the USA at the Johns Hopkins University, Baltimore (1954). He was the first to advocate the concept of placebo effect through his famous paper "A study of the placebo response" (1954) – the article was considered as one of the 27 most notable achievements in medical history by the *Lancet* in 1997.
- Lasagna played a major role in the institution of the Kefauver-Harris Amendment (1962) of the Food, Drug, and Cosmetic Act – which vouched for superior efficacy requirements for new drug approvals.
- He stressed for updating the Hippocratic Oath relevant to the current settings in the medical field around the globe and the same was put forth by him in 1964. Currently, the Lasagna's abridged version (Modern Physicians' Oath) is recited in many medical schools during graduation.

Fig. 1.41 Louis C. Lasagna. (From Wright (2003). Copyright © 2003 Tufts University; reproduced with permission)

- Lasagna established the Center for the Study of Drug Development (CSDD) at the University of Rochester in Rochester, New York, in 1976. The Center was later moved to Tufts University School of Medicine in Boston, Massachusetts (1984), along with Lasagna. There at the Tufts University, he was appointed as the first Dean to the newly formed Sackler School of Graduate Biomedical Sciences.
- The "Lasagna Committee" constituted under the chairmanship of Lasagna was instrumental in bringing up the Competitiveness Council FDA Reform Proposal by the then President George H.W. Bush (1991). The committee is also known as the "National Committee to Review Current Procedures for Approval of New Drugs for Cancer and AIDS."
- He had published around 650 articles, chapters, and books; the notable books are *The Doctors' Dilemmas* (1962) and *Life, Death and the Doctor* (1968). Apart from the placebo effect, he had written many papers on other clinical pharmacology-related areas including adverse effects and informed consent in clinical trials. Lasagna's contribution to the field of clinical pharmacology had begotten him the sobriquet the *Father of Clinical Pharmacology*.

1.3 Conclusion

From the magical potions and herbal remedies in 3000 BC, through the development of crude drugs and their derivatives in the nineteenth century and to the synthetic development of drugs including monoclonal antibodies, gene, and stem cell therapeutics, the sphere of pharmacology is burgeoning at a rapid pace currently.

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Indian Contributions to Pharmacology

2

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Wherever the art of medicine is loved, there is also a love of humanity.

Hippocrates

Cure sometimes, treat often, comfort always.

Hippocrates

Abstract

The history and knowledge of Indian pharmacology can be traced back to more than thousands of years. Ayurveda, the foundation of the Indian medical system, dates back to sixth century BC. The Unani system of medicine entered in the medieval period between the eighth and eighteenth century. The modern era of medicine was marked by the British invasion from the sixteenth century. Eminent Indians who worked in the field of pharmacology during this era include Sir Upendranath Brahmachari, Ram Nath Chopra (the *Father of Indian Pharmacology*), Prof. M. L. Shroff (the *Father of Pharmacy in India*), Yellapragada SubbaRow, Prafulla Chandra Ray, U. K. Sheth, Asima Chatterjee, Har Gobind Khorana and Ranjit Roy Chaudhury. Indian scientists like Sastry and Pandey have also contributed to the research in receptor pharmacology significantly at various institutes around the world. The Central Drug Research Institute (CDRI) has developed many drugs like α,β-arteether, centchroman, dalzbone, consap, gugulipid, bacosides and others. Since 1990, a large number of pharmaceutical companies have emerged in India and have set up their research and development centres, but still there exists a lacuna in the field of drug discovery and development.

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2.1 Introduction

Pharmacology is an interdisciplinary science which originated in collaboration with the preclinical and clinical disciplines. The word *pharmacology* was not used in print until the seventeenth century; however, around fourth century, the word *pharmacum* was used to denote a medicine or drug. In the late 1600s, Walter Harris in his book the *Course of Chemistry* adapted this late Latin term to *pharmacologia*.

2.1.1 The Vedic Era of Medicine

- The knowledge of pharmacology has been recorded for more than thousands of years across the world. The earliest known documentation of medical materials is the prehistoric Indian Ayurvedic essay from the sixth century BC. Two historical explications, considered to be the most important writings of ancient medicine, are *Charaka Samhita* and *Sushruta Samhita* which are considered the base of Indian system of Ayurvedic system (Indian traditional medicine); the writings in these treatises are followed even today by the current Ayurvedic practitioners as a basis for their practices.
- The origin of Hindu Vedas, namely, *Rig Veda*, *Sam Veda*, *Yajur Veda* and *Atharva Veda*, deals with various other aspects including health.
  - The Rig Veda, the earliest source of Ayurveda, means *panchamahabhuta*, i.e. the five basic elements of entire creation, the three doshas of primary forces of prana. These are the basics for Ayurveda in therapeutic science.
  - The Sam and Yajur Vedas assembled later during 4000 to 3000 BC and broadly associated with mantras and rituals which are considered important for maintaining good health.
  - Later, from 3000 to 2000 BC, Atharva Veda was compiled with the subset of Ayurveda (which means the *science of life*) which is now considered as the fifth Veda.
- The Sushruta Samhita is one of the foundational texts of Ayurveda. The Charaka Samhita's information on drug sources includes collection, preparation, extraction, storage and uses. The surviving text contains descriptions of 1120 illnesses and 700 medicinal plants with special emphasis on safety, efficacy, dosage and benefits. The surgery-related part of Ayurveda is *Dhanvantari* which emerged from *Lord Dhanvantari* (Lord Vishnu) who is worshiped as the *God of Health*; he is also surnamed as *Divodasa Dhanvantari* as a *King of Kasi* for his knowledge about surgery-related health.
- Sushruta, the follower of Divodasa, described surgery-related fractures, burns, amputation, wounds, plastic surgery and prosthetic surgery in his Sushruta

Samhita. Vital body points similar to Chinese acupuncture, human anatomy and vital parts surgery like the brain and eyes due to glaucoma and pterigium were also mentioned in Sushruta Samhita. All these works were revised by Nagarjuna (third or fourth century AD) and Dalhana (tenth century AD).

- Nagarjuna's knowledge of Siddha system of medicine from South India was significantly added to Ayurveda, and the golden period of Ayurveda was considered to be between 600 BC and 5 AD.
- Ayurveda also elaborated moral and ethical code for physicians similar to the Hippocrates oath. Friendship to all compassion for the ailing, devotion to professional duties and a philosophical attitude to diseases with fatal endings were the cornerstones on medical practice with Ayurveda.
- Across the Indian Ocean, trade and exchange of medicinal plants and knowledge of their uses have been occurring for many centuries between the Indian subcontinent, West Asia and other surrounding countries. The native Ayurvedic healers were largely influenced by the practices of the physicians hailing from Persia and other neighbouring regions. Moreover, the Ayurvedic treatises were also translated into Persian, Arabic, Tibetan and Chinese languages.

2.1.2 The Medieval Era of Medicine

- During the Medieval period between the eighth and eighteenth century AD which also marked the beginning of colonial domination, the growth of Ayurvedic medicine was sluggish owing to the political situations and foreign invaders.
- During the eighth and fourteenth centuries, a popular philosophical and religious movement appeared called *Tantrism*. When Tantrism started its development, alchemy played an integral part. Alchemy refers to the art and science of the use of metallic compounds, particularly of mercury and sulphur; use of iron, silver, tin and lead came in later.
- Though alchemy was mainly focused on basic metals like gold from mercury, it also has given some important contributions to the medicinal world through the preparation of various metallic products of medicinal use, especially the compounds of mercury that later on used as the Ayurvedic products by physicians for treating various diseases.
- Various formulations of copper, zinc and iron were considered to be very important from a medicinal point of view. As per Tantras undecaying and enduring of the body can be possible with the use of mercury preparations, yogic breathing and exercises and also by the use of gold prepared from the transmutation of metals.
- The elicitation of pulse was mentioned for the first time in the works of Tantric cult. Tantric treatises also elaborated about nervous system involvement in connection with the description of yogic exercises prescribed for devotees of the cult.
- While in Tantras, the seat of consciousness was connected with brain or cerebrospinal system, in Charaka and Sushruta, the heart was considered as the centre of

consciousness. So the Nadis of Tantra are the distinctive contribution to the Indian medicine, and they might have revived the idea of magic, miracles, rites and mysticism which could have been responsible for stagnation and decline of Indian medicine.

- During this period, the Unani or Greco-Arabic medicine system entered India along with the Muslims during their establishment in the eleventh century; the system also garnered enough backing for its growth. Exchange occurred with Indian and Arabic medicine as Indian physicians were invited to the court of the Arabian scholars, and they assisted the Arabic scholars in translating the Arabic medicine (Unani). Ali ibn Rabban in the book *Firdous al-Hikmah* ('Paradise of Wisdom') elucidated the complete system of Greek medicine as well as the Indian medical knowledge. In addition, many foreign scholars and physicians were invited from Persia, Khorasan and Europe by the Mughal Emperors and were encouraged to support in the development of Indian medicine.

2.1.3 The Modern Era of Medicine

- The modern era of medicine was marked by the British invasion in India, during which there occurred a stagnation of Indian medicine as the modern scientific civilization and its related-system of medicine emerged.
- The European physicians had arrived in India from the sixteenth century onwards as employees of the European trading companies, and they were trained in the current medical practices in Europe and took care of the medical needs of the company soldiers and officials, and these physicians later introduced the Western system of medicine in India. Western medicine was generally known as *Doctory* in India, and the practitioners were called *doctors*.
- The British East India Company dominated in eighteenth century employed many surgeons trained in Europe. The examinations conducted in India were for the advancement in service and for British aspirants in India. The Indian soldiers recruited by the company were the first Indians to receive medical treatment from British surgeons.
- Since the anatomical concepts in the Indian systems did not match European observations based on human dissections, the advances in European medicine in the seventeenth and eighteenth centuries led the British surgeons to believe that Western medicine was superior to the Indian systems.
- However, the Indian–European interactions continued despite these conceptual differences. Many European and Indian botanists and scholars also worked together in many Indian medicinal plants during this period, and this helped them to identify and classify indigenous drugs in scientific lines.

2.2 Eminent Personalities Who Worked in the Field of Pharmacology

2.2.1 Sir Upendranath Brahmachari (1873, Jamalpur, Bihar–1946) (Fig. 2.1)

- Upendranath Brahmachari started his career as a mathematician in 1893. He went on to complete his Masters in Chemistry. He then joined Calcutta Medical College and obtained his degree in Medicine and Surgery in 1899.

Fig. 2.1 Upendranath Brahmachari. (From https://upload.wikimedia.org/wikipedia/commons/6/6d/Upendranath_Brahmachari.jpg. Public Domain; reproduced)

Fig. 2.2 Ram Nath Chopra. (Courtesy: CSIR-Indian Institute of Integrative Medicine, From https://www.iiim.res.in/. reproduced)

- Subsequently, he worked with determination in a mean equipped facility in the Campbell Hospital to discover a new drug to cure the disease kala-azar. After 5 years of continuous laborious work, urea stibamine was finally discovered and became very essential for the treatment of kala-azar.

2.2.2 Ram Nath Chopra (1882, Gujranwala, Punjab–1973, Srinagar, Kashmir) (Fig. 2.2)

- Ram Nath Chopra, known as the *Father of Indian Pharmacology*, had his early education at Jammu, Srinagar and Lahore.
- After his studies in England, he returned to India and became the first professor of pharmacology in the newly established Calcutta School of Tropical Medicine in 1921.
- Before his arrival, only materia medica was taught in medical schools. There was no separate discipline of pharmacology.
- He had piloted many studies on general pharmacology and pharmacotherapy, along with many surveys on drug addiction.
- He was the first to establish a centre of study and research in pharmacology in India, at the Calcutta School of Tropical Medicine. The Central Drug Research Institute (CDRI) was set up in Lucknow largely through his efforts.
- Chopra was helped by B.N. Ghosh in his teaching and experimental work. His work has encouraged many research activities at various institutions, on the medicinal plants available in India.
- As a result of his work, many indigenous substances like ispaghula, kurchi, rauwolfia, psoralea and cobra venom were included in the Indian Pharmacopeia. Many books authored by Chopra and his associates entitled *Indigenous Drugs of India*, *Glossary of Medicinal Plants of India* and *Poisonous Plants of India* have been very popular encyclopaedia of Indian medicinal plants.
- His report (1931) on the restriction of the profession of pharmacy to qualified persons paved way for the establishment of many standard pharmacy institutions like L.M. College of Pharmacy, Ahmedabad, Madras Medical College and Birla College among others.
- Many pioneers in the pharmacy industry like Prof. M. L. Shroff (*Father of Pharmacy in India*) and Prof. P. C. Dandiya contributed towards the development of pharmacy colleges and post-graduation in pharmacology by science students.

2.2.3 Yellapragada Subbarow (1895, Bhimavaram, Andhra Pradesh – 1948, New York, USA) (Fig. 2.3)

- Yellapragada Subbarow was an ill-fated scientist who failed to acquire due recognition to his contributions in drug discovery.
- He worked at Harvard Medical School until 1940, followed by Lederle Laboratories for research. His extensive research led to the discovery of polymyxin, an antibiotic which is still in use.

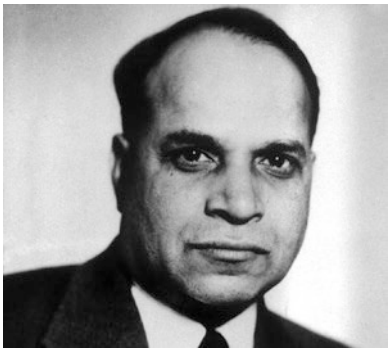

Fig. 2.3 Yellapragada Subbarow. (From https://madrascourier.com/biography/yellapragada-subbarow-the-immigrant-scientist-who-served-humanity/. Public Domain; adapted)

- Aureomycin, the first of tetracyclines, was discovered by him. His discovery of phosphocreatine and adenosine triphosphate as cellular energy sources has contributed to many research developments in the field of biochemistry.
- Fiske-Subbarow method of colorimetric determination of phosphorus in blood and urine has been one of the most cited papers.
- Many other discoveries like that of folic acid, aminopterin and vitamin B_{12} (the antipernicious factor) are also credited to him. A major breakthrough was the discovery of methotrexate, a derivative of aminopterin, which is still being used in various clinical conditions including malignancy.
- Another major discovery was that of diethylcarbamazine, the drug which is being used till date for the treatment of filariasis.

2.2.4 Prafulla Chandra Ray (1861, Jessore District, Bengal Presidency–1944, Calcutta, Bengal Presidency) (Fig. 2.4)

- Prafulla Chandra Ray was the one who set up the first chemical factory in India, with very minimal resources.
- In 1901, his pioneering effort ensued the formation of the Bengal Chemical and Pharmaceutical Works Ltd.
- After retirement, he donated all his salary for the rest of his service in the University to the development of the Department of Chemistry and also paved way for the creation of two research fellowships.
- Prafulla Chandra Ray, as a scientist and an industrial entrepreneur, can be considered as the *Father of the Indian Pharmaceutical Industry*.

2.2.5 Uttamchand Khimchand Sheth (1920, Mumbai, Maharashtra–2000, Mumbai, Maharashtra) (Fig. 2.5)

- U. K. Sheth was a physician turned pharmacologist, who lit the spark of Clinical Pharmacology at Seth G.S. Medical College in Mumbai, and he is rightly called as the *Father of Indian Clinical Pharmacology*. Following this clinical pharma-

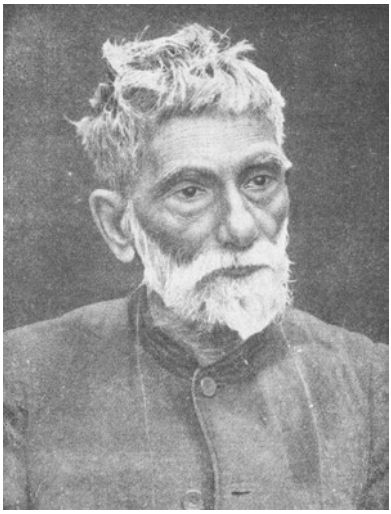

Fig. 2.4 Prafulla Chandra Ray. (From https://upload.wikimedia.org/wikipedia/commons/4/42/%E0%A6%86%E0%A6%A4%E0%A7%8D%E0%A6%AE%E0%A6%9A%E0%A6%B0%E0%A6%BF%E0%A6%A4_%28%E0%A6%AA%E0%A7%8D%E0%A6%B0%E0%A6%AB%E0%A7%81%E0%A6%B2%E0%A7%8D%E0%A6%B2%E0%A6%9A%E0%A6%A8%E0%A7%8D%E0%A6%A6%E0%A7%8D%E0%A6%B0_%E0%A6%B0%E0%A6%BE%E0%A6%AF%E0%A6%BC%29_005.tif. Public Domain; reproduced)

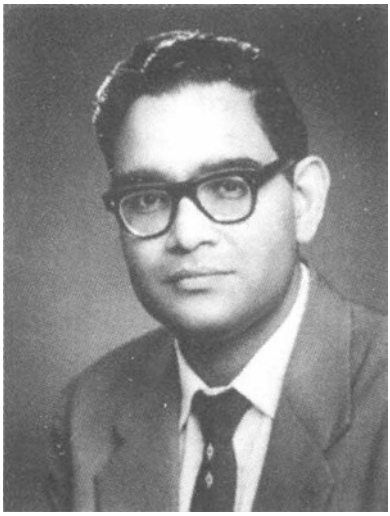

Fig. 2.5 Uttamchand Khimchand Sheth. (Courtesy: The National Medical Journal of India. From http://archive.nmji.in/archives/Volume-13/issue-6/obituaties-2.pdf; adapted)

cology services were started in various other institutes like Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, Nizam's Institute of Medical Sciences (NIMS), Hyderabad and Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry.

- He has played a vital role in the research and development of several drugs like triazine diuretics, triamterene and diethylcarbamazine. The most important

research activity of U.K. Sheth was that of his active role in the clinical development of mefloquine for malaria, which has saved many patients suffering from chloroquine-resistant malaria.

- U. K. Sheth, B. N. Dhawan, V. N. Sharma and few others helped Govinda Achari to establish the *Indian Pharmacological Society (IPS)* and made it an independent society in India. R. N. Chopra was elected as the founder president of IPS, and the first conference of IPS was held in Patna.
- He was also awarded the B. C. Roy Award in 1978.

2.2.6 Asima Chatterjee (1917, Kolkata, India–2006, Kolkata, India) (Fig. 2.6)

- Asima Chatterjee proved that Indian women can make an important scientific contribution to medicine by doing research on periwinkle-derived alkaloids (commonly known as the vinca alkaloids) that have anticancer properties. She also contributed to the development of few antimalarial and antiepileptic drugs.

2.2.7 Har Gobind Khorana (1922, Raipur, Punjab–2011, Massachusetts, USA) (Fig. 2.7)

- Har Gobind Khorana obtained his Ph.D. degree from the University of Liverpool. At the age of 46, his contribution towards elucidating the genetic code was recognized, and he shared the Nobel Prize for Physiology or Medicine in 1968 with Robert W. Holley and Marshall W. Nierenberg.
- In 1972, at the Massachusetts Institute of Technology, Har Gobind's team had a major accomplishment in chemical biology by describing the complete chemical synthesis of a functional tRNA gene of yeast. He was the first to synthesize an artificial gene into a living cell.

Fig. 2.6 Asima Chatterjee. (Courtesy: Feminism in India (FII). From https://feminisminindia.com/2017/11/02/asima-chatterjee-pioneer-medicinal-chemistry/; reproduced with permission)

Fig. 2.7 Har Gobind Khorana. (Courtesy: The Asian Age. From https://dailyasianage.com/news/102986/har-gobind-khorana; reproduced)

- Khorana's methods of extension of DNA polymers using enzymes like polymerase and ligase are extensively used in all biological laboratories for sequencing, cloning and engineering new organisms.

2.2.8 Ranjit Roy Chaudhury (1930, Patna, Bihar–2015, Chennai, Tamil Nadu) (Fig. 2.8)

- Brought up from an elite family, Ranjit Roy Chaudhury pursued his medical education at the prestigious Prince of Wales Medical College at Patna (now rechristened as Patna Medical College). He undertook D.Phil at the Oxford University where Sir John Vane, the Nobel Laurette, was one of the academic members.
- After a short stint as Assistant Professor at the All India Institute of Medical Sciences (AIIMS) for 2 years, Ranjit joined the CIBA Company, a modern drug discovery centre located at Goregaon, Mumbai.
- Once again, his academic pursuit made him join the PGIMER, Chandigarh, and was instrumental in setting up the first super-speciality course in Pharmacology (not only in India but also as of Asia), namely, the Doctor of Medicine (D.M.) course in Clinical Pharmacology in 1978. Later, he rose to the position of Dean of the reputed institute.
- Ranjit also held the post of Chairman of the Pharmacology and Toxicology panel in the Indian Council of Medical Research (ICMR); he also served other national organizations and also the WHO at various capacities.
- He was the founder of 'Delhi Society for Promotion of Rational Use of Drugs (DSPRUD)' through which the 'Delhi Model' of Rational Use of Medicines was rolled out (mid-1990s). The model made provision for access to free medicines for nearly 90% of the drugs prescribed in the government hospitals of Delhi.

Fig. 2.8 Ranjit Roy Chaudhury. (From Vaidya (2016). http://www.jomrjournal.org/text.asp?2016/3/1/54/184183; CC BY; reproduced)

- He was part of various national committees involved in policy-making. Ranjit was a member of the committee which was involved in drafting the first ethical guidelines on research in human subjects by ICMR (1980).
- Prof. Ranjit Roy Chaudhury expert committee was constituted to formulate policy and guidelines for approval of new drugs, clinical trials and banning of drugs. The committee's report was released in July 2013, and the same was accepted by the Government of India.
- Another committee to reform the Indian Medical Council Act, 1956, was constituted in July 2014 with a Group of Experts (GoE) under the patronage of Prof. Ranjit Roy Chaudhury. The committee's report was submitted in February 2015, and the same was scrutinized by the Parliamentary Standing Committee on Health and Family Welfare for further processing.
- One of the major highlights of the Prof. Ranjit Roy Chaudhury Committee's outcomes is the proposal to quash the autonomous body, namely, the Medical Council of India (MCI), and to replace the same with the proposed National Medical Commission (NMC) with the following four different vertical boards:
  - Under-Graduate Medical Education Board (UGMEB)
  - Post-Graduate Medical Education Board (PGMEB)
  - Medical Assessment and Rating Board (MARB)
  - Board for Medical Registration (BMR)
- The highest post he held was the Advisor to the Ministry of Health and Family Welfare, Government of India (2014).

2.3 Indian Contribution to Receptor Pharmacology

Indians have contributed to the research in pharmacology significantly. Indian investigators have contributed to the knowledge of drugs at various laboratories in several academic institutions and pharmaceutical industries including many private and government agencies throughout the world.

2.3.1 Sastry

Sastry has explored a series of asymmetric analogues of acetylcholine. He and his colleagues proposed the various orientations of main functional groups of acetylcholine which interact with muscarinic and nicotinic receptors. They also put forth the molecular conformations that take place during the activation of cholinergic receptors. Khorana and his colleagues at MIT systematically investigated these molecular conformational changes in detail.

2.3.2 G. N. Pandey

G. N. Pandey and his colleagues, at Chicago, have performed exhaustive research on the biogenic amines and their involvement in the pathogenesis of mental disorders. In depressed patients with suicidal intentions, they delineated abnormalities of alpha-2 adrenergic receptors and the associated adenylate cyclase system, and they also demonstrated the increased expression of $5HT_{2A}$ receptors on the platelets in the same group of patients. They also found an increased expression of benzodiazepine receptors and also a decrease in phospholipase C and protein kinase C enzymes in the brain tissues of teenage suicide victims.

2.3.3 C. Prasad

C. Prasad and his colleagues, at the Louisiana State University, New Orleans, have documented a new cyclic dipeptide cyclo (His-Pro) in the nervous system. They also showed the existence of a different endogenous enterostatin-like peptide in the brain and about its vital role in the modulation of insulin secretion and appetite**.**

2.3.4 S. Dutta and B. H. Marks

S. Dutta and B. H. Marks**,** at the Ohio State University, had studied in detail about the mechanism of action of cardiac glycosides and related drugs. Owing to the sustained interest in natural products, by the researchers group at Ohio, elaborate studies were performed on the pharmacology of compounds like the thalictrum alkaloids.

2.3.5 Gulati

Gulati, in Chicago, has done extensive research on endothelin agonists and antagonists, to unravel their functional roles. His studies showcase the involvement of the endothelin modulators in central cardiovascular effects and also in the pathogenesis of hypertension.

2.3.6 Kohli and Goldberg

Kohli and Goldberg were attributed with the blood flow increasing capacity of dopamine in kidney to the dopamine receptors. Subsequently, many subtypes of dopamine receptors were discovered. Kohli et al. reported the D_1 agonist activity of dihydrexidine. Supplementary dopaminergic actions in kidney were more comprehensively studied by Lokhandwala and co-workers.

2.3.7 P. Dasgupta and S. Basu

P. Dasgupta and S. Basu made a breakthrough discovery stating that dopamine does not just work as a mood enhancer but can also assist in killing tumour cells.

2.3.8 Bhattacherjee and Paterson

Bhattacherjee and Paterson have investigated the distribution of 3H-prostaglandin (PGE_2) and its binding to ocular tissues of rabbit and bovine membrane.

2.3.9 Patel

Patel and co-workers, at the University of Tennessee, examined the association between the epidermal growth factor and the increase in cAMP concentrations. Murad along with Mittal and other coinvestigators purified guanylate cyclase, the important regulator of cyclic GMP. They also confirmed that nitric oxide or sodium nitroprusside can activate the guanylate cyclase, which formed the basis of the Nobel Prize for Murad.

2.3.10 G. Krishna

G. Krishna and his colleagues have studied about the production of nitric oxide by inducing the nitric oxide synthase expressed in human retinal, cerebellar and skeletal muscle tissues.

2.3.11 Verma

Verma from the Salk Institute has contributed enormously to our understanding of the signal transduction pathways of the NF-Kappa B family of transcription factors which assist in cellular growth, differentiation and apoptosis.

2.3.12 Sengupta et al.

Sengupta et al. studied the role of sensory nerves in rat colon. Colorectal distension mediated by opioid agonists on these sensory nerves was investigated by him.

2.3.13 Swamy

Swamy, Triggle and co-workers have studied the role of calcium in vascular activity of drugs. They have determined that nifedipine, a calcium channel antagonist, was a potent inhibitor of both norepinephrine- and potassium-induced responses.

2.3.14 Lal

Lal and others made an interesting observation with regard to the regulation of vascular tone by vitamin B_6, which inhibited the influx of Ca^{2+} across the cell membrane. He also stated that on supplementation with vitamin $B_{6,}$ the blood pressure of hypertensive rats can be reduced.

2.3.15 Bhagat

Bhagat has done research on the diminished responsiveness of endotoxin-treated guinea pig atria to norepinephrine. It was shown that the endotoxin could be acting at level of heart cell membrane.

2.3.16 Kumar et al.

Kumar et al. have studied and reported about gels as ophthalmic delivery systems. He has proposed that it can be produced by a mixture of carbopol and methylcellulose with the aid of cone and plate viscometry.

2.4 History and Contributions of Central Drug Research Institute (CDRI) in Pharmacology

The Council of Scientific and Industrial Research (CSIR), New Delhi, established the Central Drug Research Institute (CDRI) at Lucknow as the National Laboratory for Drug Research. The then Prime Minister and the President of CSIR, Pt. Jawaharlal Nehru, inaugurated the Institute on 17 February 1951. The spectrum of activities includes phase I to IV clinical and pharmacokinetic studies of CDRI-developed drugs, limited clinical studies with other selected compounds, bioavailability and bioequivalence studies and in vitro studies on human tissues.

Table 2.1 Drugs developed in the Central Drug Research Institute (CDRI)

Drug/product	Use	Licensee with year of license
α β-Arteether	Antimalarial	Themis Medicare Ltd., Mumbai (1997)
Centchroman	Contraceptive	HLL Lifecare Ltd., Thiruvananthapuram (1990); Torrent Pharma. Ltd., Ahmedabad (1991)
	Dysfunctional uterine bleeding	
Bacosides Enriched Standardized Extract of *Bacopa* (BESEB)	Memory improvement	Lumen Marketing Co., Chennai (2002)
Dalzbone	Rapid fracture healing	Pharmanza Herbal Pvt. Ltd., Gujarat (2015)
Centimizone	Antithyroid	Unichem Lab. Ltd., Mumbai (1972)
Elubaquin	Anti-relapse antimalarial	Nicholas Piramal India Ltd., Mumbai (1999)
Centpropazine	Antidepressant	Merind Ltd., Mumbai (1996)
Chandonium iodide	Neuromuscular blocker	Ranbaxy Labs Ltd., New Delhi (1987); Cipla Ltd., Mumbai (1995)
Consap	Spermicidal cream	HLL Lifecare Ltd., Thiruvananthapuram (2004)
Centbutindole	Neuroleptic	Chemosyn Pvt. Ltd., Mumbai (1987)
Centbucridine	Local anaesthetic	Themis Chemical Ltd., Mumbai (1987)
Gugulipid	Hypolipidemic	Cipla Ltd., Mumbai (1987); Nicholas Piramal India Ltd., Mumbai (2000)
Isaptent	Cervical dilatation	Unichem Lab. Ltd., Mumbai (1972)

Adapted from https://cdri.res.in/newDrugs_cdri.aspx

Box 2.1: Drugs Developed in the Central Drug Research Institute (CDRI) Assigned with International Non-proprietary Names (INNs)

Drug	Activity	INN
Centbucridine	Local anaesthetic	Bucricaine
Centbutindole	Neuroleptic	Biriperone
Centchroman	Contraceptives	Ormeloxifene
Centimizone	Antithyroid	Mipinazole
Compound 80/53	Antimalarial	Elubaquin

- Phase I clinical studies of more than 27 products have been conducted in CDRI which includes natural products, vaccines and synthetic compounds.
- Phase II clinical studies have been conducted in more than 31 products which included many diagnostic kits.
- Phase III clinical studies have been conducted in more than 20 products till date. The list of drugs licensed and marketed after the clinical studies are mentioned in Table 2.1.
- WHO assigns International Non-proprietary Name (INN) to selected new drugs. Few of the drugs developed by CDRI have achieved this distinction and were awarded INN by WHO (Box 2.1).

- CDRI has created many pilot plants for chemical and fermentation technology primarily to bulk produce its own drugs for toxicology and clinical trials. They have also scaled up and refined the technology to make it suitable for transfer to the pharmaceutical industry.
- CDRI offers technology transfer to new CDRI drugs, for other generic drugs and for other drug intermediates. CDRI is a major clearinghouse for drug-related information in the country. It is one of the National Patent Centres of the country.
- CDRI is perhaps the only national laboratory having in-house facility for total development of new drugs beginning with the identification of 'New Chemical Entity' from synthetic or natural source and ending in clinical studies.

2.5 Role of Pharmaceutical Industry in the Development of Pharmacology in India

- The Indian pharmaceutical industry has been making tremendous progress in the domestic and international market. Before 1960, there was no industrial house in India, for doing real-world pharmacological research. In 1963, a multidisciplinary research centre was opened by Ciba Research Centre in Bombay, followed by Sarabhai chemicals in 1964, Hoechst AG in 1972, Smith, Kline and French and Boots pharmaceuticals in 1981 and Astra in 1984.
- The objective of these industrial centres was to develop new chemical entities with relevance to Indian market. After 1990, a large number of pharmaceutical companies have emerged in India and have set up their research and development centres in India. It includes companies like Sun Pharma, Ranbaxy, Dr. Reddy's Laboratory, Zydus Cadila, Lupin, Wockhardt, Dabur, Torrent, Glenmark and others.
- While the Indian Pharmaceutical Industry has made excellent progress over the past few decades in the field of drug production and formulation, there is no equal amount of contribution in the field of drug discovery and development.

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Part II

General Pharmacological Principles

Sources and Nature of Drugs

3

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Abstract

Drugs are substances or products that are used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient. It is only logical that these drugs might arise from several sources. The sources of drugs have travelled through a complete arc, being derived mainly from natural sources in the early centuries to being synthetically manufactured today. There is also a grey area in between, which constitutes the semi-synthetic sources. While natural alkaloids and glycosides are still used today, the synthetically manufactured drugs generally have higher yields that are significantly associated with quality, purity and low cost. This chapter deals with almost all the sources of drugs known to man. The nature of drugs relates to the physical or chemical properties of drugs, in general. These properties including drug matter, drug size, drug shape and drug bonds, and others are also explained in brief at the end of this chapter.

Keywords

Chemical name · Generic name · Proprietary name · Alkaloids · Glycosides

3.1 What Is a Drug?

- A drug is the active chemical entity that is present in a prescribed medicine, which is used for diagnosis, prevention, treatment or cure of a disease. However, this definition excludes contraceptives and drugs used for improvement of health.
- The term "drug" is derived from a French word "drogue", meaning "dried herb".
- In 1966, WHO defined "drug" as "any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient".

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3.2 Nomenclature of Drugs

Every drug has three different names, as follows:

3.2.1 Chemical Name

- Chemical name depicts the drug's chemical nature. However, this is too cumbersome to remember, and hence never used practically when prescribing. For example, (RS)-2-(4-(−2-methylpropyl) phenyl) propanoic acid is the chemical name of ibuprofen, a common NSAID. As evident from this example, chemical names are long, confusing and troublesome to use.

3.2.2 Non-proprietary or Generic Name

- The non-proprietary or generic name is the one that is authoritatively accepted by a scientific body. The scientific body may be country-specific, and hence, different countries might end up having different names for the same drug.
- To avoid ambiguity, all member nations of the WHO signed an agreement to use a single recommended International Non-proprietary Name (rINN) for each drug. For example, ibuprofen is a non-proprietary name.
- Despite the rINN agreement, due to widespread use, a few of the older drugs still have more than one non-proprietary name, e.g. lignocaine–lidocaine and pethidine–meperidine.

3.2.3 Proprietary or Brand Name

- Proprietary name is the name that is owned and designated by the manufacturer. For example, ibuprofen is marketed under various brand names like Brufen®, Combiflam®, Unafen®, Advil®, etc.
- Unlike the chemical and non-proprietary names, the proprietary names are easy to remember, short, catchy and most often suggestive of the drug component.

3.3 Sources of Drugs

Drugs have been in use from at least as early as 2700 BC in Asia and the Middle East regions. Until the advent of the twentieth century, every drug was derived from natural sources, of which plants took up the major share. With the introduction of technology, most drugs today are manufactured synthetically in the laboratory.

The major sources of drugs can be grouped into the following.

3.3.1 Natural Sources

3.3.1.1 Plants as Drug Sources

Among the natural sources, plants contribute to a vast majority of drugs. Extracts from plants may either be used without further processing (crude drugs) or with technical processing (prepared drugs). Although the earliest plant source for drugs was the leaf, other parts of plants were also later exploited for drug extraction. A few drugs can be extracted from more than one plant part (Box 3.1).

The pharmacologically active principles derived from plants may be of different categories, as mentioned below:

- **Alkaloids**
  - Alkaloids (meaning "alkali-like") are complex, organic, bitter-tasting, alkaline- and nitrogen-containing compounds, mostly obtained from plants. They may also be obtained from animal sources.
  - These are readily soluble in alcohol but sparingly in water. However, their salts (produced when alkaloids are combined with acid) are water-soluble.
  - Names of alkaloid-based drugs are suffixed with "-ine".
  - Alkaloids can be classified based on the source, as follows:
    Belladonna alkaloids – atropine, scopolamine (hyoscine)
    Cinchona alkaloids – quinine, quinidine
    Cocaine alkaloids – cocaine, cegonine
    Ergot alkaloids – ergotamine, ergometrine
    Opium alkaloids – morphine, codeine
    Rauwolfia alkaloids – reserpine, cevadine
    Xanthine alkaloids – caffeine, theophylline
- **Glycosides**
  - Glycosides are non-nitrogenous, colourless, crystalline solids.

Box 3.1: Drugs Obtained from Various Parts of the Plant

Leaves	**Flowers**	**Fruits**	**Seeds**
Digoxin, digitoxin (from *Digitalis purpurea*/foxglove plant); atropine (from *Atropa belladonna*)	Vincristine, vinblastine (from *Vinca rosea*)	Physostigmine (from *Physostigma venenosum*/calabar bean)	Strychnine (from *Nux vomica*); physostigmine (from *Physostigma venenosum*/calabar bean)
			Morphine (from *Papaver somniferum*)
Roots	**Bark**	**Stem**	
Emetine (from *Cephaelis ipecacuanha*); reserpine (from *Rauwolfia serpentina*)	Quinine (from *Cinchona*); atropine (from *Atropa belladonna*)	Tubocurarine (from *Chondrodendron tomentosum*)	

  - They are comprised of a sugar component (glucose) and a non-sugar component (aglycone or genin). The non-sugar part is the part that is responsible for its pharmacological activity. The sugar component is responsible for the pharmacokinetic properties of the drug.
  - Classical examples of glycosides include digoxin and digitoxin (called as "cardiac glycosides"), salicylic acid and others.
  - However, "aminoglycosides" are not grouped under glycosides, although they contain a glycosidic bond (-O-) in their chemical structure.
  - Similarly, nitrogen-containing glycosides are called "cyanogenic" glycosides (e.g. amygdalin) and not always grouped under glycosides.
  - Glycosides are generally classified based on the sugar component present in them.
    Glucosides: glucose-containing glycosides
    Galactosides: galactose-containing glycosides
    Fructosides: fructose-containing glycosides
- **Saponins**
  - Saponins are non-nitrogenous compounds (mostly glycosylated steroids), with the characteristic quality of "frothing".
  - On hydrolysis, they get split into a sugar and an aglycone (sapogenin), e.g. senegin and glycyrrhizin.
- **Oils**
  - Oils are liquids that are insoluble in water. The three major types of oils are:
    Essential oils are concentrated hydrophobic oils, containing aromatic volatile compounds. They do not leave stains on evaporation, e.g. clove oil, eucalyptus oil and peppermint oil. Essential oils are subdivided into hydrocarbons (terpenes) and oxygenated compounds.
    Fixed oils are esters of higher fatty acids (oleic acid, palmitic acid, stearic acid) and glycerines. They are non-volatile and leave a permanent stain on evaporation, e.g. castor oil, olive oil and chaulmoogra oil.
    Mineral oils are colourless, odourless and light mixtures of higher alkanes from mineral sources, e.g. liquid paraffin, hard paraffin and soft paraffin.
- **Waxes**
  - Waxes are esters of higher fatty acids and higher monohydric alcohols. They have high melting points, e.g. beeswax.
- **Gums and Mucilages**
  - These are complex, amorphous, colloidal, secretory hydrocarbon polysaccharides of plant origin.
  - Gums readily dissolve in water, while mucilages become slimy. For example, agar, acacia and psyllium seeds are gums, while tragacanth is mucilage.
- **Resins**
  - Resins are amorphous, bitter, water-insoluble polymers of volatile oils, e.g. asafoetida, benzoin, shellac and colophony.
- **Tannins**
  - Tannins are non-nitrogenous, bitter, water-soluble phenolic derivatives, most commonly used as astringents, as they precipitate surface proteins, e.g. tannic acid.

- **Toxins**
  - These can be simply defined as poisons of plant origin, although they might have therapeutic effects, e.g. Botulinum toxin.

3.3.1.2 Animals as Drug Sources

Drugs from animal sources are lesser in number. However, a few vital drugs have animal sources. Animal sources may be any of the following:

- **Whole Animals**
  - Hirudin and heparin are obtained from the European medical leech (*Hirudo medicinalis*) and the Mexican medical leech (*Hirudo manillensis*), respectively.
- **Organs of Animals**
  - Cod liver oil from *Gadus* spp., insulin from bovine or porcine pancreas (though recombinant insulins are used nowadays), vitamin B_{12} extracts from liver and various antitoxic sera.
- **Glandular Products or Extracts**
  - Vaccines, sera, antitoxins, antidotes and hCG.

3.3.1.3 Microbiota as Drug Sources

Several life-saving drugs have been historically derived from micro-organisms (Table 3.1).

3.3.1.4 Minerals as Drug Sources

Minerals have been used as drugs since ancient times. Both metallic and non-metallic minerals are commonly used as drugs (Table 3.2).

3.3.2 Semi-synthetic Sources

- When the nucleus of the drugs obtained from natural sources is kept intact while altering the chemical structure, the resultant products are semi-synthetic drugs.
- Homatropine (from atropine), heroin (from morphine), bromoscopolamine (from scopolamine) and ampicillin (from penicillin) are some of the examples. As evident from these examples, semi-synthetic drugs can be extensions of plant or animal sources.

Table 3.1 Drugs from microbial sources

Drug	Micro-organism
Penicillin	*Penicillium notatum*
Chloramphenicol	*Streptomyces venezuelae*
Streptomycin	*Streptomyces griseus*
Neomycin	*Streptomyces fradiae*
Bacitracin	*Bacillus subtilis*

Table 3.2 Minerals as drug sources

Metallic or non-metallic	Metallic mineral	Use
Metallic	Ferrous sulphate	Haematinic
	Magnesium sulphate	Purgative
	Bismuth subnitrate	Antiseptic
	Aluminium hydroxide	Antacid
Non-metallic	Potassium iodide	Expectorant
	Hydrogen peroxide	Antiseptic
	Iodine	Antiseptic
	Radio-isotopes of iodine	Theranostics of thyroid disorders

3.3.3 Synthetic Sources

- Unlike semi-synthetic drugs, synthetic drugs are those in which both the nucleus and the chemical structure are altered or modified.
- These are exclusively prepared in the laboratory. One of the earliest synthetic drugs was sulphonamide, which began with the synthesis of prontosil dye. A vast majority of the modern-day drugs are manufactured synthetically.

Synthetic drugs have many advantages over natural and semi-synthetic sources of drugs, as mentioned below:

- Chemical purity
- High quality, which can be manually controlled
- Improved safety profile, particularly in terms of less antigenicity
- Cost-effective preparation methodology

A subcategory of synthetic drug sourcing is the "biosynthetic" category. Biosynthetic source (genetically engineered drug) is a relatively new field that has been developed by mixing discoveries from molecular biology, recombinant DNA technology, DNA alteration, gene splicing and immunopharmacology, e.g. recombinant Hepatitis B vaccine, recombinant insulin and others.

3.4 Nature of Drugs

Drugs can be functionally defined as products that bring about changes in biological functions through their actions. In a vast majority of cases, drugs either act as agonists (activators) or antagonists (inhibitors) at target molecules called receptors. Drugs may either be produced within the physiological system (e.g. hormones) or be administered from outside (xenobiotics).

For a drug to bring about such changes, it should exhibit a few characteristics, which broadly define the physical and chemical nature of that drug.

3.4.1 Drug Matter

- Solid (e.g. aspirin), liquid (e.g. ethanol) and gaseous (e.g. nitrous oxide) states are the three distinct states of matter in which drugs can exist. This nature of a drug helps in deciding the ideal route of administration.

3.4.2 Drug Size

- Most drugs have a molecular weight (MW) between 100 and 1000 units.
- When the drug is too small, it may end up being highly non-selective at the intended receptor (e.g. lithium with a MW of 7 units).
- On the other hand, with a very large size, permeation becomes difficult, meaning the drug has to be administered directly at the site of action (e.g. alteplase with a MW of 59,050 units).

3.4.3 Drug Reactivity or Bonding

The chemical force of bonding between the drug and the receptor can be of three types – covalent, electrostatic and hydrophobic.

- Covalent bonds are the strongest and hence, irreversible in most cases (e.g. aspirin binding to cyclooxygenase enzyme in platelets).
- Electrostatic bonds may vary in strength from strong to weak (hydrogen bonds) to very weak (van der Waals bonds).
- Hydrophobic bonds are quite weak and more relevant for lipid-soluble drugs.
- To make a highly specific drug, the bonding has to be weak (and not strong). This is simply because of the logical fact that stronger the bond, more will be the number of receptor types binding to the drug. If the bond is weak, then there is only one receptor that can ideally bind to it. Hence, drug specificity and strength of bonding exhibit an inverse relationship (Table 3.3).

Table 3.3 Bond types and their strength

Bond type	Bond strength (kcal/mol)
Covalent	40–140
Reinforced ionic	10
Ionic	5
Hydrogen	1–7
Ion-dipole	1–7
Dipole-dipole	1–7
van der Waals	0.5–1
Hydrophobic	1

3.4.4 Drug Shape

- The shape of a drug should be in line with that of the receptor to which it is to be bound, analogous to the concept of "lock and key". A concept that is closely associated with shape of the molecule is chirality.
- Some drugs (mostly those with asymmetric carbon atom) have essentially the same chemical and molecular formulae, but they are arranged differently in space ("enantiomers"). These forms are also called as optical isomers, as they are mirror images of each other. They are commonly denoted by R/S ("Rectus" and "Sinister") configuration. They are also denoted by their optical rotation, as (+) and (−) or d- (dextro) and l- (levo).
- Different enantiomers can have significantly different physiological activities. For example, let us consider carvedilol. One enantiomer, the (S)(−) form, is a potent beta blocker, while the (R)(+) form is 100 times weaker. When a drug is given such that both chiral forms are present, then it is known as a "racemic mixture".

3.4.5 Transitory Properties

- The drugs must have the necessary characteristics to be able to travel to the receptor or the site of action from the site of administration.

3.4.6 Post-action Profile

- Once the desired action has been executed, the drug must be easily inactivated or excreted from the body.

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Principles and Modes of Drug Administration

4

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Abstract

It becomes more than essential for a clinical pharmacologist to understand the various principles and modes of drug administration, since these are clinically relevant in therapeutics, and helps in avoiding any potential harm to patients receiving these drugs. While there are several principles of drug administration, the five important ones are: the right patient, the right drug, the right dose, the right time and the right route of administration. Modes or routes of drug administration vary from the widely followed oral route to parenteral and inhalational routes. There also exist certain specialized routes and modes of drug delivery, like the liposomal delivery, prodrug delivery and others. Each of these routes of administration has its own pros and cons, which have to be weighed against each other before choosing the same. This chapter deals about the key principles and routes or modes of drug administration.

Keywords

Drug delivery systems · Drug administration · Liposomes · Prodrugs · Drug principles

4.1 Principles of Drug Administration

The first and foremost principle of drug administration follows the "Do No Harm" oath. Drug administration is governed by five "rights".

- Right patient
- Right drug
- Right dose
- Right time
- Right route

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Other than the traditional "rights", there are a few other added "rights", as follows.

- Right documentation
- Right assessment
- Right response to the drug
- Patient's right to be educated
- Patient's right to refuse the drug

4.2 Preparations of Drugs and Dosage Forms

The preparations are summarized in Box 4.1.

4.3 Routes of Drug Administration

Drugs can be administered through several routes, which are sometimes also the site of action, but most commonly transitory passages.

The major routes of administration include:

- Oral
- Sublingual
- Rectal
- Application to other epithelial surfaces like skin, cornea, vaginal and nasal mucosa
- Inhalation
- Injection
  - Subcutaneous
  - Intramuscular
  - Intradermal
  - Intravenous
  - Intra-arterial
  - Intrathecal
  - Intravitreal

4.3.1 Oral Route

Oral administration is arguably the most ancient method of drug use. It is still the most common and the most convenient route of administration even in the modern era. It has several advantages over its parenteral counterparts (Box 4.2).

Various factors govern the effect of orally administered drugs. While most of the oral drugs are intended for systemic use, drugs like vancomycin and mesalazine

Box 4.1: Dosage Forms and Their Description

Type	Description
Tablet	Compressed drug in the form of a hard disc. Tablets can be plain, scored (with a line for easy breaking), coated (film, sugar or enteric), dispersible, osmotic (e.g. prazosin), chewable (e.g. vitamin C) and effervescent (e.g. aspirin)
Capsule	A gelatinous container to hold drugs in solid or liquid formulation
Caplet	A coated solid form, shaped like a capsule (Capsule + Tablet)
Syrup	A sugar-containing aqueous solution; drugs that have a bad inherent taste are usually preferred to be marketed as syrups
Cream	A non-greasy, semi-solid preparation intended for topical skin application
Ointment	A greasy oil-based semi-solid preparation intended for topical skin application
Lotion	Liquid suspension intended for topical use
Liniment	Drugs mixed with alcohol, oil or soapy emollient intended for topical use
Gel/jelly	A clear or translucent semi-solid preparation intended for topical use; usually liquefies on application to the skin surface
Paste	A semi-solid ointment-like preparation, but thicker and thus less penetrating beyond the skin surface
Lozenge	A solid preparation that releases the drug when held in the oral cavity; may be for local (for sore throat) or systemic use (nicotine lozenges)
Troche	Similar to lozenges, but usually only for local use (clotrimazole for oral candidiasis)
Aqueous solution	One or more drugs "completely" dissolved in water
Aqueous suspension	One or more drugs "partially" dissolved in water
Aerosol spray/foam	A liquid, powder or foam deposited at the intended site by air pressure
Elixir	A sweet aromatic alcoholic solution commonly used as a vehicle
Extract	A concentrated form of drug usually derived from natural sources
Powder	Finely ground preparation of drugs, either for external or internal use
Suppository	One or more drugs prepared with a firm base such as gelatin; can be for application into the rectum, vagina (pessary) or urethra (bougie)
Tincture	An alcoholic solution; usually plant-derived
Transdermal patch	A semipermeable membrane shaped as a patch that contains the drug to be absorbed across the skin layers

are used locally for gut pathologies like *Clostridium difficile*-associated pseudomembranous colitis and inflammatory bowel disease, respectively.

- Orally administered drugs usually pass through the gut wall and liver, which contain several inactivating enzymes. This process is called "pre-systemic" or "first-pass" metabolism. This indicates that only a fraction of the administered

Box 4.2: Advantages and Disadvantages of Oral Route

Advantages	Disadvantages
Safe	Slow onset of action, hence unsuitable in emergencies
Convenient	Inconvenient in comatose or unconscious patients or those with persistent emesis
Non-invasive	Variable and erratic absorption
Painless	Unpalatable drugs may be difficult to be ingested by this route
No external assistance needed	Drugs degraded by GIT or liver cannot be given orally
Inexpensive in most cases	
No sterile precautions needed	
Both solids and liquids can be administered	

drug actually reaches the systemic circulation (this fraction is referred to as the oral "bioavailability" of a drug).

4.3.2 Sublingual Route

- Oral mucosal veins drain directly into the superior vena cava, thereby bypassing the portal circulation and, thus, the first-pass metabolism in the liver and the intestine (bioavailability ≈ 100%).
- Since there is rapid absorption into the systemic circulation, onset of action becomes accelerated as compared to the oral route of administration, and hence, this route may be preferred for administration of drugs used in emergency conditions, e.g. nitroglycerin (glyceryl trinitrate), buprenorphine, and desamino-oxytocin.
- Lipid-soluble drugs can be given by this route. Also, it is to be noted that highly irritant drugs or drugs with bitter taste cannot be administered sublingually. Other than placing the tablet below the tongue, the tablet can also be crushed and spread all over the oral mucosa. This route is called the buccal route.

4.3.3 Rectal Route

- Unlike the oral route, drugs with irritant or unpalatable nature can be administered through the rectum. Rectal route can also be preferred when the patient has persistent vomiting or is unable to swallow. Also, this route can be used for systemic drug administration in addition to the local administration.
- Bioavailability of drugs given via this route will be higher than that of the oral route. This is because almost 50% of the drug amount that is absorbed through the external haemorrhoidal veins escape the first-pass metabolism in the liver

(the other 50% is absorbed through the internal haemorrhoidal veins, which pass through the hepatic first-pass metabolism).

- The other advantage is that CYP3A4 (a prominent metabolizing enzyme) levels are higher in the upper intestine and not very common in the lower intestine, meaning that more amount of the active drug is available for action when compared to the oral route.
- However, absorption can be highly irregular and incomplete. Also, this route might cause discomfort to the patient, leading to non-compliance. For example, paracetamol and diazepam can be given rectally for their systemic action, while anti-inflammatory drugs can be given for their local action in ulcerative colitis.

4.3.4 Skin, Cornea, Vagina and Nasal Mucosa

- Cutaneous administration of drugs is mainly utilized for local effect. However, absorption into the systemic circulation is very common and can lead to adverse effects. Sometimes, this systemic absorption is made use of, for its therapeutic value.
- Normal epidermis acts a barrier to the entry of drugs and allows only the entry of lipid-soluble drugs. Another factor that decides the rate and amount of absorption is the surface area of application (directly proportional).
- On the other hand, dermis allows free entry of several drugs. This is the reason why abraded or burnt skin favours better absorption.
- Rubbing the skin after topical application and using oily substrates are other methods to increase absorption through the skin.
- Since the rate and extent of absorption become highly variable, transdermal patches have been developed.
  - Transdermal patch or transdermal therapeutic system (TTS) is an adhesive patch that delivers the drug contained within it at a constant rate into the systemic circulation. This patch has multiple layers, as follows.
    a. Innermost adhesive layer (usually with a priming dose): attaches to the skin.
    b. Rate-controlling micro-pore membrane: controls the rate of delivery to the skin surface. The rate is usually set to a rate that is slower than the slowest rate of absorption from the skin to avoid unnecessary backlog or wastage on the skin surface.
    c. Main drug reservoir layer: contains the active drug to be delivered.
    d. Outer backing film: an occlusive supporting layer.
  - These patches usually last for 1–3 days, sometimes even longer. Convenience is a major advantage over other routes of administrations. High cost and local irritation are a few demerits.
  - Although the patches can be applied anywhere over the body, chest, abdomen, upper arm, lower back, buttock or mastoid regions are preferred. These regions are usually thin-skinned or highly vascular.

  - Drugs like fentanyl, nitroglycerin, hyoscine, clonidine, estradiol and nicotine are delivered through skin patches.
- Topically applied ophthalmic drugs are predominantly intended for local use. Systemic absorption through the nasolacrimal canal might result in unwanted adverse effects, particularly with drugs like corticosteroids and beta-blockers.
- Corneal abrasions or infections can accelerate the rate and extent of absorption. Ocular suspensions and ointments help in delivering drugs over a longer period of time. Ocular inserts can aid in delivering small amounts of the drug.
- Intra-vaginal delivery is majorly for local and extended (cervix) action. Administration can be done using various formulations like tablets, creams, gels, pessaries and rings. Progesterone, oestrogens and anti-infectives are some common examples.
- Nasal mucosa is another site that can bypass first-pass metabolism. Other than the obvious locally acting drugs, systemic effects have been successfully seen with desmopressin, GnRH analogues, insulin and calcitonin. Absorption into the systemic circulation occurs through the nasal-associated lymphoid tissue.

4.3.5 Inhalational Route

- Drug delivery by inhalation is a common route, both for local and for systemic actions. Volatile liquids and gases can be administered by this route.
- Since the surface area of the alveoli is large, absorption into the systemic circulation is rapid. This results in rapid onset of action. Another advantage is that plasma concentration can be rapidly adjusted as well.
- The pulmonary system functions as the route for both administration and excretion. Newer devices like metered dose inhalers (MDIs) and colloidal pulmonary carrier devices have further strengthened the use of this route, e.g. salbutamol as a local bronchodilator and nitrous oxide as a general anaesthetic agent.

4.3.6 Parenteral Routes

- Limitations seen with oral drug administration are circumvented with parenteral injections, as the drug almost directly reaches the circulation, without having to cross the enteral mucosa or the liver. A few limitations do exist with parenteral routes, as listed below.
  - The formulation to be injected has to be sterile.
  - Injections are invasive.
  - Injections are painful and associated with local reactions.
  - Assistance of another person is often required.

- **Subcutaneous Route**
  - The drug gets deposited in the nerve-rich (irritant drugs cannot be injected) vessel-poor (absorption is slower than that of intramuscular injections) thin (only small volumes can be injected) subcutaneous layer.
  - A major advantage is that injection by self is easy. Subcutaneous route is to be avoided in patients of shock since there exists severe vasoconstriction, resulting in delayed absorption.
  - Drugs commonly administered through subcutaneous route are insulins and heparin. Novel modalities of subcutaneous drug delivery include pellet implantation, device implantation and dermojets.

    Pellet or device implantation can be sialistic (non-biodegradable) or biodegradable. Sustained release of the drugs occurs over several weeks or months. Non-biodegradable pellet shells have to be removed after the action of the drug, e.g. testosterone and etonogestrel.

    Dermojets are high-velocity jets of drug solution projected into the subcutaneous tissue, enabling virtually painless administration. Since there are no needles involved, this technique is suitable for mass injections.
- **Intramuscular Route**
  - The drug is injected into the fibres of one of the larger muscles like the deltoid, gluteus maximus, triceps, rectus femoris and vastus lateralis.
  - Irritant drugs that cannot be administered subcutaneously can be given by this route.
  - In comparison with subcutaneous route, a larger amount of drug can be injected intramuscularly.
  - It is less painful, but injection by self is impractical since the depth of injection is greater.
  - Patients at risk of developing haematoma should not be given intramuscular injections.
  - Rate and extent of absorption depend on factors like bulk of the muscle, vascularity, local temperature and nature of the injected drug.

- **Intradermal** injections are not very commonly used, except in scenarios like BCG vaccination and sensitivity testings. The most common technique of administration is by raising a bleb by injecting the drug into the skin.
- **Intravenous Route**
  - Intravenous injections are rapid-acting since the drug directly reaches the systemic circulation. Bioavailability is truly 100% in this case. This is the route of choice in emergencies.
  - The drug can either be administered in a bulk dose (bolus) or as a steady infusion.
  - Any intravenous injection has to be done under close monitoring of the patient's vitals.
  - Only aqueous solutions can be given (suspensions are contraindicated as they can cause embolism).

  - Oily vehicles, drugs that cause haemolysis or precipitation should not be given through this route.
- **Intra-arterial** injections are rarely used when the action is required to be localized at a particular organ or site, as in the case of head and neck tumours. This route is also employed for diagnostic applications.
- **Intrathecal** route is used when a local action is required at the meninges or cerebrospinal axis. The drugs are injected into the spinal subarachnoid space or ventricular space. Blood-brain barrier and blood-CSF barriers are the reasons why intravenous drugs usually do not reach the cerebrospinal axis, e.g. methotrexate and baclofen.
- **Intravitreal** injections are commonly used by ophthalmic surgeons to treat conditions like age-related macular degeneration, e.g. ranibizumab.

4.4 Specialized and Targeted Drug Delivery Systems

While the routine drug delivery modalities are effective, efforts are regularly put in to improve selectivity and specificity of drug delivery. These novel or special approaches are listed below.

4.4.1 Prodrugs

- Prodrugs are inactive (or less active) precursors that are metabolized to (more) active metabolites. In most cases, prodrugs have an advantage over the active metabolite in that they have better pharmacokinetic properties. For example, levodopa is absorbed from the GI tract and crosses to blood-brain barrier to get converted to the active metabolite, dopamine, which, by itself, cannot cross the barrier. Another example is famciclovir, which is a prodrug of penciclovir, and has better bioavailability than its active metabolite.
- Ideal prodrugs should have no pharmacological activity against a particular target but should be able to be metabolically transformed into a compound with the desired activity. Prodrugs are of two types, type I (intracellular bioactivation) and type II (extracellular bioactivation), each with further subtypes (Table 4.1).

Table 4.1 Prodrugs based on the tissue location of bioactivation

Type	Tissue location of bioactivation	Examples
IA	Therapeutic target tissues/cells	Aciclovir, cyclophosphamide
IB	Metabolic tissues like liver, lung and GI mucosa	Carbamazepine, captopril
IIA	GI fluids	Sulfasalazine
IIB	Systemic circulation, other ECF compartments	Bambuterol, fosphenytoin
IIC	Therapeutic target tissues/cells	ADEPTs, GDEPTs, VDEPTs[a]

[a]*ADEPT* Antibody-directed enzyme prodrug therapy, *GDEPT* Gene-directed enzyme prodrug therapy, *VDEPT* Virus-directed enzyme prodrug therapy

- Prodrugs have to be differentiated from "co-drugs", which are essentially two or more chemically linked synergistic drug molecules in order to improve the delivery characteristics of one or more of the drugs. For example, sulfasalazine is a combination of sulfapyridine and 5-aminosalicylic acid. In simple terms, these drugs can be referred to as "mutual prodrugs".

4.4.2 Beaded Delivery

- The beaded delivery pattern consists of several minute beads composed of polystyrene-like inert substances. These beads are overlaid with the active substance and encased within delivery capsules.
- The delivery is usually made to be acid-sensitive, thus releasing the drug on exposure to gastric acid. Drug levels become dependent on the amount of gastric acidity in the stomach. For example, tolterodine has been administered via beaded delivery.

4.4.3 Liposomal Nanoparticle-Based Delivery

- Liposomes are concentric vesicular vehicles that are roughly 0.1–1.0 μm in diameter. These vesicles are formed by sonication of an aqueous suspension of phospholipids.
- They are used chiefly as vehicles for non-lipid-soluble drugs, which get trapped in the central portion and get released on rupture of the liposomal structure.
- Lipid-soluble drugs can be carried using bilayered liposomal delivery devices, as they can be trapped between the hydrophilic head and the hydrophobic tail of the layer (hence, called amphiphilic liposomes).
- Liposomal carriers are taken up by the reticuloendothelial cells in the liver (and other tissues to a minor degree). Malignant cells are also liable to take up these carriers, thus making this modality an effective anticancer drug delivery system. For example, amphotericin B is formulated as a liposomal drug, which is less nephrotoxic and better tolerated than the conventional formulation; doxorubicin (pegylated liposomal formulation) is used for myeloma and ovarian carcinoma management.
- Liposomes can be classified based on the size and the number of bilayers into unilamellar vesicles (ULV) and multilamellar vesicles (MLV). They can also be classified based on their composition into conventional, cationic, immunosensitive and pH-sensitive.
- Uses of liposomal drug delivery modality include:
  - Site-specific targeting
  - Site-avoidance delivery
  - Intracellular delivery
  - Sustained-release delivery
  - Enhancement of response to vaccine antigens

Table 4.2 Nanoparticle class, materials and their application

Class	Material	Application
Natural products and their derivatives	Liposomes	Drug delivery
	Chitosan	
	Dextran	
	Gelatine	
	Alginates	
	Starch	
Dendrimers	Branched polymers	Drug delivery
Fullerenes	Carbon-based molecules	Drug delivery
Polymer carriers	Polylactic acid	Drug or gene delivery
	Polycaprolactone	

- Other nanoparticles in drug delivery are represented in Table 4.2.

4.4.4 Antibody-Drug Conjugates

- The conjugates of antibody and drug (ADC) are commonly used in oncotherapy, where an anticancer drug is tagged with an antibody with the help of a linker.
- The antibody is specific against a particular protein expressed only on cancerous cells. Thus, ADCs are very specific and bind only to these cancerous cells so that the normal tissues are spared of the toxicity. Classical examples include trastuzumab emtansine (for HER-2-positive breast cancer treatment) and gemtuzumab ozogamicin (for AML).

4.4.5 Coated Implantable Devices

- Drug-eluting stents (DES) can be peripheral or coronary stent devices that release drugs at a slow and sustained pace.
- These stents classically have three parts – a platform, a drug and a polymer coating that binds the drug to the platform. Common drugs used as stents include sirolimus and paclitaxel. These agents conventionally block cell proliferation, thus prevent the risk of restenosis in the coronary circulation. However, stents are associated with the risk of stent thrombosis.
- Another class of implantable devices is the hormonal intrauterine devices (IUDs), which are used as contraceptive devices.

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Drug Absorption and Bioavailability

5

Abialbon Paul

Abstract

Absorption is the process of drug movement from the site of drug administration to the systemic circulation. Various processes underlie the successful absorption of drugs. They include passive diffusion, facilitated diffusion, active transport, and endocytosis. Drug absorption is quantified in terms of bioavailability. Bioavailability is the extent to which absorption occurs. In other words, bioavailability is the fraction of the administered drug that reaches the systemic circulation in the unchanged form. Various factors impede or enhance absorption. The lipid solubility, pH of the medium and the presence of and the density of membrane transporters have a greater effect on the rate of absorption. Various routes of drug administration are employed to maximize the amount of drug absorbed and hasten the onset of action of drugs. The intravenous route lacks a phase of absorption as the drug is directly injected into the systemic circulation. Quantification of the bioavailability by studying the structure and the presence of chemical groups is called Quantitative structure-bioavailability relationship (QSBR). Various novel models have been proposed to improve drug absorption and increase systemic exposure to drugs with low oral bioavailability.

Keywords

Absorption · Bioavailability · Route of administration · Transport

5.1 Definition

- Absorption is the movement of drug from the site of drug administration to the systemic circulation.

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- Bioavailability is the extent to which absorption occurs. In other words, bioavailability is the fraction of the administered drug that reaches the systemic circulation in the unchanged form.

5.2 Mechanisms

Drug absorption can take place through passive and active processes.

5.2.1 Passive Transport

Does not require energy.

- **Passive Diffusion**
  - The movement of drug molecules is driven by a concentration gradient from a higher concentration to a lower concentration.
  - Lipid-soluble drugs are able to diffuse easily through biological membranes. The ionization of ionic drugs, however, is affected by the pH.
  - There is no specificity for the type of drug molecules transported.
  - The rate of diffusion is directly proportional to the concentration gradient, the surface area available for diffusion, and the lipid-water partition coefficient of the drug.
- **Facilitated Diffusion**
  - Carrier proteins are involved in facilitated drug transport.
  - The movement of the drug molecules is driven by an electrochemical gradient from a higher to lower gradient.
  - There is a high specificity for the type of drug molecules transported as carrier proteins are specific to the drug/type of drug they carry.
- **Paracellular Transport**
  - The drug molecules move through intercellular gaps in the endothelium of capillaries and postcapillary venules.
  - There is no specificity of the drug molecules transported; however, the drug must be of lower molecular weight.
  - This process is highly limited in organs with tight junctions.

5.2.2 Active Transport

Energy in the form of ATP is required.

- **Primary Active Transport**
  - The drug molecules move from a lower concentration to a higher concentration against the gradient.
  - There is a high specificity for the type of drug molecules transported.

  - The transporter protein directly uses energy in the form of ATP for drug transport.
- **Secondary Active Transport**
  - The process is similar to primary active transport except that ATP is used indirectly to maintain the concentration gradient of the drug, i.e., the movement of target substance achieved by the cotransport of another substance is of the same (symport) or opposite (antiport) directions.
  - The electrical gradient thus established then facilitates the transport of drug molecules.

5.3 Quantifying Drug Absorption

5.3.1 Bioavailability

- With the exception of the intravenous route of drug administration, the amount of drug administered and the amount of drug reaching the systemic circulation are often different. The amount of drug reaching the systemic circulation is likely to be less due to absorptive losses and pre-systemic or first-pass drug metabolism.
- Bioavailability is represented as F having values between 0 and 1, where 0 signifies no absorption while 1 signifies complete absorption.
- By definition, because we inject the drugs directly in the systemic circulation, the bioavailability of an i.v. dose is considered to be 100% or $F = 1$. Hence, the i.v. dose acts as a reference to compute the bioavailability of other routes of drug administration.
- The bioavailability of a drug for any route of administration can be calculated by
- $F = AUC$ for the route of administration/AUC for the i.v. route. Here, AUC stands for area under the curve.

5.3.2 Rate of Absorption

- The rate of drug absorption determines the onset of action in an acute setting.
- Drugs which are highly lipid soluble are absorbed fast and will have an earlier onset of action.
- The rate of absorption also determines peak concentration achieved for some drugs.
- Drugs which are absorbed fast tend to accumulate in the vascular compartment before they can be distributed throughout the body during the second phase of drug distribution. This could result in a higher peak concentration (C_{max}).
- Drugs which are absorbed slower (e.g., drugs given as slow i.v. infusions) will start distributing before the T_{max}, and hence the C_{max} achieved will be lower.
- The rate of drug absorption generally is not clinically very important after the drug has reached the steady-state concentration.

5.4 Factors Affecting Absorption

5.4.1 Host Factors

- **pH and Ionization**
  - Passive transport of ionic substances is affected by the degree of ionization because the unionized form diffuses easily through biological membranes. This is in turn affected by the pH of the milieu.
  - Drugs exist as either weak acids or weak bases which ionize and exist as ionic (lipid insoluble) and nonionic (lipid soluble) forms.
  - pKa of the drug is the pH at which the drug is 50% ionized and 50% nonionized.
  - For weak acids, at pH lower than their pKa, they do not ionize readily, and hence a higher fraction of the drug stays in the nonionized form. At higher pH, they readily give out an H^+ ion to form an ion. Hence, for a weak acid, at acidic pH, the fraction of nonionic form is more, while at basic pH, the fraction of the ionic form is more.
  - For weak bases, at pH lower than their pKa, they ionize readily by accepting an H^+ ion in the medium, and hence higher fraction of the drug stays in the ionic form. At higher pH, they tend to stay unionized. Hence, for a weak base, at acidic pH, the fraction of ionic form is more, while at basic pH, the fraction of the nonionic form is more.
  - The nonionic (lipid soluble) form is readily absorbed by passive diffusion.
  - Mnemonic: AAA [acids are absorbed better in the acidic medium.]
- **Membrane Transporters**
  - Various highly specialized transporters are present throughout the gastrointestinal tract. Some of these transporters are influx transporters, while others are efflux transporters that extrude foreign molecules out of the system to reduce toxic exposure.
  - Genetic variations in the transporters can explain a part of the interindividual variability of drug absorption.
  - Example of some transporters:
    PepT1 – H^+/dipeptide transporter present in the apical surface of the enterocyte
    PepT2 – present in the basolateral membrane of the enterocyte
    P-Glycoproteins – a family of ABC proteins termed as MRP (multidrug resistance protein)
    Organic cation transporters
    Organic anion transporters

5.4.2 Routes of Drug Administration

Depending on the route of drug administration, various factors can influence the rate and extent of drug absorption.

- **Oral Administration**
- Drug administration through the oral route can be very unpredictable as a lot of factors might influence absorptive processes. Some of the factors that modulate absorption are:
  - Blood flow. The rate of blood flow to the gastrointestinal tract affects drug absorption. It can vary physiologically (e.g., blood flow increases after a meal) or pathologically (e.g., congestive heart failure results in stasis of blood and a decrease in the rate of drug absorption).
  - Absorptive surface area. The surface area of the intestines can be variable in patients who have undergone intestinal resection surgeries. Because the intestinal surface is significantly larger than the gastric surface area, a large proportion of the drug is absorbed from the intestine despite the differences in ionization caused by gastric pH.
  - Gastric emptying time can be variable due to the presence of food, its amount, composition, osmolarity, pH along with diurnal, and interindividual variations.
  - Gender. The gastric emptying time is generally slower for women in the reproductive age group and those who take estrogen replacement therapy.
  - Emesis. Either as a result of drug irritation on the gastric mucosa or due to systemic pathology, emesis will result in incomplete drug absorption.
  - Digestive enzymes can affect drug stability and dissolution rate. They also affect the degree of ionization. The gastric pH may be altered due to many physiological (e.g., the gastric acid output changes diurnally) and pathological processes (e.g., patients with pernicious anemia will have lower gastric acid output).
  - Presence of other food substances can alter the rate of absorption by influencing the gastric emptying rate (e.g., lipid content of the food delays gastric emptying) or chemically interacting with the drug (e.g., heavy metals chelate tetracyclines).
  - The intestinal microbiota can result in a structural breakdown of certain drugs, and the composition of the intestinal flora is highly variable.
  - Certain drugs which get absorbed in the upper part of the intestinal tract will achieve lower levels in the colon where their effects might be needed (e.g., 5-aminosalicylic acid in inflammatory bowel disease).
  - Highly ionic and water-soluble drug will have a negligible amount of oral absorption that oral dosing is completely ineffective (e.g., aminoglycosides, vancomycin).
- **Sublingual Absorption**
  - Drug absorbed through the sublingual vein bypasses the portal circulation. Hence the bioavailability is increased for a drug which has a significant first-pass metabolism when given sublingually compared to oral dosing.
- **Intravenous Injections**
  - The absorption through parenteral routes is generally more predictable than the enteral routes.
  - The rate of absorption is fast as the phase of absorption is bypassed resulting in a quicker onset of drug action.

  - Some drugs metabolized in the lungs will experience first-pass metabolism reducing the bioavailability.
  - Continuous i.v. administration can be affected by the patency of the veins. Thrombosis and extravasation of fluid into local capillary beds will reduce the rate of drug absorption into the systemic circulation.
- **Subcutaneous Injections**
  - The absorption from the subcutaneous site is generally dependent on the blood flow to the area.
  - A large particle size of the drugs will reduce the speed of absorption.
  - Complex formation with proteins or other substances added to the drug can result in reduced and protracted drug absorption phase. This can be used clinically to prolong the duration of action of the drug.
  - If the drug causes vasoconstriction or if a vasoconstrictor is added to the drug mixture, it will reduce the drug absorption (e.g., epinephrine added to local anesthetics).
  - Local pH can affect the degree of ionization of the drugs. The unionized form is absorbed faster compared to the ionized fraction.
- **Intramuscular Injections**
  - The rate of blood flow to the injected muscle affects the rate of drug absorption. This can be increased by the application of local heat, massage, or exercise.
  - The rate of drug absorption from the gluteus is generally faster in males than in females. This is due to the fact that females tend to have more fat which is relatively less perfused.
- **Inhalational Route**
  - The rate of absorption is generally rapid because of the large surface area of the lung. Disease conditions of the lung that reduce the perfusion or ventilation can reduce the rate of drug absorption.
  - First-pass metabolism by the liver is bypassed.
- **Topical Application**
- Absorption through the skin or mucosa can be highly variable. The following factors increase the rate of drug absorption.
  - Larger surface area of application.
  - Perspiration opens up the pores.
  - Increase in contact time.
  - Abraded skin/mucosa.
  - Rubbing.
- **Rectal Administration**
  - Rectal absorption is often incomplete and unpredictable.
  - Drug absorbed from the lower part enters the systemic circulation directly bypassing the portal circulation, while drug absorption from the upper part enters the portal circulation and will be subject to first-pass metabolism in the liver.

5.4.3 Drug Factors

- **Solubility**
  - The solubility of the drug is defined as the extent to which the molecules from the solid surface have been removed by the solvent and dissolved into the solution.
  - The solubility of the drugs determines the availability of the drug at the absorptive surface/membrane.
  - Ionized drugs generally tend to be better soluble in aqueous solutions. Since the ionization of drugs is heavily influenced by the pH, the local pH also affects drug solubility.
  - Improving drug solubility is important in improving extent and rate of drug absorption for drugs which have a solubility-limited absorption profile.
- **Dissolution**
  - The process of dissolution is different from solubility in that dissolution is a kinetic process that describes the rate of drug molecules moving from the solid into the solution phase.
  - Increasing the surface area of the particles can increase dissolution. This can be done by a process called micronization for drugs which are poorly water-soluble (e.g., griseofulvin).

5.5 Quantitative Structure-Bioavailability Relationship (QSBR)

The chemical structure of the drug molecules can affect bioavailability, and this can be quantified by QSBR.

- **Factors that Decrease Bioavailability**
  - No of hydrogen donors
  - Heavy atoms
  - Presence of tetrazole, aminopyridine, and benzoquinone groups
- **Factors that Increase Bioavailability**
  - No of hydrogen acceptors
  - Low molecular weight
  - Presence of azide, salicylic acid, and amide groups
- **Physicochemical Interactions**
  - The drug particles can interact with other molecules in the surrounding and form complexes and aggregates or chemically react with them.
  - These interactions decrease the availability of the drug at the membrane where absorption takes place and reduces bioavailability.

5.6 Pharmaceutical Equivalents and Bioequivalence

- When two drugs contain the same active ingredients, in the same concentration or strength and in the same dosage form, they are called pharmaceutical equivalents.
- Pharmaceutical equivalents are said to be bioequivalent when the rate and extent of bioavailability are similar (within 80–120% usually).
- The parameters that are commonly studied to check bioequivalence are the AUC of the concentration-time plot and the maximum concentration achieved in plasma (C_{max}).

5.7 Novel Methods to Improve Absorption

- Drug-eluting stents to deliver drugs directly to the site of stenotic lesions
- Nanoparticle drug delivery systems

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Drug Distribution

6

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Abstract

The process of drug movement after absorption into various body compartments like the interstitial space and the intracellular space is called drug distribution. Distribution is an important process which results in exposure of the target organ to the drug. The initial phase is highly influenced by the amount of blood flow to various organs and is responsible for the acute onset action of the drugs. The second phase is a slower phase where the drug equilibrates with the muscle, skin, and fat. The second phase can be responsible for the termination of action for some of the drugs. The cardiac output, local regional blood flow, alterations in capillary permeability, plasma protein binding, the local pH, and affinity to tissue proteins are some of the factors that influence drug distribution. Increase in plasma protein binding results in lower volume of distribution, longer duration of action, and slower onset of action. Plasma protein binding is responsible for displacement reactions and drug interactions. Excessive tissue protein binding can result in organ toxicities. To some extent, distribution can be quantified by the apparent volume of distribution. The volume of distribution helps us determine the loading dose of drugs which are required in emergency settings.

Keywords

Distribution · Protein binding · Volume of distribution · Redistribution · Drug displacement

6.1 Introduction

Following drug absorption into the systemic circulation, the drug molecules equilibrate between the vascular compartment and other body compartments like the interstitial space and the intracellular space. This process is termed as drug

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distribution. Factors that influence drug distribution affect the rate and delivery of drugs to various organs.

Understanding drug distribution is important in establishing:

- The amount of drug available to the target organ to produce action
- The amount of drug distribution to nontarget organs that could potentially result in an adverse drug reaction
- The loading dose of drugs

6.2 Phases of Drug Distribution

6.2.1 Initial Phase

- The drug is initially distributed to highly vascular organs; the organs liver, kidney, and brain receive most of the drug.
- In the brain, drug distribution is determined by the permeability of the blood-brain barrier (BBB) to the drug molecules.
- This phase is responsible for the acute onset of action of abused drugs and anesthetic drugs.

6.2.2 Second Phase

- The muscle, skin, and fat receive drug slowly during the second phase.
- It accounts for most of the extravascular drug distribution.
- It is responsible for the phenomenon of redistribution seen with some drugs.

6.3 Factors Affecting Drug Distribution

- Cardiac output
- Local blood flow
- Capillary permeability
- Plasma protein binding
- Local pH
- Tissue volume
- Disease states affecting any of the parameters above

6.4 Plasma Protein Binding

Some drugs bind avidly to plasma proteins and exist in two forms: the bound form and the unbound form. The unbound drug is the active form. The ratio of the bound form and the unbound form alters drug distribution.

6.4.1 Characteristics of Binding

- Drugs commonly bind to the following plasma proteins:
  - Albumin binds acidic drugs.
  - Alpha-1 acid glycoprotein binds basic drugs.
  - Hormone carrier proteins (e.g., thyroid binding globulin, sex hormone-binding globulin).
- The binding is reversible.
- The bound-unbound fraction is affected by:
  - Drug concentrations.
  - Affinity to the binding protein.
  - Disease-induced alteration of protein levels like hypoalbuminemia decreases the bound fraction of drugs, while inflammatory conditions increase acute phase proteins resulting in an increase of the bound fraction.
  - Physiological alterations in the protein levels, e.g., during pregnancy.
  - Presence of other drugs that compete with the binding sites.
- Drugs that bind to plasma proteins have a lower aVd than drugs that do not.
- Binding to plasma proteins might limit their glomerular filtration and slow down drug metabolism pathways.

6.4.2 Clinical Implications of Plasma Protein Binding

Alterations in the plasma protein binding generally do not cause significant clinical complications. This is owing to the fact that the alterations in the unbound fraction are transient and the drug concentration equilibrates throughout the various compartments in which the drug is distributed. The risk of clinical complications is high when the drug has the following properties:

- High clearance (so mild alterations in the renal clearance will be significant)
- Low therapeutic index (higher risk of toxicity)
- Rapidly acting (toxic effect can manifest before the drug reaches new equilibrium)

Lignocaine is one of the drugs that satisfy most of these conditions, and hence more clinical caution is needed with its use.

6.4.3 Toxicity

- Toxic reactions can be precipitated as displacement of drugs occurs due to the presence of other drugs competing for the same binding sites on the plasma proteins.

- For example, precipitation of kernicterus occurs in neonates with hemolytic jaundice who are administered with sulfonamides. Sulfonamides displace the delta fraction of bilirubin (bilirubin bound to albumin) thereby making the bilirubin to cross the poorly developed BBB.

6.4.4 Drug Measurements

- Special precautions are needed while measuring drugs that have a high affinity to plasma proteins.
- The free unbound fraction of the drug must be measured to make more meaningful clinical judgments. If this is not possible, then every attempt must be made to estimate the protein levels in the blood, and adjusted drug levels must be calculated whenever possible.

6.5 Tissue Binding

Similar to how drugs bind to plasma proteins, drugs also reversibly bind to proteins and phospholipids in tissues.

- The tissues to which drugs bind act as a reservoir of these drugs. They keep releasing the drug into the central compartment slowly and increase the elimination half-life of the drug.
- Local accumulation can cause acute tissue toxicity, e.g., nephrotoxicity/ototoxicity by aminoglycosides and cardiotoxicity by digoxin.
- Some drugs can get accumulated in tissues and remain for a very long time. For example, tetracyclines accumulate in the bones and thyroids.

6.6 Redistribution

- Redistribution refers to the change in the plasma drug concentration which is significant enough to cause alteration/termination of drug action. The alteration of plasma concentration occurs due to the second phase of drug distribution.
- The commonest example is the redistribution of thiopentone. Due to its lipid solubility, it readily penetrates the BBB during the initial phase of drug distribution. This ensures a very rapid onset of action. During the second phase of drug distribution, the drug redistributes to the adipose tissue resulting in termination of action in the brain.
- This phenomenon is commonly seen in drugs that are highly lipid soluble and are administered via the inhalation or the intravenous route.

6.7 Barriers to Drug Movement

- Certain organs have highly specialized inter-endothelial junctions that prevent drug movement into the organs reducing the exposure to potentially toxic substances.
- For example, the BBB is a continuous tight junction.
- Drugs that overcome this barrier require high lipid solubility, should be unionized, must be of a smaller molecular size, and should not be bound to plasma proteins.
- Other organs can have barriers to drug uptake as well (blood-testis barrier).
- Diseases such as meningitis or encephalitis can disrupt the barriers and might increase the influx of the drug and might result in enhanced therapeutic effect or toxicity.

6.8 Apparent Volume of Distribution (aVd)

- The apparent volume of distribution (aVd) is the volume needed to dissolve the drug to reach a concentration measured in the plasma after the drug has equilibrated within the body compartments and steady-state plasma concentration has reached.
- Amount of drug administered/aVd = C_{ss}.
- Hence,

$$\text{aVd} = \text{Amount of drug administered} / C_{ss}$$

- where C_{ss} is the steady-state plasma concentration and *aVd* is the apparent volume of distribution.
- aVd is calculated as the ratio of the dose administered to the steady-state plasma concentration.
- If a higher proportion of the drug stays within the vascular space, due to binding to plasma protein or high molecular weight, the drug will have a lower volume of distribution.
- If a lower proportion of the drug stays in the vascular space, due to binding to tissue proteins, it will have a higher volume of distribution.
- If a drug has a large volume of distribution, it does not necessarily mean extensive spreading to large areas in the body. Due to the way we define aVd, we can only conclude that such a drug leaves the vascular space. It is possible that a drug has a high volume of distribution because of selective binding to localized tissue proteins.
- Certain drugs have a very large volume of distribution.
  - Chloroquine has an aVd of 1500 L owing to its high lipid solubility.
  - Digoxin has an aVd of about 667 L as it binds to proteins in the heart.

6.9 Types of Volumes of Distribution

Four different types of volumes of distribution can be calculated. Each has its own advantages and disadvantages:

- Initial volume of distribution
- Extrapolated volume of distribution
- Non-compartmental volume of distribution
- Steady-state volume of distribution

6.9.1 Initial Volume of Distribution

- This is the volume calculated immediately following an i.v. bolus of the drug and is denoted as $V_{initial}$.
- It does not take into account any phase of drug distribution.
- This volume can be used to calculate the physiological volume of the central compartment and to adjust the loading dose to avoid any toxicity due to peaking drug concentrations.

6.9.2 Extrapolated Volume of Distribution

- The extrapolated volume of distribution is calculated after the initial phase of distribution has taken place. This method takes the slow late stages along the concentration/time curve (the terminal elimination phase) and extrapolates a line of best fit from them to estimate the initial concentration. It is denoted as V_{extrap}.
- It tends to be highly inaccurate as there is a higher chance of overestimating the loading dose with this method. Hence, this is not used to provide insight into clinical practice.

6.9.3 Non-compartmental Volume of Distribution

- This method considers only one compartment at a time. The area under the curve of the concentration-time plot and the terminal elimination time constant is used to calculate the volume of distribution.
- The calculated volume of distribution is denoted as V_{area}.
- Since the terminal elimination time constant is used, this method assumes the excretion rate to be constant over time, and the average is taken into consideration.
- Any alteration to the elimination that affects the clearance of the drugs, like in renal failure, will result in a slower rate of drug elimination resulting in a false low extrapolated initial concentration resulting in false high volumes being calculated.

6.9.4 Steady-state Volume of Distribution

- This method uses the steady-state plasma concentration of the drug and the weight of drug administered to calculate the volume of distribution *(V_{ss})*.
- This method is most useful clinically. It is used to calculate the volume of distribution and consequently the loading dose for a patient.

6.10 Factors Affecting the Volume of Distribution

6.11 Modeling Factors

- Timing of drug measurements.
  - If the drug concentrations are estimated before distribution is complete, a higher plasma concentration and a lower volume of distribution will be estimated.
- Pharmacokinetic model used.
  - Based on the pharmacokinetic model used (e.g., $V_{initial}$, V_{extrap}, V_{ss}, or V_{area}), the estimations will vary.
- Drug measurement.
  - Measurement of free unbound drugs and the total drugs impacts the estimated volume.

6.11.1 Drug Factors

- Molecular size – smaller molecules have a high aVd.
- Molecular charge – uncharged molecules have a high aVd.
- pKa – alters the ionization of drugs.
- Affinity to tissue proteins – binding to tissue proteins decreases the amount of drug in the central compartment resulting in an increase in aVd.
- Lipid solubility is the most important factor. Lipid-soluble drugs have high volumes of distribution.
- Aqueous solubility – water-soluble drugs tend to be ionic and have lesser volumes of distribution.

6.11.2 Patient Factors

- Total body volume – Alterations in the total body volume either physiological (e.g., age and gender) or pathological (e.g., hypovolemic shock) will alter the volume available for the drug to get distributed into.

- Age – With increasing age, the water content of the body decreases. The muscle mass also decreases resulting in lower tissue binding for some of the drugs. Often, the aVd is found to decrease for many drugs. The loading doses must be calculated with caution in the elderly.
- Gender – Females tend to have a larger volume of distribution.
- pH – Alters ionization of drugs.
- Protein levels – Lower protein levels result in larger than expected volumes of distribution for drugs with significant plasma protein binding.
- Displacement – Presence of other drugs that compete for protein binding sites will alter the distribution of drugs.
- Pregnancy – Increases the aVd for many drugs.
- Edema, ascites, and effusions – Increases the aVd for many drugs as the excess fluid can act as reservoirs of the drug.

6.12 Volume of Distribution: Practical Implications

6.12.1 Dialysis

Drugs with a low volume of distribution are better extracted with hemodialysis/hemoperfusion as the drug resides predominantly in the vascular compartment. Hence knowledge of the apparent volume of distribution of the drug can help make clinical decisions in the management of poisoned patients.

6.12.2 Estimating the Loading Dose

Knowing the apparent volume of distribution can help us estimate the loading dose of drugs. The loading dose is given to saturate the volume the drug is distributed into so as to achieve the required plasma concentration and produce action in an acute setting. Drugs with larger volumes of distribution need to have larger loading doses.

6.13 Rate of Distribution of Drugs

- The rate of drug distribution will influence the concentration of the drug attained in the plasma.
- A drug which takes a longer time to distribute from the central volume to the tissues will attain a higher concentration in the plasma. This will also be influenced by the mode of drug delivery. An i.v. bolus dose will increase the concentration in the central vascular compartment fast, whereas an i.v. infusion will result in lower C_{max} owing to the drug getting distributed before the drug administration is complete.
- Disease states can affect the rate of distribution as well. For example, stasis in the vascular system either due to shock or cardiac failure will result in substantially

slower distribution resulting in alteration of the C_{max} and eventually increased toxicity.

6.14 Drug Distribution to the Fetus or Newborn

- The drug distribution to the fetus is determined by:
  - Lipid solubility
  - Plasma protein binding
  - pKa of the drug and ion trapping
- The fetal pH (7.0–7.2) is slightly more acidic than the maternal plasma (7.4). Weak bases which cross the placenta will ionize, and the ionic form will not be able to diffuse back freely into the maternal plasma resulting in increased fetal exposure.
- The mother's milk has a lower pH as well and has a higher concentration of lipids. The composition of milk changes postpartum and also within a feeding cycle (foremilk and hindmilk). These changes contribute to the time- and phase-dependent variation in drug movement into maternal milk.
- Drug distribution into the mother's milk is determined by:
  - pKa and ionization
  - Low plasma protein binding
  - Low molecular weight
  - High lipophilicity

6.15 Compartment Models in Drug Distribution Kinetics

- Compartment models mathematically simulate absorption and distribution. It is an oversimplification of the distribution process that helps us understand and attribute changes in drug pharmacokinetic profile.
- A pharmacokinetic compartment is "a mathematical concept which describes a space in the body which a drug appears to occupy." It does not need to correspond to any specific anatomical space or physiological volume.
- A single compartment model is the most commonly used model to simulate drug distribution. It tends to be a heavy oversimplification and is often the least accurate. The single compartment model considers the whole human being as a single homogenous compartment that allows for uniform mixing of substances.
- Two-compartment models simulate drug movement between the central and peripheral compartments. This model incorporates the initial and second phases of drug distribution and is usually better in predicting pharmacokinetic parameters than the one compartment model.
- A three-compartment model is better at predicting anesthetic drugs as they distinguish between the central compartment, slow distributing fat compartment, and fast redistributing muscle compartment.

- Complex multi-compartments are better at predicting and understanding drug distribution; however, they require a lot of computations. The model that closely predicts the real-life conditions is chosen to represent the drug pharmacokinetics.

6.15.1 Limitations of Compartment Modeling

- The central compartment is considered to be the only compartment from which drugs can be eliminated. This may not be true as drugs can get metabolized or excreted by other tissues apart from the liver and the kidney.
- The use of complex multi-compartment models might not improve the accuracy of prediction while increasing the complexity of the model.
- The models do not correspond to real physical volumes within the body. Even when they do (e.g., brain compartment), it is difficult to confirm the drug levels experimentally in humans. There is more reliance on calculated (simulated) data in modeling.

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Drug Metabolism

7

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Abstract

Metabolism is the major pathway for the elimination of the majority of xenobiotics and endogenous molecules from the body. Metabolism of the drug can occur predominantly in the liver and kidney with a minor contribution from the GI tract, lungs, skin, and plasma. In the liver, the cytochrome P450 enzymes play a major role in the metabolism of drugs and have a significant role in "drug interaction" due to enzyme induction and inhibition during multiple drug administration. Usually, the drug or any xenobiotic undergoes phase I metabolism wherein the toxic compound is structurally converted to non-toxic compound followed by phase II wherein modified metabolite is conjugated with endogenous molecules to make it water soluble for ease of excretion. During metabolism, a drug can be inactivated or activated from a prodrug state or can produce inactive or active metabolites. Knowledge about the organ metabolizing the drug is important for physician while administering multiple drugs in disease-states like liver and renal failure for the "fine-tuning" of the dose of a drug and avoiding further damage to the failing organ. As various factors like age, gender, food intake, disease status of the metabolizing organ, circadian rhythm, and genetic polymorphism can influence the drug metabolizing capacity, basic knowledge about the drug metabolism is crucial for effective pharmacotherapy.

Keywords

Xenobiotics · Drug metabolism · CYP450 · Metabolite · Enzyme induction · Enzyme inhibition

7.1 Introduction

All the organisms that ever exist in this world are constantly exposed to various foreign chemical substances from the environment throughout their lifetime. Any foreign substance (xenobiotic), absorbed into the body by various routes

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intentionally or accidentally, has to be eliminated either unchanged or modified. Numerous xenobiotics exert longer duration of action by higher protein-binding nature or increased lipophilicity. Therefore, if termination of action of a xenobiotic is solely dependent on elimination, it would be difficult to remove all the xenobiotics. Metabolism is an alternative process that converts lipid-soluble substance to water soluble and makes the elimination of these substances faster.

7.2 Definition

Metabolism, also known as biotransformation, is a process of chemical alteration of the structure of xenobiotics and various endogenous molecules inside an organism.

The importance of biotransformation in different fields of medicine is shown in Table 7.1.

7.3 Sites of Drug Metabolism

The sites of metabolism can be broadly divided as that is happening at the organ and cellular levels.

Table 7.1 Importance of biotransformation in different fields of medical science

S. no.	Field	Importance of biotransformation
1	Pharmacology	Metabolism of various active drug into inactive metabolites in order to terminate the biological effect of the drug
		Formation of active drug from the inert prodrugs
		Generation of active secondary metabolites leading to increased therapeutic effects and adverse drug reactions
2	Toxicology	Accumulation of toxic metabolites generated from the relatively innocuous compound leading to organ toxicity in overdosage
		Knowledge about enzyme involved in biotransformation provide development of a specific antidote for the toxins
3	Pharmaceutical drug development	Requirement of the separate study protocol for the assessment of efficacy and safety testing of a metabolite from the parent drug
		Development of metabolite as a separate new drug
		Difference in the expression of enzyme metabolizing the drug between the humans and animals leading to limitation of usage of animal models
		Long-term assessment of animal models for the generation of data regarding carcinogenicity and mutagenicity potential of metabolites
4	Clinical pharmacology	Drug-drug interactions at the level of metabolism (two drugs competing for getting metabolized by the same enzyme)
		Microsomal enzyme induction or inhibition by a drug leading to therapeutic failure or toxicity of another drug
		Variation between the individuals with regard to drug-metabolizing capacity resulting in therapeutic failure or toxicity

7.3.1 Sites of Metabolism at the Organ Level

- **Intestinal Mucosa**
  - Epithelial cells of the GI tract are considered as the first organ involved in the metabolism of oral medications. Both the phase I and phase II metabolic reactions occur in the intestinal mucosa. For many drugs, first-pass metabolism occurs at intestinal mucosa. *First-pass metabolism* refers to the metabolism of a drug during its passage from the site of absorption into the systemic circulation which can happen at the intestinal lumen, wall, or liver; it is also at times referred to as "pre-systemic metabolism."
  - Alcohol dehydrogenase is present in the stomach wall mucosa, and it inactivates a part of ingested ethanol at the level of the stomach before the liver.
  - Many CYP3A subfamily enzymes (CYP3A4, CYP2E1, CYP2D6, CYP1A1, and CYP3A5) and intestinal monoamine oxidase are located in the villous tips of enterocytes and are responsible for phase I oxidation of various drugs. For example, sumatriptan, phenylephrine, epinephrine, opioids, and β_2 adrenergic agonists contain amine and are oxidized to inactive metabolites.
  - Cyclosporine, midazolam, saquinavir, erythromycin, ethinyl estradiol, and tacrolimus have significant intestinal first-pass metabolism than the liver first-pass metabolism.
  - Furthermore, esterase and amidase enzymes are located in the GI tract and are responsible for complete metabolic destruction of local anesthetics.
  - Phase II reactions also occur in the intestinal mucosa. Morphine, actively secreted after i.v. administration, undergoes glucuronide conjugation in the small intestinal mucosa.
  - Estrogen sulfotransferase, dehydroepiandrosterone sulfotransferase, phenol sulfotransferase, and monoamine-metabolizing sulfotransferase are responsible for sulfation of oral contraceptive pills, progestins, paracetamol, and terbutaline, respectively, at intestinal level.
  - Sulfasalazine is metabolized to 5-aminosalicylic acid (5-ASA) and sulfapyridine by pre-systemic acetylation by *N*-acetyltransferase located in the intestinal mucosa.
  - 6-mercaptopurine and captopril are conjugated with methyl group by *S*-methyl transferase and thiopurine methyltransferase at the small intestine. This rate of conjugation reaction is equal to that seen in the liver.
- **Liver**
  - The liver, the organ with the highest metabolically active tissue per gram, is responsible for the metabolism of the majority of drugs administered by oral and various parenteral routes.
  - The larger size of the liver, its higher blood perfusion (both enteral and systemic), and hepatocytes with the highest concentration of drug-metabolizing enzymes make the liver as the major organ for drug metabolism.
  - The enzymes present in the liver responsible for drug metabolism are as follows:

Cytochrome P 450 (CYP) monooxygenase enzyme family ➔ most common and responsible for phase I oxidation reactions involving carbon and oxygen atoms and dealkylation reactions. More than 55 isoenzymes have been identified in humans. All the CYP450 enzymes have hemoprotein (iron-protoporphyrin IX complex) in its structure. When a homogenate solution of microsomes is prepared and exposed to carbon monoxide, the ferrous form of hemoprotein binds with carbon monoxide and creates a complex which absorbs 450 nm wavelength of light. Hence in CYP450, P represents "pigment" or "porphyrin" and "450" represents the wavelength (450 nm). CYP3A4 (30% of liver CYP450 content), CYP2C9 (20%), CYP1A2 (15%), CYP2E1 (10%), CYP2D6 (5%), CYP2A6 (4%), and CYP2B6 (1%) are the most common and important enzymes under the CYP superfamily.

Flavin-containing monooxygenases (FMOs) ➔ responsible for phase I oxidation reaction involving nitrogen, sulfur, and phosphorous atoms.

Monoamine oxidases (MAOs) ➔ responsible for phase I oxidative deamination of monoamines.

Diamine oxidases (DAOs) ➔ responsible for phase I oxidative deamination of diamines.

NADPH CYP450 reductase enzymes.

NADPH-quinone oxidoreductase ➔ responsible for the biotransformation of quinones.

Xanthine oxidase and xanthine dehydrogenase.

Alcohol and aldehyde dehydrogenase (ADH and ALDH).

Epoxide hydrolase responsible for hydrolysis of epoxides.

Esterases and amidases.

Various transferase enzymes of phase II reactions are uridine diphosphate-glucuronosyl transferase (UGT), *N*-acetyltransferase (NAT), methyltransferase (MT), glutathione *S*-transferase (GST), glycine transferase, sulfotransferase (SULT), xenobiotic acyl-CoA ligase, and amino acid *N*-acyl transferase.

– Since the majority of drugs are metabolized by the liver, any decompensated liver status can significantly affect the therapeutic efficacy of the drug and can lead to toxic reactions with catastrophic drug interactions during multidrug therapy.

- **Kidneys**
  - The occurrence of renal failure due to the conversion of drugs into reactive electrophilic intermediates and causing oxidative damage in renal tissues signifies the fact that the kidney also plays a vital role in drug metabolism.
  - Metabolic enzymes are located in renal cortex and medullary tissues. Among various CYP enzyme families, CYP3A4, CYP3A5, CYP2B6, CYP2C8, and CYP2C9 are present in the kidneys. Other drug-metabolizing enzymes like NAT, SULT, UGT, and GST are functional in the kidneys.
  - Among various types of UGT present in humans, UGT1A6 (metabolizing paracetamol); UGT1A9 (conjugating furosemide, mycophenolic acid, propo-

fol, raloxifene, and sulfinpyrazone); and UGT2B7 (conjugating carbamazepine, fenoprofen, ketoprofen, naproxen, morphine, codeine, valproic acid, and zidovudine) are expressed in the kidneys.
  - The rate of conjugation of mycophenolic acid with glucuronic acid by renal UGT is the same as that of the liver. The metabolism of propofol and morphine into their glucuronide conjugates by the kidneys is three to four times higher than that of the liver.
  - Kidneys also play a major role in the biotransformation of endogenous molecules like prostaglandins, leukotriene, and steroids.
  - Hence it is evident that kidneys apart from excreting a wide range of xenobiotics and their metabolites possess a significant role in biotransformation.
- **Lungs**
  - Xenobiotics inhaled from the environment are metabolized in the respiratory tract itself as a part of the natural defense. Metabolism of drugs in lungs is significantly different from that by liver and GIT in turns of low and variable expression of drug-metabolizing enzymes.
  - The major CYP enzymes in the liver like CYP3A4, CYP1A2, CYP2CP, and CYP2C19 are expressed in low degrees in the lungs. However, minor enzymes in the liver like CYP1B1, CYP2B6, CYP2E1, and CYP3A5 are the most common enzymes in the lungs. CYP1A1 is induced by smoking in the lungs. SULT, UGT, GST, esterases, cyclooxygenases, and FMOs are also present in the alveolar epithelial lining.
  - Drugs like budesonide, ciclesonide, salmeterol, fluticasone propionate, and theophylline are metabolized by the lungs.
  - Ciclesonide is a prodrug and converted to an active form by the esterases present in the lungs.
  - In a similar way, the prodrug beclomethasone dipropionate is converted to active and potent metabolite 17-beclomethasone monopropionate by the esterase enzyme present in the lungs.
  - NADP-dependent microsomal conversion of theophylline to less potent metabolite named theonosine (theophylline-7β-D-ribofuranoside) occurs in the lungs.
  - Among various anesthetic agents, propofol and prilocaine undergo insignificant metabolism in the lungs.
  - The lungs are also involved in inactivation of various endogenous hormones. Amines like serotonin and norepinephrine are inactivated by MAO and catechol-*O*-methyltransferase (COMT) in pulmonary endothelial cells. Peptides like bradykinin, atrial natriuretic peptide (ANP), and endothelin are inactivated by angiotensin-converting enzyme (ACE) in pulmonary endothelial cells. Prostaglandins like PGD_2 and $PGF_{2\alpha}$ and purines like adenosine triphosphate (ATP), adenosine monophosphate (AMP), and adenosine are also inactivated in the lungs.
- **Skin**
  - The skin also contains enzymes like CYP1A1, CYP2B6, CYP2E1, and CYP3A5 to oxidize various xenobiotics absorbed from the environment. Skin

cells express mRNA of conjugating enzymes like GST, SULT, and NAT. Expression of UGT in skin cell is not documented till date.
  - After application of methyl salicylate topically, the skin metabolizes it into salicylate, and analgesic effect is obtained.
  - Drugs like *p*-aminobenzoic acid, dapsone, and sulfamethoxazole are metabolized by NAT to its respective *N*-acetyl metabolite in fibroblasts of the skin after topical application.
  - Minoxidil, the drug which is applied topically for hair growth by causing vasodilation, is converted to its active metabolite minoxidil sulfate by the SULT present in keratinocytes of the skin.
  - Betamethasone, a topical steroid, has less skin penetration. Hence, the prodrug betamethasone 17-valerate was designed with a higher penetration rate. After application, betamethasone 17-valerate is converted to active drug betamethasone by esterase enzymes located in the skin.
  - When applied topically, nitroglycerine undergoes 15 to 20% first-pass metabolism in the skin which is low when compared to the oral route and explains the moderate efficacy of transdermal nitroglycerine patch in angina.
- **Plasma**
  - Blood esterases, like red cell esterase, plasma esterase, and pseudocholinesterase, are responsible for inactivation and activation of various drugs by metabolic cleaving of the molecule into two groups.
  - Esmolol and remifentanil are metabolized to an inactive metabolite by red cell esterases.
  - Suxamethonium, mivacurium, etomidate, and ester-containing local anesthetic agents are cleaved into inactive products by plasma pseudocholinesterase enzyme.
  - Few prodrugs are activated by plasma esterase. Dabigatran etexilate and irinotecan are converted to active compounds as dabigatran and SN-38, respectively, by plasma esterases. Ramipril is activated to ramiprilat by plasma esterase activity.
  - Deficiency of esterases in plasma can lead to catastrophic effect during the administration of skeletal muscle relaxants during surgery (succinylcholine apnea).

7.3.2 Sites of Metabolism at the Cellular Level

- Enzymatic metabolism at the cellular level can be classified into two types, namely, microsomal and non-microsomal based on the location of enzymes.
- Microsomal enzymes include CYP enzyme family, mixed function oxidases (monooxygenases), UGTs, and epoxide hydrolases. Microsomal enzymes can be induced or inhibited by various substrates.
- Non-microsomal enzymes are located in the cytoplasm, mitochondria, and plasma. Flavin protein oxidases, enzymes involved in conjugation (except for UGT, esterases, and amidases), are non-microsomal enzymes. Though these enzymes are not inducible, these enzymes may show significant genetic polymorphisms in expression.

7.4 Phases of Metabolism

Broadly the metabolism of drugs can be classified into two phases as follows:

- Phase I is called as *non-synthetic or functionalization reaction* wherein new functional groups (like –OH, –C=O, or $–NH_2$) are created in the parent molecule so that the functional group can be attached with additional molecules in further steps. The metabolite generated may be inactive or active in this phase. Only a little effect will be seen in the water solubility of the molecule after phase I biotransformation, but a dramatic effect will be observed in its biological activity.
- Phase II is also called as *synthetic or conjugation reaction* wherein various molecular groups like alkyl, aryl, various amino acids, and glucuronyl are attached to the phase I metabolite in order to make it water soluble. Metabolite generated in this phase is mostly inactive.

7.5 Types of Phase I Reactions

7.5.1 Oxidative Reaction

- Oxidative reactions are the most common phase I reactions, and they involve enzymes like CYP450 and FMN monooxygenase inside the microsomes and enzymes like MAO, DAO, and xanthine oxidase (XO) outside the microsomes (non-microsomal oxidation).
- In oxidative reactions, one molecule of oxygen is inserted into the chemical structure with or without the removal of hydrogen molecule from the parent molecule. The following are the various types of oxidative reactions.
- Hydroxylation (R–H to R–OH), dehydrogenation (R–C–OH to R–C=O), deamination (R–C–NH_2 to R–C=O), dealkylation (R–CH_3 to R–H), carboxylation (R–C=O to R-COOH), *S*-oxidation, and *N*-oxidation are the types of oxidative reactions (Fig. 7.1)**.**

- **Aromatic Hydroxylation**
  - One oxygen molecule is inserted without the removal of the hydrogen molecule in the aryl structure resulting in the formation of a hydroxyl group.
  - Drugs containing "ring structures" like benzene, phenyl, and naphthalene undergo aromatic hydroxylation. Hydroxylation group will not be formed directly in one step as direct oxidation of arene is highly impossible. Hence, an intermediate compound called "arene oxide" is formed which rearranges to hydroxyl group later. This is called as *NIH (National Institutes of Health) shift*.
  - Examples:
    Phenytoin ➔ CYP2C9 ➔ *p*-hydroxy phenytoin
    Propranolol ➔ CYP2D6 ➔ 4-OH propranolol

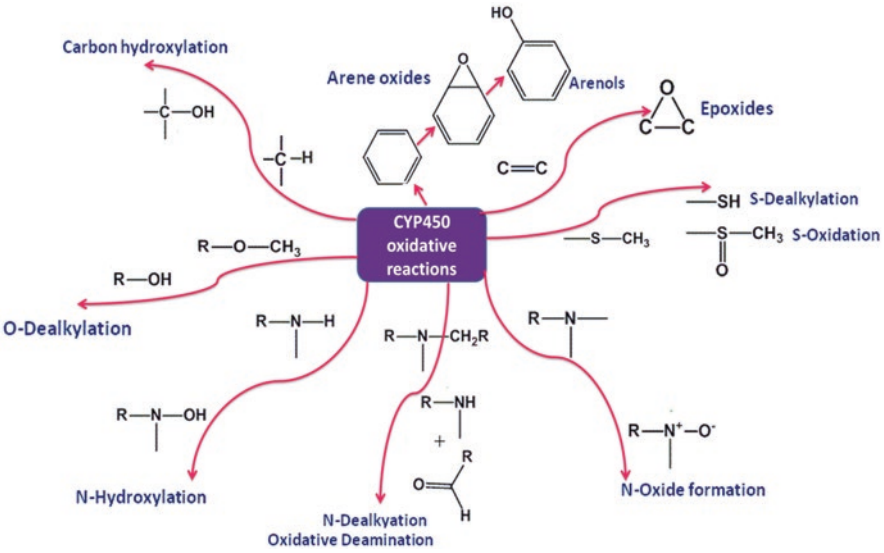

Fig. 7.1 Various pathways of oxidative reactions by CYP450 enzymes

Amphetamine ➔ CYP2D6 ➔ 4-OH amphetamine
S-warfarin ➔ CYP2C9 ➔ 7-OH *S*-warfarin
Imipramine ➔CYP2D6 ➔ 2-OH imipramine
Desipramine ➔CYP2D6➔2-OH desipramine

- **Aliphatic Hydroxylation**
  - Drugs with straight chain, branched chain, and chains linked with ring structure (alicyclic compounds like cyclohexane or cyclopentane) undergo aliphatic hydroxylation.
  - Usually, hydroxylation for aliphatic drugs occurs at terminal carbon (ω) or just one carbon next to it called as "penultimate carbon" (ω-1). In the case of alicyclic compounds, hydroxylation occurs at third or fourth carbon resulting in the formation of "cis" and "trans" configuration. The metabolites will be usually "alcohol" in both scenarios.
  - Examples:
    R-Ibuprofen ➔ CYP3A4, CYP2C9, CYP2C8 ➔ 2-OH ibuprofen
    S-Ibuprofen ➔CYP2C19 ➔ 3-OH ibuprofen
    Valproic acid ➔CYP2A6 ➔3-hydroxy valproic acid
    Valproic acid ➔CYP2C9, CYP2B6 ➔ 4-OH or 5-OH valproic acid
    Midazolam ➔CYP3A4 ➔ 4-OH or 1-OH midazolam
- **Oxidative Deamination**
  - Drugs with primary aliphatic or arylamines in their structure undergo oxidative deamination mediated by CYP450 enzymes. They have at least one α-carbon-hydrogen bond for hydroxylation at the carbon atom resulting in the formation of carbinolamine intermediate. Carbinolamine again breaks down to aldehyde or ketone with the release of ammonia.

  - Example:
    Amphetamine ➔CYP2D6 ➔phenyl acetone
- ***N*-Oxidation**
  - Drugs with the secondary and tertiary amine and amide in its structure undergo *N*-oxidation to form a stable *N*-oxide. In the case of drugs with tertiary amine structure, an intermediate carbinolamine is formed by rearrangement of *N*-oxide which in turn collapses to form secondary amine structure.
  - Examples:
    Chlorpheniramine ➔flavin monooxygenase ➔chlorpheniramine *N*-oxide
    Dapsone ➔ CYP2C9 ➔ dapsone hydroxylamine (toxic compound)
    Meperidine ➔ CYP3A4, CYP2B6, CYP2C19 ➔ meperidine *N*-oxide
- ***N*-Dealkylation**
  - Drugs with secondary and tertiary amine structures undergo *N*-dealkylation resulting in the removal of alkyl group along with nitrogen atom.
  - Substitutions like methyl, ethyl, phenyl, isopropyl, n-butyl, and allyl are commonly dealkylated from the nitrogen atom in the liver. The conversion of tertiary amine to a secondary amine by dealkylation is faster when compared to the conversion of secondary to primary amines.
  - Metabolites are often labeled with a prefix of "des" or "nor" and frequently have biological activity.
  - Examples:
    Imipramine ➔CYP3A4, CYP1A2 & CYP2C19 ➔desipramine
    Diazepam ➔CYP3A4 & CYP2C19 ➔nordiazepam
    Morphine ➔CYP3A4 & CYP2C8 ➔ normorphine
    Caffeine ➔CYP3A4, CYP2E1 & CYP2C8 ➔ theophylline
    Theophylline ➔CYP1A2 ➔1-methylxanthine
- **O-Dealkylation**
  - The oxidative mechanism is similar to that of *N*-dealkylation wherein the carbon atom attached to the oxygen is hydroxylated and cleaved resulting in the formation of alcohol and aldehyde/ketone.
  - Mixed-function microsomal oxidases are involved in *O*-dealkylation.
  - The drugs with ether, aliphatic, and aromatic ethers in their structure undergo *O*-dealkylation.
  - Few thioethers also utilize this oxidative pathway (e.g., 6-methylthiopurine), whereas the majority of thioethers undergo *S*-oxidation.
  - Examples:
    Codeine ➔CYP2D6 ➔morphine
    Indomethacin ➔CYP2C9➔ *O*-des-methyl-indomethacin
    Dextromethorphan ➔CYP2D6➔dextrorphan
- ***S*-Oxidation**
  - Almost majority of *S*-oxidation reactions are carried out by flavin monooxygenase enzymes and few by CYP450 enzymes.
  - Drugs with thiols, thioethers, disulfides, and sulfides in their structure undergo *S*-oxidation.
  - Examples:

Cimetidine➔CYP3A4, FMO➔cimetidine sulfoxide
Ethionamide ➔FMO➔ethionamide sulfoxide (hepatotoxic)
Omeprazole ➔CYP3A4➔omeprazole sulfone
Thioridazine➔CYP3A4 & CYP2D6 ➔thioridazine-2-sulfoxide

- **Dehalogenation**
  - Halogen-containing drugs like chloramphenicol, isoflurane, sevoflurane, enflurane, and halothane utilize oxidative dehalogenation pathway.
  - CYP2E1 enzyme mediates most of the dehalogenation reactions.
  - Dehalogenation reaction can create metabolite like acid chloride, acid fluoride, or trifluoroacetate which can bond with proteins resulting in the formation of "haptens." Haptens that induced immune reaction and hypersensitivity leading to organ failure are well documented.
  - Examples:
    Halothane ➔ CYP2E1➔trifluoroacetic acid
    Sevoflurane ➔CYP2E1➔hexafluoroisopropanol
- **Non-microsomal Oxidative Reactions**
  - These oxidative reactions occur outside the microsomes (plasma, cytosol, and mitochondria). Various endogenous molecules utilize these oxidative pathways commonly.
  - Examples:
    Xanthine ➔xanthine oxidase➔uric acid in plasma
    Ethanol ➔alcohol dehydrogenase ➔acetaldehyde in the cytoplasm
    Methanol ➔alcohol dehydrogenase ➔ formaldehyde in the cytoplasm
    Adrenaline ➔monoamine oxidase ➔vinyl mandelic acid

7.5.2 Reduction Reactions

- Reduction reactions are less common than oxidative reactions. When a hydrogen atom is added with or without removal of an oxygen atom, then it is called as a reduction reaction.
- Drugs may undergo reduction reactions such as dehydroxylation (R-OH to R-H), hydrogenation (R-C=O to R-C-OH), decarboxylation (R-COOH to R-C=O), amination (R-NO_2 to R-NH_2), and methylation (R-C-H to R-CH_3).
- Examples:
  - *P*-nitro benzoic acid ➔nitro-reductase ➔*p*-aminobenzoic acid (amination)
  - Chloramphenicol ➔nitro-reductase ➔ amine of chloramphenicol (amination)
  - Prontosil ➔azoreductase ➔ sulfanilamide (hydrogenation)
  - Sulfasalazine ➔azoreductase ➔sulfapyridine (hydrogenation)
  - Chloral hydrate ➔ aldehyde reductase ➔trichloroethanol (hydrogenation)

7.5.3 Hydrolysis

- Drugs with esters and amides in their structure undergo cleavage metabolism by the hydrolysis pathway. In this reaction, a water molecule is added resulting in the formation of two separate metabolites from the parent molecule.
- Enzymes like pseudocholinesterase, arylcarboxylesterase, liver microsomal carboxylesterase, and paraoxonase are involved in this reaction.
- Examples: procaine, succinylcholine, procainamide, aspirin, and enalapril.

7.5.4 Cyclization

- The drug with a straight-chain structure is converted to a ring structure. This approach is now exploited for the development of novel prodrugs called as "cyclization-activated prodrugs."
- Often cyclization occurs as a single step or as a second step after initial common oxidative metabolism.
- Examples:
  - Proguanil ➔CYP2C19 ➔ cycloguanil
  - *N*-dealkylated methadone ➔ pyrrolidine
  - N-dealkylated hydralazine ➔ methyl-*S*-triazolophthalazine
  - Ramipril ➔ diketopiperazine metabolite

7.6 Phase II Reactions

- Only after conjugation reactions (phase II), the water solubility of the drug metabolites is increased thereby favoring easy excretion through the kidneys.
- Phase II reactions utilize mainly "transferase" enzymes in order to transfer polar molecules to a functional group created in phase I reaction. Increasing the polarity of the drug metabolites in phase II results in poor cellular diffusion and low affinity for the receptor. Hence the metabolites of phase II often are biologically inactive. The types of phase II reactions are as follows:

7.6.1 Glucuronidation

- Glucuronidation is the most common phase II reaction in humans.
- The glucuronic acid is highly available in the liver, and many functional groups like alcohol, phenol, amine, and carboxylic acids undergo glucuronic acid conjugation.
- Glucuronidation is mediated by *uridine diphosphate-glucuronosyl transferase (UGT)*, and in humans two subfamilies of UGT, namely, UGT1 and UGT2, are present. UGT is present in the intestine, lung, nasal mucosa, brain, and kidneys.

- Drugs conjugated with glucuronic acid with a high molecular weight (>300 kDa) are excreted in bile which then can undergo significant "enterohepatic circulation" after deconjugation by the gut microbes.
- Example:
  - Acetaminophen and morphine (*O*-glucuronidation)
  - Ibuprofen (acyl glucuronidation)
  - Para-aminosalicylic acid (*N*-glucuronidation)
  - Morphine (*O*-glucuronidation)

7.6.2 Sulfation

- Sulfation is the second most common phase II reaction involving the enzyme *sulfotransferase (SULT)*.
- Endogenous compounds like steroids, catecholamines, thyroxine, and bile acids utilize sulfation as the major pathway for conjugation and excretion. Drugs with phenol moiety readily undergo sulfonation.
- SULT1 and SULT2 are two isoforms of sulfotransferases present in humans.
- Example:
  - Acetaminophen ➔SULT1AI➔ acetaminophen sulfate
  - Methyldopa ➔SULT1A3➔ α-methyldopa-mono-*O*-sulfate

7.6.3 Acetylation

- Acetyl CoA can be transferred to the primary aliphatic amines, aromatic amines, and hydrazines in the structure of drug metabolites. Metabolites with secondary amine structure are not acetylated.
- *N-acetyltransferase (NAT)* is the enzyme responsible for acetylation reaction.
- Acetylation reaction often produces arylamines which have carcinogenic potential and hydroxylamines which have hapten-induced hypersensitivity potential.
- Genetic polymorphism in the acetyltransferase enzymes results in differences in the rate of acetylation reaction, namely, "fast acetylators" and "slow acetylators."
- Examples:
  - Hydralazine ➔NAT ➔hydralazine acetone hydrazine
  - Procainamide ➔ NAT ➔ *N*-acetyl procainamide (a hapten causing SLE)
  - Benzocaine ➔ NAT ➔ acetyl benzocaine

7.6.4 Methylation

- Methylation differs from other conjugation reactions in terms of generation of active metabolites in phase II.

- *O*-Methyl metabolites formed by this reaction in some cases have higher lipophilicity and increased biological activity.
- *Methyltransferase* (MT) is the enzyme involved, and it requires *S*-adenosyl methionine as a cofactor. COMT, phenol-*O*-methyltransferase (POMT), TPMT, thiol methyltransferase (TMT), histamine *N*-methyltransferase (HNMT), and nicotinamide *N*-methyltransferase (NNMT) are the various types of methyltransferase enzymes.
- Examples:
  - Norepinephrine ➔ COMT ➔epinephrine (active metabolite)
  - Azathioprine ➔TPMT➔ *S*-methyl-azathioprine
  - Thioguanine ➔TPMT➔ methyl-thioguanosine
  - Histamine ➔HNMT ➔*N*-methyl histamine

7.6.5 Glutathionylation

- The metabolites with reactive electrophile nature are prevented from interacting with endogenous molecules by the conjugation reaction with glutathione.
- *Glutathione S-transferase (GST)* mediates this reaction and utilizes glutathione as a substrate. GST exists in both microsomal and cytosolic state; xenobiotics are metabolized by the former, while endogenous molecules are metabolized by the latter.
- Examples:
  - N-acetyl-*p*-benzoquinone imine, NAPQI (a toxic metabolite of paracetamol), busulfan, Adriamycin, and fosfomycin.

7.6.6 Glycine Conjugation

- *Glycine N-acyl transferase (GLYAT)* is the enzyme involved in this reaction. Glycine conjugation is utilized by very few drugs and not significant clinically.
- GLYAT utilizes acyl-CoA as a cofactor for the transfer of the glycine group to the xenobiotics.
- Examples: PABA, salicylates, permethrin, and phenylacetic acid.

7.7 Nonenzymatic Degradation

- Spontaneous rearrangement of molecules in the parent drug leading to breakdown can occur in certain drugs. This is called as *Hofmann elimination*. This property is helpful in the patient with both renal and liver failure since the drugs undergo spontaneous metabolism without the help of enzymes in the organs.
- Example: atracurium.

7.8 Microsomal Enzyme Induction and Inhibition

- Ribosomes, the protein factory of the cell, are an integral part of microsomes. Hence the microsomal enzymes can be synthesized at a higher or lower rate by altering the transcription of CYP450 mRNAs by different endogenous molecules or xenobiotics on prolonged administration.
- When the CYP450 enzymes are expressed at higher concentrations by a xenobiotic, then it is called as an *inducer* of that particular enzyme, and when the same is expressed at a lower concentration by a xenobiotic, then that particular xenobiotic is an *inhibitor* of the enzyme.
- The induction is carried out by stimulation of particular nuclear receptors by the xenobiotics resulting in a cascade of pathways culminating in increased expression of CYP450 mRNAs.
- Nuclear receptors like aryl hydrocarbon receptor (stimulated by omeprazole), constitutive androstane receptor (stimulated by phenobarbital), pregnane X receptor (stimulated by rifampin), and PPAR (stimulated by fibrates and pioglitazone) are involved in enzyme induction.
- When the substrate induces its own metabolizing enzyme, then it is called as "auto-induction" (e.g., cyclophosphamide for CYP2B, hexobarbital for CYP2C9, and ethanol for CYP2E1), and when the substrate inhibits its own metabolizing enzyme, then it is called as *auto-inhibition* (e.g., ritonavir and erythromycin for CYP3A).
- Table 7.2 illustrates the various CYP450 enzymes with their substrates, inducers, and inhibitors.

7.8.1 Clinical Significance of Microsomal Enzyme Induction and Inhibition

- Microsomal induction induces the failure of therapy due to increased inactivation of the drug by the enzyme, e.g., rifampicin induces CYP3A4. As progestins are the substrates of CYP3A4, induction of CYP3A4 leads to failure of oral contraceptives.
- Microsomal induction induces drug toxicity even at normal dosage due to increased generation of active drug from the prodrug, e.g., increased generation of oxazepam from diazepam via CYP3A4 on chronic use of glucocorticoids resulting in excessive sedation.
- Microsomal inhibition induces accumulation of active drug leading to toxicity and extended pharmacological activity, e.g., increased incidence of *torsades de pointes* due to poor amiodarone inactivation in the presence of CYP3A4 inhibition by grapefruit.
- Increased muscle relaxant effects of vecuronium are noted in patients taking cimetidine, while the relaxant effects of vecuronium, pancuronium, and rocuronium are noted in patients with chronic phenytoin therapy.

Table 7.2 Substrates, inducers, and inhibitors of CYP450 enzymes

S. No.	CYP enzyme	Substrate	Inducers	Inhibitors
1	CYP1A2	Paracetamol, theophylline, warfarin, caffeine	Smoking, omeprazole Charred foods, carbamazepine	Fluvoxamine
2	CYP2A6	Coumarins, nicotine	Rifampin, phenobarbital	Tranylcypromine
3	CYP2B6	Ketamine, methadone, propofol, sertraline, efavirenz, ticlopidine	Phenobarbital cyclophosphamide	Ticlopidine Clopidogrel
4	CYP2C9	Flurbiprofen, ibuprofen Losartan, phenytoin, S-warfarin	Barbiturates Carbamazepine Rifampin	Sulfaphenazole
5	CYP2C19	Diazepam, naproxen Omeprazole, propranolol	Barbiturates Rifampin	Fluconazole
6	CYP2D6	Clozapine, codeine Dextromethorphan Fluoxetine, haloperidol Risperidone, selegiline Tamoxifen, timolol	Ethanol Ritonavir Primidone Phenytoin Phenobarbital	Quinidine paroxetine
7	CYP2E1	Paracetamol, enflurane	Ethanol, isoniazid	Disulfiram
8	CYP3A4	Halothane, ethanol Paracetamol, amiodarone Cisapride, cocaine Cyclosporine, dapsone Diazepam, diltiazem Indinavir, lignocaine Macrolides, midazolam Miconazole, mifepristone Nifedipine, progestins Ritonavir, saquinavir Tacrolimus, tamoxifen Testosterone Verapamil	Barbiturates Carbamazepine Clotrimazole Erythromycin Glucocorticoids Pioglitazone Phenytoin Rifampin St. John's wort (hyperforin)	4-Methyl pyrazole Clarithromycin Diltiazem Erythromycin Fluconazole Grapefruit Itraconazole Ketoconazole Ritonavir

- Analgesic effect of codeine is increased when carbamazepine and rifampicin are co-administered, and decreased effect is noted for codeine in the presence of tricyclic antidepressants (TCAs) and quinidine.
- Due to auto-induction, tolerance to a particular drug may be developed soon, e.g., carbamazepine via CYP3A4.
- Porphyria may be precipitated by enzyme induction leading to increased porphyrin synthesis by barbiturates.
- Phenytoin can increase the metabolism of endogenous steroids by induction of CYP3A4 and hence can be used in the management of Cushing's syndrome.
- Since bilirubin also utilizes the UGT pathway for elimination enzyme induction with phenobarbitone, it is helpful in reducing jaundice in congenital non-hemolytic jaundice of neonates.
- Clearance of ropivacaine is decreased due to CYP1A2 inhibition by fluvoxamine resulting in convulsions and arrhythmias.

7.9 Factors Influencing Drug Metabolism

- **Age:** Pediatric and geriatric populations are slow metabolizers when compared to adults due to immature and loss of enzyme activity in the respective populations.
- **Sex:** Males metabolize drugs faster than females. Drugs like ethanol, propranolol, and estrogens are metabolized faster in males than females.
- **Liver size and liver function capacity:** Metabolism of drugs is significantly affected in active liver diseases leading to toxic reactions and failure of therapy. Males and adults with bigger liver size metabolize the drugs faster than females and children.
- **Body temperature:**
  - Hyperthermia increases blood flow to the organs including the liver and kidney, and one can expect drug clearance by metabolism at a faster rate. However, on the contrary, the metabolism of majority of drugs is reduced due to decreased function of CYP450, FMOs, and other enzymes.
  - Interleukins (IL 1, IL 4, and IL6), INF-y, and TNF-α secreted during fever decrease the activity of drug-metabolizing enzymes.
  - Drugs like α-methyldopa, salicylamide, antipyrine, and sulfonamides are proven to undergo decreased metabolism during fever.
- **Diet:**
  - Since CYP450 enzymes, especially CYP3A4, can be induced or inhibited by various dietary compounds, type of food intake has a potential role in drug metabolism.
  - Grapefruit juice inhibits CYP3A4, and hence midazolam, cyclosporine, and diazepam toxicity occur.
  - Cruciferous vegetables (cabbage, cauliflower, and others) induce CYP1A1 and CYP1A2 leading to therapeutic failure of warfarin, carbamazepine, and theophylline therapy.
  - Ethanol induces CYP2E1 and hence increases carcinogen generation by oxidative reaction in alcoholics.
  - Polycyclic aromatic hydrocarbons in barbecued meat induce CYP1A2 and significantly affect the theophylline metabolism.
- **Environmental factors:**
  - Insecticides and aromatic hydrocarbons are known to induce or inhibit CYP enzymes.
  - Exposure to chlorinated hydrocarbon insecticides like lindane and DTT significantly decreases the plasma half-life of antipyrine by microsomal induction.
  - Cigarette smoke and other plastic burnt smoke contain benzopyrene which induces CYP1A1 and CYP1A2.
  - Heavy metal exposure like mercury, cobalt, nickel, and arsenic results in inhibition of drug-metabolizing enzymes directly by forming protein adducts.
  - Various animal studies have proven that high-altitude hypoxia upregulates CYP2D6 activity and downregulates CYP1A2.

- **Co-administration of other drugs and comorbidity:** Hypothyroidism decreases the expression of various CYP450 enzymes. Presence of diabetic microvascular complication affects drug metabolism in the kidneys. Drugs taken for other comorbid conditions can cause significant drug-drug interactions.
- **Genetic polymorphisms:**
  - ***CYP2A6* polymorphism:** CYP2A6 is responsible for activation of various procarcinogens, metabolism of nicotine, and warfarin. *CYP2A6*4* and *CYP2A6*2* are nonfunctional alleles and hence offer protection from carcinogens. However, accumulation of TCAs, haloperidol, and selective serotonin reuptake inhibitors (SSRIs) can lead to toxicity due to decreased metabolism by nonfunctional alleles.
  - ***CYP2C9* polymorphism:** CYP2C9 metabolizes *S*-warfarin, phenytoin, and various NSAIDs. *CYP2C9*2* and *CYP2C9*3* alleles have reduced enzyme activity that can cause bleeding disorder when *S*-warfarin is administered.
  - ***CYP2C19* polymorphism:** Diazepam, omeprazole, and various NSAIDs are metabolized by CYP2C19. In this enzyme, *CYP2C19*2* is more common than other alleles and has no enzymatic capacity.
  - ***CYP2D6* polymorphism:** Three different polymorphisms such as extensive metabolizers (EM), under metabolizers (UM), and poor metabolizers (PM) exist in CYP2D6. In poor metabolizers, codeine efficacy is reduced due to poor conversion to morphine. Nortriptyline is required in a higher dose than the normal individuals in UM type of CYP2D6.
  - **Slow and fast acetylators:** Based on the polymorphism in the *NAT2* gene, there are three sets of populations, namely, fast, intermediate, and slow acetylators. *NAT2*5/*7* and *NAT2*6/*7* genotypes are slow metabolizers of isoniazid resulting in drug-induced hepatotoxicity.

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Drug Elimination

8

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Abstract

The process by which the drug and/or its metabolite(s) are transferred permanently from the internal to the external environment is called excretion of the drug. The major route of drug elimination is kidney, followed by the liver, lungs, skin, salivary glands, mammary glands, and semen constituting the nonrenal pathways for drug elimination. Size, solubility, polarity, and protein binding nature are the major determinants for the elimination of drugs by the organ. The kinetics of elimination defines the half-life of the drug and thereby determines the dosing frequency of the drug. The drug can undergo linear (*first order*) kinetics or nonlinear (*zero order or saturation*) kinetics. Elimination is one of the sites for "drug interaction" wherein two drugs can compete for excretion resulting in toxicity or failure of therapy. Elimination of drugs in breast milk is a major concern while prescribing the drug for lactating mother. In clinics, the knowledge about the drug elimination gains significance in multidrug therapy, in the presence of organ failure and other co-morbid conditions.

Keywords

Renal drug elimination · Nonrenal drug elimination · Clearance · Half-life · First-order kinetics · Zero order kinetics

8.1 Introduction

Homer William Smith (1895–1962), a renowned renal physiologist, quoted that "Superficially, it might be said that the function of the kidneys is to make urine; but in a more considered view one can say that the kidneys make the stuff of physiology itself." It is not hyperbolizing when we say "kidneys make the stuff of Pharmacology itself." Drug elimination (excretion) occurs principally in the kidneys, and elimination requires biotransformation by various organs including the kidneys.

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Excretion of drugs is defined as a process through which the drug and its metabolite(s) are permanently transferred to the external environment from the internal milieu. Apart from the kidneys, drug elimination occurs via various "nonrenal" pathways like the liver, intestine, lungs, skin, salivary glands, lacrimal glands, mammary glands, and semen.

8.2 Renal Drug Elimination

8.2.1 Property of the Drug (Metabolite)

The drugs or their metabolites that undergo renal route of elimination should be:

- Water soluble
- Non-volatile
- Small molecular size (<500 Da)

8.2.2 Process of Renal Drug Elimination

The principal processes that determine urinary excretion of a drug are:

- Glomerular filtration
- Active tubular secretion
- Active or passive tubular reabsorption

8.2.3 Glomerular Filtration

- Glomerular filtration is a nonselective and unidirectional process whereby maximum compounds are filtered. The plasma protein or blood cell-bound drugs and drugs with large molecular size (>500 Da) are exceptions.
- Though the negative charge in the glomerular basement repels anionic drugs, the main driving force for filtration of drugs is hydrostatic pressure of the blood flowing in glomerular capillaries. Creatinine, inulin, mannitol, and sodium thiosulfate are used to estimate the GFR.
- Majority of the drugs are excreted by glomerular filtration. Macromolecular drugs like biologicals (monoclonal antibodies) cannot be filtered and hence utilize nonrenal pathways for elimination.
- Glomerular filtration of drugs depends upon renal blood flow and status of protein binding of the drugs.

8.2.4 Active Tubular Secretion

- Around 80% of the reabsorbed drug enters peritubular capillaries of the proximal tubules and is actively secreted into the tubular lumen. Hence, tubular secretion of drugs is the most effective method of renal drug elimination.
- Active secretion is a carrier-mediated process which requires energy for transportation against a concentration gradient. Therefore, active secretion can achieve maximal drug clearance.
- Two active tubular secretion mechanisms have been identified:
  - System for secretion of organic acids or anions (organic anion transport): penicillin, salicylates, furosemide, indomethacin, probenecid, and metabolites conjugated with glucuronic acid, glycine, and sulfates
  - System for secretion of organic bases or cations (organic cation transport): morphine, mecamylamine, hexamethonium, amiloride, triamterene, quinine, and endogenous amines such as catecholamines, choline, and histamine
- Para-aminohippurate (PAH) and iodopyracet are used to determine active secretion by the kidneys. Drugs that undergo active secretion have clearance >500 ml/min. This is due to the occurrence of both glomerular filtration and tubular secretion.
- Changes in pH and status of plasma protein binding will not affect the active secretion as the bound drug dissociates rapidly once the unbound drug gets excreted. Hence, active secretion is independent of the status of plasma protein binding.
- Drugs with same ionic charge and carrier-mediated process for excretion compete with each other. Few examples are:
  - Probenecid competes with penicillin in active tubular secretion of an organic acid. This property can be exploited in conditions where a high dose of penicillins is required (like gonococcal infection), wherein, along with the administration of probenecid, the requirement of a penicillin dose is nearly halved.
  - Probenecid also competes with para-aminosalicylic acid (PAS), PAH, and 17-ketosteroids secretion doubling their plasma concentrations.
  - Inhibition of secretion of nitrofurantoin by probenecid is detrimental in the case of urinary tract infection where nitrofurantoin fails to reach the site of action.
  - Metformin and pyrimethamine compete with each other for active secretion via hepatic organic cation transporter 2 (hOCT2), and hence, the area under the curve (AUC) of metformin is increased 1.4 times than metformin alone. Dolutegravir also increases the AUC of metformin 2.5 times upon coadministration by the same mechanism.
  - Cisplatin-induced nephrotoxicity can be decreased by coadministration of cimetidine wherein both the drug utilizes OCT and cimetidine prevents the accumulation of cisplatin in the nephrons.

8.2.5 Tubular Reabsorption

- Tubular reabsorption can be either an active process or passive process.
- Active tubular reabsorption is seen with endogenous substances such as electrolytes, glucose, vitamins, amino acids, and uric acid and few drugs like oxipurinol.
- Passive tubular reabsorption is seen with a large number of drugs and few endogenous substances.
- The determinants of passive tubular reabsorption are as follows:
  - Lipophilicity of drugs
  - pH of urine
  - pK_a of drug
  - Urine flow rate
- The passive tubular reabsorption of drugs is based on the polarity of the drug which in turn depends on the pH of urine and pK_a of the drug. As most of the drugs are either weak acids or weak bases, the pH of urine and pK_a of drug play a major role in influencing the tubular reabsorption of the drug.
- The parameters urine pH, drug pK_a, and degree of ionization are related to each other by the "Henderson–Hasselbalch equation" which is as follows:
  - For weak acids, $pH = pKa + \log\left[\dfrac{\text{ionized}}{\text{unionized}}\right]$ which on rearranging will give

    $$\%\ \text{of drug ionized} = \frac{\left[10^{pH-pKa}\right]}{\left[1+10^{pH-pKa}\right]} \times 100$$

  - For weak bases, $pH = pKa + \log\left[\dfrac{\text{unionized}}{\text{ionized}}\right]$ which on rearranging will give

    $$\%\ \text{of drug ionized} = \frac{\left[10^{pka-pH}\right]}{\left[1+10^{pKa-pH}\right]} \times 100$$

- Physiologically, the pH of human urine will be in between the range of 4.5–7.5. Hence with this range of pH in urine:
  - Very weak acidic nonpolar drugs like phenytoin (pKa >8.0) or very weak basic nonpolar drugs like propoxyphene (pKa <6.0) remain unionized mostly in the entire range of human urine pH. Hence, these drugs are extensively reabsorbed passively in all values of urinary pH.
  - On the other hand, drugs like cromoglycic acid (cromoglycate) with strong acidic nature (pKa <2.0) or drugs with strong basic nature like guanethidine (pKa >12.0) are totally ionized in the entire range of urinary pH. Hence, these drugs are never reabsorbed and are excreted at a higher rate and do not depend upon the urinary pH.
  - Drugs which are polar in their unionized form also will not be reabsorbed and are excreted at a higher rate. Their rate of excretion is the sum of filtration rate and rate of active secretion, e.g., penicillins and gentamicin.

  - Finally, acidic drugs with pKa between 3.0 and 8.0 (e.g., NSAIDs) and basic drugs with pKa between 6.0 and 12.0 (e.g., morphine, TCAs) depend greatly on the urinary pH for the extent of reabsorption. Since their pKa values are within the range of human urinary pH, their reabsorption magnitude can be as minimum as 0% and as maximum as 100%.
- When the urinary flow rate is considered, the passive reabsorption of polar drugs is not affected by the urine flow rate. However, in weak acidic and weak basic drugs, the extent of reabsorption is inversely proportional to the urine flow rate.
- Since most of the drugs used clinically are weakly basic and weakly acidic in nature, the principle of "forced diuresis" is used in a poisoning situation. Forced diuresis with increased urinary flow rate will decrease the passive reabsorption of drugs.
- Moreover, based on the nature of polarity and pKa of drugs, urine can be acidified or alkalinized resulting in the formation of ionized drugs in the tubular lumen in order to prevent its passive reabsorption.

8.3 Nonrenal Drug Elimination

8.3.1 Hepatic Drug Elimination

- The process of biliary drug excretion is similar to active renal secretion because bile secretion is capacity-limited and can be saturated.
- The capacity of the liver to clear the plasma from drug via bile (biliary clearance) can be expressed as $\text{Biliary clearance} = \text{Bile flow} * \frac{\text{Bile drug concentration}}{\text{Plasma drug concentration}}$
- Biliary clearance of the drug is very less for drugs whose biliary concentration is less than that in plasma, and drugs with higher biliary concentration than plasma will have higher biliary clearance. Hence, based on the bile or plasma concentration, the compounds that are excreted in bile can be divided into:
  - Group A: the ratio of bile to plasma concentration is approximately 1, e.g., glucose, sodium, and potassium ions.
  - Group B: the ratio of bile to plasma concentration is >1 (10–1000), e.g., bile salts, bilirubin, and creatinine.
  - Group C: the ratio of bile to plasma concentration is <1, e.g., sucrose, inulin, phosphates, and mucoproteins.
- The factors influencing the biliary clearance of drugs are:
  - The polarity of drugs (greater the polarity better the excretion).
  - Size of the molecule (molecular size above 500 Da are excreted mainly in bile).
  - Drug metabolism (biotransformation converts drugs into more polar nature, and conjugation with glucuronic acid and glutathione increases molecular size facilitating the biliary excretion).

  - Drugs with highly polar functional groups like – COOH (cromoglicic acid) or – NH_4^+ result in unchanged excretion in bile.
  - Route of administration (oral route of drug administration has more biliary excretion than the parenteral route of drug administration).
  - Presence of food (protein- and fat-rich food increases the bile secretion and, thereby, increases the biliary clearance of drugs).
- Several drugs which are excreted unchanged in bile, and drugs with glucuronide and glutathione conjugation after hydrolyzing the conjugates can be reabsorbed via intestine and undergo enterohepatic circulation.
- Enterohepatic circulation is observed with endogenous compounds like vitamin B_{12}, vitamin D, folic acid, steroids, and bile salts. Drugs like carbenoxolone, oral contraceptives, indomethacin, mycophenolic acid, rifampicin, and chlorpromazine have prolonged half-life due to the enterohepatic circulation. Any alteration in microbial flora by oral antibiotics will significantly affect the enterohepatic circulation process and thereby causes failure of therapy.
- The compound sulfobromophthalein is commonly used to assess the active biliary excretion capacity of the liver.

8.3.2 Pulmonary Drug Elimination

- The pulmonary drug elimination is an important aspect in anesthesiology, as gaseous and volatile substances are eliminated by the pulmonary system.
- Systemic drugs are excreted by simple passive diffusion from plasma with higher concentration into the alveolar space with lower concentration, e.g., anesthetic inhaled agents like halothane, nitrous oxide, fluranes, and ethanol.
- Elimination of drugs by the lungs depends upon the respiratory rate, pulmonary blood flow, pathological state of the lungs, solubility of drugs in blood, and physiochemical nature of the drugs.
- Drugs with high blood solubility like ethanol are excreted slowly by the lungs, and drugs with low blood solubility like nitrous oxide are excreted at the same rate of absorption.
- Topically administered drugs are eliminated by local mucociliary clearance, macrophage uptake, and enzymatic degradation.
- The lungs also contribute to the metabolism of drugs (refer to Chap. 7) to some extent.

8.3.3 Salivary Drug Elimination

- The excretion nature of drugs via saliva is by passive diffusion and active secretion process. The pH of saliva varies from 5.8 to 8.4, and hence the salivary excretion of drugs can be predictable by pH-partition hypothesis.
- Lipid-soluble drugs and unionized drugs are excreted passively in the saliva.

- Basic drugs are excreted more in saliva as compared to the acidic drugs because the mean salivary pH is around 6.4 which is acidic when compared to the plasma.
- The ratio of salivary concentration to plasma concentration of drugs is almost constant always for drugs like carbamazepine, theophylline, and caffeine. Hence, based on the salivary concentration, the approximate concentration of drug in the blood can be estimated.
- Lithium, penicillin, and phenytoin are actively secreted in saliva, and hence, the concentration of drugs is two- to threefold higher than the plasma concentration.
- Drugs with dye nature are excreted unchanged in the saliva and cause discoloration of the oral mucosa and tongue, e.g., rifampicin.
- Similar to biliary drug excretion, drugs excreted via saliva also undergo enterohepatic cycling, e.g., sulfonamides and clonidine.

8.3.4 Drug Elimination by Mammary Gland

- The pH of human milk ranges from 6.4 to 7.6 with a mean pH of 7.0.
- Majority of drugs are excreted into the breast milk by passive diffusion. However, drugs like cimetidine and ranitidine utilize active transport for the cations.
- Acidic drugs like nitrofurantoin achieve 26 times higher concentration in milk than the plasma indicating the involvement of the active cationic transport. Benzylpenicillin concentration in the milk can be reduced by coadministration of probenecid suggesting the blockade of active secretion of penicillin by the probenecid in the mammary gland.
- The ratio between the concentration of drug in milk to that of plasma or serum is called as MP ratio or MS ratio. This MP ratio is a time-dependent parameter and can be influenced by various parameters like:
  - Drug ionization and plasma protein binding (drugs that are more likely to be transferred into breast milk are free and unbound to proteins)
  - Molecular weight (low molecular weight drugs are highly transferred into breast milk)
  - Solubility of drugs in lipids (lipid-soluble drugs enter into breast milk at a higher rate)
  - pH of milk and plasma (weakly alkaline drugs achieve a higher concentration in the breast milk than the weak acidic drugs)
- The property of elimination of drug via breast milk gains significance when lactation for an infant is considered. The exposure of the infant to the drug via breast milk can be estimated by exposure index (%).
- Exposure index (%) = 100 × MP ratio × (milk intake by infant/infant drug clearance).
- Drugs with low rates of clearance can result in a significant degree of drug exposure in infants via breast milk (>10% of exposure index).

8.3.5 Drug Elimination by the Skin

- Drug and its metabolite elimination by skin are usually by passive diffusion. In some cases, this route of elimination is responsible for urticaria, dermatitis, and other hypersensitivity reactions due to the drugs.
- Some drugs excreted via skin are benzoic acid, methamphetamine, rifampicin, salicylic acid, alcohol, and antipyrine and heavy metals like lead, mercury, and arsenic.
- Palmar-plantar erythrodysesthesia which occurs in doxorubicin therapy could be attributed to excretion of doxorubicin by sweat glands, penetration of the drug into stratum corneum, and forming the free radical-induced damage.
- Methamphetamine can be detected in sweat within 2 h of ingestion and will be excreted more than 1 week after stopping from multiple uses. This property can be used as an alternative method for the detection of abuse of methamphetamine.
- Moreover, many drugs with abuse potential like ethanol, buprenorphine, cocaine, and cannabinoids are also excreted via sweat glands and can be quantified for abuse activity.

8.3.6 Drug Elimination by Gastrointestinal System

- Water soluble and ionized forms of drugs are excreted in feces.
- Drugs like codeine and morphine are actively secreted through the gastric mucosa at a pH of 1–3. Gastric lavage is done in parenteral opioid poisoning to prevent reabsorption of morphine from gastric lumen.
- P-glycoprotein expressed in the intestinal mucosa is responsible for excretion of anticancer drugs like docetaxel.
- In rats, intestinal secretion by P-glycoprotein is the major route of elimination for the anthelminthic drug ivermectin. Verapamil, an inhibitor of P-glycoprotein, reduces the fecal concentration of ivermectin.

8.3.7 Drug Elimination in Semen

- Various drugs are excreted in the male genital system through the prostate and seminal vesicles and hence can be recovered in semen.
- Antibiotics like methicillin, cephalexin, cephalothin, tetracycline, ciprofloxacin, norfloxacin, sulfamethoxazole, and clindamycin attain a higher concentration in semen than that of plasma.
- Antibiotics like ampicillin, oxacillin, erythromycin, chloramphenicol, nalidixic acid, aztreonam, kanamycin, and metronidazole attain very least concentration in the semen.

- Though antiepileptic drugs like phenytoin and sodium valproate are excreted in semen, significant changes in sperm motility are not observed even on chronic use.

8.4 Kinetics of Elimination

8.4.1 Clearance

- Clearance (CL) is defined as the volume (hypothetical) of body fluids containing drug from which drug is cleared completely in a specific time. The unit of clearance is ml/min.
- Mathematically, clearance can be expressed as Clearance = Elimination rate/Plasma drug concentration. Clearance can be different for different organs. Renal clearance is an important parameter in determining the other factors of the kinetics of elimination like half-life and maintenance dose of drugs.
- Physiologically, renal clearance can be defined as:

$$\text{Renal clearance} = \frac{\text{Rate of filtration} + \text{Rate of secretion} - \text{Rate of reabsorption}}{\text{Plasma drug concentration}}$$

- Renal clearance can be as less as zero (theoretically) and as maximum as renal blood flow (650 ml/min for PAH). The ratio of clearance of drug to that of creatinine is called as *renal clearance ratio* which ranges from 0 to 5.
- The relationship between the renal clearance and the mechanism of clearance is presented in Table 8.1.
- The following are the factors which determine the clearance of the drug:
  - Physicochemical properties of the drugs: molecular size, pKa, and lipid solubility.
  - Plasma concentration of the drug: glomerular filtration and passive tubular reabsorption are dependent on the plasma concentration of the drug and show saturation point, while active secretion shows a linear relationship with the plasma concentration.

Table 8.1 Mechanisms involved across different ranges of renal clearance

S. No.	Renal clearance (ml/min)	Renal clearance ratio	Mechanism of renal clearance	Example
1	0 (least)	0	Complete reabsorption of filtered drug	Glucose
2	0.1–130	0.01–0.99	Partial reabsorption of filtered drug	Lipophilic drugs
3	130	1	Only drug filtration	Inulin
3	130.1–649	1.01–4.99	Drug filtration with active secretion	Polar, ionic drugs
4	650 (peak)	5	Clearance equals renal blood flow	PAH

  - Plasma protein binding: free drugs are easily excreted in the urine and bound drugs cannot.
  - Blood flow to the kidneys: glomerular filtration and active secretion are dependent on the renal blood flow. Elimination of the drugs is enhanced by increasing the contact of the drug with the secretory sites which in turn is dependent on the renal blood flow.
  - Sex: clearance is lesser in females than males by approximately 10–20%.
  - Age: newborn and elderly population have a clearance of 30–40% lesser than that of the adults.
  - Drug interactions leading to changes in the active secretion, urine pH, and renal blood flow can alter the drug clearance.
  - Renal disease status.
- Clearance is the single most parameter which determines the maintenance dose of any drug. This is because, at steady-state concentration, the dosing rate should be equal to the rate of elimination, i.e., Dosing rate = Rate of elimination = Clearance × Target plasma concentration.
- Hence, if the target concentration is fixed with the clearance value, the dosing rate can be calculated.

8.4.2 Elimination Half-Life ($t_{1/2}$) or Beta Half-Life

- The elimination half-life of drug (also called as the biological half-life) can be simply stated as the time interval in which the amount of drug in the body is reduced to one-half from its initial value.
- The half-life of the drug is commonly misunderstood as the "equivalence of clearance" which is completely inaccurate. The half-life of the drug is a "meta-meter" which takes the account of drug clearance with its volume of distribution (V_d) after dosing. Hence, half-life of the drug is a predictor of drug fluctuation and accumulation in plasma concentration.
- Mathematically, $t_{1/2}$ of the drug can be calculated as $\text{Half} - \text{life of drug} = \left[\frac{0.693}{k}\right]$ wherein K is the elimination constant which is the ratio of clearance to the volume of distribution. Hence, $t_{1/2} = [0.693 \times V_d]/\text{CL}$.
- The important use of half-life in the clinical setting is that the time to reach the steady-state plasma concentration of drug can be determined using it. Generally, it takes 4–5 half-lives for the drug to reach the steady-state plasma concentration. The half-life used in this setting is called as the *steady-state half-life*.
- In a situation where the drugs are administered in prolonged dosing, the drug may enter into the peripheral compartment from plasma, and after stopping the infusion, the drugs will be slowly diffusing back into plasma. This slow equilibration creates the prolongation of the half-life of the drug. This is referred to as the *terminal half-life* of the drug.

- The steady-state half-life of the drug markedly differs from terminal half-life in drugs like gentamicin (2–3 h after single administration versus 53 h on continuous infusion), indomethacin (2.4 h versus 120 h), and various anesthetic agents.
- In the case of radionuclides used in radiotherapy, the half-life of the drug is the combination of both physical (radioactive decay) and the biological half-life. This half-life is called as the *effective half-life*.
- The half-life of drugs depends upon the clearance and volume of distribution, and hence, $t_{1/2}$ may change when either or both of the parameters change.

8.4.3 Absorption Half-Life

- Physiologically multiple compartments exist in the human body, and hence drug can get distributed in various compartments. Using multi-compartment modeling, the half-life of the drug can be calculated during the distribution phase (in i.v. administration) or in absorption phase (in oral administration). This half-life is called as *absorption or alpha half-life*.
- Clinically, absorption half-life plays a minor role except in anesthetic drug usage. However, absorption half-life plays a major role in the pharmacokinetic estimation in drug development.

8.4.4 Linear and Nonlinear Kinetics of Drugs

- Drugs can follow linear or nonlinear pharmacokinetics upon administration. On chronic administration the pharmacokinetics parameters like clearance, half-life, and volume of distribution transform gradually indicating a shift toward nonlinear pharmacokinetics.
- Nonlinear kinetics occurs due to saturation in any of the components of ADME. Saturation of protein binding, saturation of metabolizing enzyme, and saturation of carriers or transport involved in the absorption or elimination lead to nonlinear kinetics.
- Very few drugs like ethanol, tolbutamide, phenytoin, warfarin, theophylline, and aspirin follow the true nonlinear kinetics. The following are the few examples for drug undergoing nonlinear kinetics at different levels:
  - Saturation at absorption:
    Transport saturation at the gut wall: riboflavin, gabapentin
    Saturation of intestinal metabolism: propranolol, salicylamide
  - Saturation at distribution:
    Plasma protein binding saturation: phenylbutazone, lidocaine
    Tissue binding saturation: imipramine, thiopental
  - Saturation at metabolism:
    Saturation of metabolism enzyme: phenytoin, salicylic acid
    Metabolic enzyme inhibition: diazepam

Table 8.2 Characteristics of linear and nonlinear kinetics

S. No.	Property	Linear kinetics	Nonlinear kinetics
1	Alternate terms	First-order kinetics	Zero-order kinetics
		Non-saturation kinetics	Saturation kinetics
			Capacity limited kinetics
2	Frequency	Majority of drugs	Very few drugs
3	Clearance	Clearance is constant	Clearance decreases with increasing plasma concentration
4	Half-life	Constant	Half-life increases with increase in plasma concentration
5	Rate of elimination	Proportional to the plasma concentration	Independent of plasma concentration
6	Drug excretion	Constant fraction of drug is excreted in unit time	Constant amount of drug is excreted in unit time
7	Concentration vs time profile	Superimposable for all doses	Different for different dose and, hence, nonsuperimposable
8	Area under the curve (AUC)	Directly proportional to the dose	Disproportional to the dose
9	Effect of dose on various PK parameters	No change with the increase in dose	PK parameters change with various doses
10	Example	95% of available drugs	Phenytoin, warfarin, ethanol, theophylline, tolbutamide, and aspirin

  - Saturation at elimination:
    Saturation of active secretion: PAH
    Saturation of tubular reabsorption: ascorbic acid, riboflavin, ethanol
- In nonlinear kinetics, due to saturation, the drug excretion will be in constant amount, while in linear kinetics, the drugs are excreted in a constant fraction. The few differences between linear and nonlinear kinetics are illustrated in Table 8.2.

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Mechanisms of Drug Action

9

Abialbon Paul

Abstract

Pharmacodynamics is the study of the biological effect of drugs on biological tissues and organs and the mechanism of action. While there are some exceptions, the general rule is that drugs do not work unless they bind to a receptor. A receptor is a cellular component that the drugs bind to and produce cellular action. Receptors can either be signal-transducing extracellular entities, ion channels, intracellular enzymes or intranuclear targets. Drug molecules require affinity to bind to a receptor and intrinsic activity to activate them and cause downstream signaling. The broad types of drugs that bind to the receptor are agonists, partial agonists, antagonists and inverse agonists. The effect of a drug at varying dose ranges is studied by plotting a dose-response curve. The dose-response relationship helps us in identifying the most potent and efficacious drug for a particular clinical response. Based on the site at which the drugs act and their intrinsic activity, drugs can interact with each other to cause additive or antagonistic effects. Receptors being stimulated or blocked for a long time undergo downregulation or upregulation, respectively. Various host and drug factors affect the pharmacodynamic action of drugs.

Keywords

Pharmacodynamics · Action · Receptors · Signaling · Agonists · Antagonists

9.1 What Is Pharmacodynamics?

Pharmacodynamics is the study of the biologic effect of drugs on tissues and organ systems and their mechanism of action.

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9.2 Concepts in Pharmacodynamics

9.2.1 Receptor

- The specialized cellular unit to which drugs bind to elicit responses is called a receptor. According to Paul Ehrlich's famous maxim '*Corpora non agunt nisi fixata*', drugs do not work unless they are bound.
- We now know this is not entirely true. There are few examples where drugs produce clinical benefit without binding to a receptor, e.g. osmotic diuretics and antacids, among others. However, the majority of the response to drugs used clinically is mediated to various types of receptors present in the cells.

9.2.2 Acceptor

- An acceptor is an entity to which the drug binds but do not result in a biochemical or physiological effect but can significantly alter the pharmacokinetics, e.g. binding to albumin.
- A drug might bind to a single type of receptor or bind nonspecifically to multiple types of receptors and consequently have multiple actions and multiple mechanisms to produce those actions. This depends on the:
  - Receptor specificity
  - Expression of the receptor in various tissues
  - Drugs concentration at the target tissue
  - Differential distribution of drugs to various organs
  - Pharmacogenetics
  - Presence of other interacting drugs

9.2.3 Affinity

- When drugs bind to receptors, they exist in two forms – the bound form and the unbound form. The receptor bound fraction is responsible for the biological activity of the drug. This is the basis of the *receptor occupancy theory*.
- The strength of the binding measured is expressed as the affinity of the drug to the receptor.
- For a drug to work, it must have some affinity to a receptor. The absence of affinity would result in no binding and hence no action.

9.2.4 Intrinsic Activity

- The ability of the drug to activate the receptor causes downstream signaling process to occur that is called *intrinsic activity*. Drugs might have high intrinsic activity (agonists) or no intrinsic activity (antagonist). Very rarely, a drug will

bind to a receptor and produce a response completely opposite to that of an endogenous ligand (inverse agonist).

9.2.5 Pharmacophore

The part of the drug that is responsible for binding to the receptors is called as the *pharmacophore*.

9.2.6 Drug Action

- Drugs act by binding to receptors that exist in the body to bind to endogenous ligands and bring about biochemical and physiological changes.
- This binding can either increase or decrease the endogenous response depending on the chemical nature of the drug and the site of binding.
- Drugs do not create new intracellular responses.

9.2.7 Specificity of Drug Responses

- Specificity is the ability of a drug to bind to a single type of receptors. Most of the drugs used clinically tend to have low specificity.
- Low specificity of drug-receptor binding results in off-target side effects. These are side effects arising due to drugs binding to other receptors in the body.

9.2.8 Structure-Activity Relationship

- The affinity, intrinsic activity and specificity of a drug are highly dependent on the chemical structure of the drug.
- Minor changes in the chemical composition and/or chemical structure can result in an alteration in the drug's ability to bind and activate the receptor. This is useful as focused alterations in the structure can be made to improve the clinical benefit while reducing the adverse drug reactions.

9.3 Drug-Receptor Interaction

When drugs bind to a receptor, they may modify the receptor in many ways to bring about a biological effect.

- *Agonist*: An agonist is a drug that binds to the receptor and enhances the endogenous signaling of the receptor.
- *Primary agonist*: An agonist that binds to the same site as the endogenous ligand is called a primary agonist.

- *Allosteric or allotropic agonist*: An agonist that binds to a different site on the receptor other than the site for an endogenous ligand.
- *Partial agonist*: A partial agonist binds to a receptor while only partially activating it. The response a partial agonist produces is less than the response produced with an agonist for the same receptor.
- *Antagonis*t: An antagonist is a drug that competitively binds to the receptor while not stimulating any downstream signal transduction.
- *Syntopic antagonists* bind to the same site of the primary agonist.
- *Allosteric antagonists* bind to a different site other than the site of the primary agonist and often bring about a structural change that alters the binding of the primary ligand.

9.4 Dose-Response Relationship

The objective quantification of a drug's action is represented by the *dose-response curve* (*DRC*). The percentage of maximal response is plotted in the Y-axis, and the plasma concentration of the drug is plotted in the X-axis.

There are two types of dose-response curves.

9.4.1 Graded Dose-Response Curve

- When the response is measured with a continuous variable, the Y-axis is continuous, and the plotted DRC is called graded DRC, e.g. reduction in blood pressure and change in the intraocular pressure.
- ED_{50} can be calculated from the graph, and it denotes the dose at which the drug will produce 50% of the maximal response seen with the drug.

9.4.2 Quantal Dose-Response Curve

- If the response is binary (e.g. contraceptive effect, pain relief or death), it is not possible to plot the data on a discrete Y-axis.
- The percentage of the population (study population) experiencing the effect is instead plotted as a continuous variable in the Y-axis.
- ED_{50} is the dose of the drug that produces a response in 50% of the study population.
- **DRC plotted on a normal graph**
  - Hyperbolic in shape.
  - Linearity in the dose-response relationship not obvious.
  - Low dosing ranges can be plotted.
- **DRC plotted on a semi-log graph**
  - Sigmoidal in shape.
  - Linearity observed around 30–70% of the maximal response.
  - Large dose ranges (1–10^3) can be plotted.

- **Special Situations: Biphasic DRC**
  - There are special cases where the drug causes stimulation or therapeutic effects at lower doses while inhibition or toxic effects at higher doses.
  - This results in an inverted U-shaped or J-shaped curve.
  - Alcohol is a common example of hormesis. It has been shown to produce beneficial effects at lower doses while toxicity at higher doses. Stress and physical exercise are common physiological examples of hormetic effect.
  - It is postulated that prostaglandins, endothelin and purinergic agonists have hormetic effects.

9.5 Quantifying Pharmacodynamics

9.5.1 Affinity

- Affinity can be objectively calculated by studying the concentration of the ligand [D], the concentration of the unbound receptors [R] and the concentration of the ligand-receptor complexes [DR].

$$\rightarrow [D] + [R] = [DR]$$

- Let's denote the forward rate constant as 'k+1' and the backward rate constant as 'k−1'.
- The rate of formation of drug-receptor complexes at any point in time is given by k+1 $[D][R]$, while the rate of dissociation of drug-receptor complexes is given by k−1[DR]. The difference between k+1$[D][R]$ and k−1[DR] gives the concentration of the drug-ligand complexes at a given instance of time.
- At equilibrium, the rate of formation and dissociation of [DR] is the same.
- Hence, k+1 $[D][R]$ = k–1[DR].
- Or
- k–1/k+1 = $[D][R]$/[DR]; this is defined as the equilibrium dissociation constant K_D.
- The reciprocal of K_D (i.e. $1/K_D$) is defined as the equilibrium association constant K_A.
- The fraction of receptors occupied at any given instant of time can be calculated as the ratio between the drug-ligand complex and the total number of receptors. The total number of receptors is determined by adding the concentration of unbound receptors and the concentration of the drug-ligand complexes.

$$f = [DR] / [R] + [DR]$$

- We know K_D = $[D][R]$/[DR].
- Substituting [DR] = $[D][R]/K_D$ in the equation for 'f' we get,

$$f = [D] / K_D + [D].$$

- When $[D] = K_D$,

$$f = K_D / K_D + K_D = 0.5.$$

- Hence, K_D (the equilibrium dissociation constant) is the drug concentration at which half the receptors are occupied.
- The quantification of affinity does not take into account the biologic activation of the drug. The biologic activation of the drug is determined by the intrinsic activity of the drug.

9.5.2 Potency

The potency of the drug is described in terms of the EC_{50} of its dose-response curve. When two drugs have similar responses, the drug with the lower EC_{50} is the more potent drug. Potency is a function of both the affinity and the intrinsic activity of the drug.

9.5.3 Efficacy

Efficacy of a drug is described in terms of the maximal effect a drug can produce. Efficacy describes the ability of a drug to bind to a receptor and produce cellular action, i.e. the intrinsic activity of the drug.

9.5.4 Quantifying Agonists

Agonists can be quantified based on two characteristics:

- **Half-maximally effective concentration**
  - The half-maximally effective concentration or the EC_{50} is a measure of the potency of the drug.
  - When two agonists produce the same response, the drug which produces the response at a lower dose will have a lower EC_{50} and is said to be more potent.
  - The potency of the drug depends both on affinity and efficacy of the drug.
- **Maximal response**
  - The maximal response is the function of the efficacy of the drug.
  - The drug with a higher maximal response is said to be more efficacious.

9.5.5 Quantifying Antagonists

The type of antagonism a drug exhibits can be studied by the change in the DRC of the agonist at the receptor in the presence of antagonists.

- **Competitive antagonist**
  - A competitive antagonist binds reversibly to the same site as that of the agonist on the receptors.
  - This results in a decrease in the potency of the agonist without a reduction in the maximal response.
  - A parallel shift to the right is noted in the DRC.
  - The magnitude of the shift depends on the affinity of the antagonist to the receptor and concentration of the antagonist.

    Affinity of the Antagonist to the Receptor

    The affinity of the competitive antagonist (Ki) can be estimated by radiolabeled ligand studies or functional response of the biological system to the agonist in the presence of the antagonist.

    The Ki can be estimated using the Schild equation

$$[L']/[L] = [I]/Ki$$

where '[L']' is the concentration of the agonist required to produce a particular fractional occupancy of the receptor in the presence of the antagonist, '[L]' is the concentration of the agonist required to produce the same fractional occupancy of the receptor in the absence of the antagonist, '[I]' is the concentration of the antagonist and 'Ki' is the affinity of the antagonist.

Concentration of the Antagonist

pA2 value is defined as 'the negative logarithm of the molar concentration of antagonist required to produce an agonist dose ratio equal to 2'.

- **Non-competitive antagonist**
- There are two types of non-competitive antagonism:
  - **Allosteric antagonism** occurs when the antagonist binds to a different site on the receptor and modifies its ligand binding region. This changes the affinity of the receptor to the agonist.
  - **Irreversible or pseudo-irreversible antagonism** occurs when the antagonist binds to the same site as that of the agonist but binds irreversibly or takes a longer time to dissociate.
  - Both types of non-competitive antagonism result in the reduction of the maximal response as the number of receptors mediating the response has decreased.
  - Flattening and shift to the right is noted in the DRC.

9.5.6 Quantifying Drug Interaction

- When two drugs are administered together, their responses might add up. The combination is said to exhibit additivity.
- Some combinations can result in supra-additive effects (positive synergism) or subadditive effects (negative synergism).
- The type of effect a drug combination produces can be studied by plotting an *isobologram*.

- An isobologram is used to study the type of drug interaction, i.e. whether additive or synergistic.
- The isobologram is plotted with the concentration of the drugs on either of the axes, and their EC_{50} are joined by a line.
- This line (50% isobole of additivity) represents the half-maximal response elicited by the combination if they are additive.
- If the drugs exhibit positive synergism, the isobole will fall below the line of additivity, while the line will fall above if they exhibit negative synergism.

9.6 Variability of Pharmacodynamic Responses

Drug action changes considerably even when the same individual is exposed to the same concentration of the drug. It also varies between individuals in a population. It is important to determine drug dosing ranges and concentration ranges so that efficacy is maximized, while toxicity is reduced.

Data on drug levels correlated with efficacy and toxicity must always be viewed with pharmacodynamic variability in mind.

9.6.1 Therapeutic Index

- Therapeutic index is the ratio of the LD_{50} and the ED_{50} from a quantal dose-response relationship. LD_{50} is the dose that is lethal to half the population, while ED_{50} is the dose that produces clinical benefit in half the population.
- Therapeutic index describes the safety of the drug in the population; the larger the therapeutic effect, the safer is the drug.

9.6.2 Therapeutic Window

- The therapeutic window of a drug is the range of steady-state concentrations. The lower level corresponds to the minimum concentration required to produce an effect, while the higher level corresponds to the maximum concentration that does not produce toxicity in an individual.
- Similarly, the values of the therapeutic window calculated for the whole population are termed as the population therapeutic window.
- Due to significant intra-individual variability, the population therapeutic window does not guarantee safety and efficacy. It provides the ranges where the likelihood of safety and efficacy of the drug is high.

9.7 Classification of Receptors

9.7.1 Types of Receptors

Drugs act on various receptors in the body by binding to endogenous ligands and mediate the cellular process. The types of receptors are:

- **Receptors that are signal transducers**
  - Drugs bind to G-protein coupled receptors (GPCRs) present on the cytoplasm which causes a change in a cellular process through the activation of a second messenger system.
  - For example, phenylephrine acting on the α_1 receptors.
- **Receptors that change the ionic flux**
  - Drugs bind to ion channels that maintain ionic balances for the normal functioning of cellular processes. Altering ion fluxes results in modulation (either increase or decrease) of cellular processes.
  - For example, d-tubocurarine action on the nicotinic neuromuscular receptors.
- **Receptors that are enzymes**
  - Certain drugs bind to intracellular enzymes and produce action by modulating the catalytic activity of the enzyme. Changes in the enzyme's activity will bring about a biological action by changes in the substrates or products associated with the enzyme.
  - For example, enalapril inhibiting the angiotensin-converting enzyme (ACE).
- **Receptors that affect nuclear mechanisms**
  - Drugs cross the nuclear membrane and bind to one of the four types of receptors present inside the nucleus including transcription factors. Such drugs bring about action by a change in the gene expression or altering epigenetic pathways. Such drugs are generally slow to act.
  - For example, dexamethasone acting on the glucocorticoid receptors.
- **Receptors that affect levels of neurotransmitters**
  - Drugs might alter the levels of neurotransmitters (NT) released into the synaptic cleft by one of the following mechanisms: decreasing NT storage, depletion of NT, inhibition of release of stored NT or decreased production of NT.
  - For example, botulinum toxin acts by inhibiting the fusion of the ACh-containing vesicles to the presynaptic membrane.

9.7.2 Common Features of a Signal Transduction System

- Majority of the drug response is mediated through signal transducers.
- Signal transducers are present on the surface of the cell. They receive information (in the form of a ligand binding) and cause a response (alter a cellular response).

- They have two domains:
  - **Ligand-binding domain (LBD)** – The part of the system that binds to endogenous ligands or the drugs.
  - **Effector domain** – The part of the system that is responsible for mediating the biological response after ligand binding.
- The effector domain often results in activation of intermediary cellular molecules called *transducers*.
- The enzymes or molecules that result in the mediation of signal transduction are called *second messengers*.
- The second messengers do not freely diffuse throughout the cell but are often localized by binding to proteins that help compartmentalize these cellular actions produced by the second messenger system.
- The second messenger system is integrated within the cell. For example, contraction of the cardiac muscle following β_1 stimulation involves an increase in cAMP and Ca^{2+}.
- Second messengers help in amplification of the original signal brought by the ligand. Hence the concentration of the ligand is usually low, while the second messengers help increase the magnitude of cellular response achieved by the ligand binding.

9.7.3 G-Protein Coupled Receptors

- **Common features of GPCRs**
- The G-protein coupled receptors (GPCRs) are transmembrane receptors and span the membrane seven times.
- They bind to a varied number of endogenous ligands, e.g. ACh, norepinephrine, peptide hormones, opioids, GABA, eicosanoids and many other peptide and protein ligands. They also bind the majority of clinically used drugs.
- GPCRs are a family of receptors and each may have many subtypes. The subtypes are determined by the alpha subunit of the G-protein which confers receptor selectivity. This enables subtype selective drug development to treat specific diseases.
- **G-proteins**
  - G-proteins are heterotrimeric GTP-binding proteins that differentiate the GPCRs.
  - They are the signal transducing units of the GPCRs.
  - The heterotrimeric structure includes:

    An alpha subunit:
    - Binds to GDP.
    - Confers specificity to receptors and effectors.
    - Four types: Gs, Gi, Gq and G12/13.
    - Gs always activates adenylyl cyclase; Gi subunit inhibits certain forms of adenylyl cyclase; Gq subunit activates phospholipase beta; and G12/13 subunit binds to GEFs (guanine nucleotide exchange factors).

Beta and gamma dimer:
- Binds to the alpha subunit.
- Prenylation of the gamma subunit helps in the membrane localization of the GPCR.
- The beta-gamma subunit confers signaling specificity to the G-protein.

- **Regulation of G-proteins**
  - The intrinsic GTPase activity is the primary mode of regulation of the activity of the G_{α} subunit.
  - Regulator of G-protein signaling (RGS) are proteins which increase the GTPase activity and thereby result in modulating the G-protein action.
  - Covalent modification can result in alteration of G-protein activity. Lipid attachment and phosphorylation can result in covalent modification.
  - Other factors that regulate the G-proteins are GPR-domain-containing proteins, Ric-8, RGS domain-containing proteins and GBA motif-containing proteins.
- **Second messenger systems**
  - **Cyclic AMP**

    Cyclic AMP is produced from adenosine monophosphate by the action of adenylyl cyclase.

    Adenylyl cyclase is activated by the Gs subunit, while it is inhibited by the Gi subunit.

    There are three main targets for the cAMP generated by the adenylyl cyclase:
    - cAMP-dependent PKA
    - cAMP-dependent GEFs
    - Transcription factor – cAMP response element binding protein (CREB)
  - **Protein kinase A**

    Protein kinase A (PKA) is a holoenzyme; a holoenzyme is a combination of an enzyme and a coenzyme. It is activated by increasing levels of cAMP.

    PKA has four units: two R units and two C units.

    The R units bind to the cAMP. On binding to cAMP, the C units get activated, and active C units phosphorylate the serine and threonine residues on specific proteins which they regulate.

    There are two forms of R units (alpha and beta) while three forms of C units (alpha, beta and gamma). This results in different types of PKA with different activation thresholds. They are also localized to different subcellular compartments producing unique actions in the local milieu.
  - **Protein kinase G**

    Protein kinase G (PKG) is activated by increasing levels of cGMP

    It has two isoforms:
    - PKG-I (cytoplasmic form)
    - PKG-II (membrane-bound form)

    Mediate platelet activation and relaxation of smooth muscle
  - **Phosphodiesterases**

    Phosphodiesterases (PDEs) are enzymes that hydrolyze the 3′, 5′-phosphodiester bond in cAMP and cGMP.

They are important for terminating the action of the cyclic nucleotides.
There are more than 50 different isoforms. Some inactivate cAMP, and some inactivate cGMP, while some inactivate both.
Various isoforms are modulated clinically. Some examples are given below:

- PDE3
  - Coronary arterial disease, e.g. inamrinone
  - Peripheral vascular disease, e.g. cilostazol
  - Essential thrombocytosis, e.g. anagrelide
- PDE4
  - COPD, e.g. roflumilast
  - Asthma, e.g. ibudilast
- PDE5
  - Erectile dysfunction, e.g. sildenafil

– **Exchange protein directly activated by cAMP (EPACs)**

Exchange protein directly activated by cAMP (EPACs) are novel cAMP-dependent signaling proteins.
They occur in isolation or in association with PKA.
Two isoforms are seen (EPAC1 and EPAC2).
Clinical significance:

- Sulfonylurea action is mediated by EPAC2.
- Incretin-mediated insulin secretion is also mediated by EPAC2.

– **Phospholipase C DAG-IP_3 Ca^{2+} pathway**

The phospholipase C (PLC) pathway uses Ca2+ as the messenger to activate and regulate various cellular processes like contraction, secretion, metabolism and even gene expression.
There are two sources of intracellular Ca2+:

- Extracellular Ca2+ regulated by Ca2+ channels on the plasma membrane
- Ca2+ stores in the endoplasmic reticulum regulated by SERCA

Activation of PLC by either Gq or Gi alpha subunits results in the release of calcium from the intracellular stores.
PLC is a cytosolic enzyme that hydrolyzes membrane-bound phosphatidylinositol-4,5-bisphosphate and releases inositol triphosphate (IP_3) and diacetyl glycerol (DAG), both of which can act as intracellular signaling moieties.

- DAG activates PKC enzymes.
- IP3 activates the IP3-receptor in the endoplasmic reticulum and release Ca2+.

Intracellular calcium regulates:

- Ca2+-dependent PKCs
- Calmodulin-sensitive enzymes
- Myosin light chain kinase (MLCK)

9.7.4 Ion Channels

- Ion channels are responsible for the regulation of various cellular functions:
  - Generate and transmit electrical impulses in nerves and muscles
  - Regulate secretion of substances from cells
  - Maintain secondary active transport through the gradient achieved
- The common ion channels that are drug targets are the Na^+, K^+, Ca^{2+} and the Cl^- channels. There are different types of ion channels:
  - Voltage-gated
  - Ligand-gated
  - Store-activated
  - Stretch-activated
  - Temperature-activated
- **Voltage-gated channels**
  - **Voltage-gated Na^+ channel**

    The voltage-gated Na^+ channel has two types of subunits. An alpha subunit that is responsible for the formation of pore and beta subunits which regulate the pore.

    The pore-forming subunit offers specificity for the type of ion that it allows through the channel.

    Functions:
    - Responsible for the production of an action potential in the nerve and muscle tissue.

    Clinical use:
    - Local anaesthetics block the depolarization by blocking Na^+ channels and reduce the sensation of pain.
    - Cardiac antiarrhythmics also block voltage-gated Na^+ channels in the conductive system of the heart.
  - **Voltage-gated Ca^{2+} channel**

    The voltage-gated Ca^{2+} channel has four subunits. The alpha subunit forms the pore, and the beta, gamma and delta subunit act as regulatory units.

    Functions
    - Produces the action potential from the pacemaker cells of the heart
    - Modifies the characteristics of the action potential created by fast voltage-gated Na^+ channels
    - Regulates secretory function of the cells in the central and peripheral nervous system
    - Regulates the vascular tone by altering the levels of calcium in the smooth muscle

    Clinical use
    - Calcium channel blockers are used as arterial vasodilators and antiarrhythmics.
  - **Voltage-gated K^+ channels**

    The most common type of channel encountered in a cell.

    The Kv channel is voltage-sensitive. The other K^+ channels are voltage-insensitive.

Functions
- Responsible for the maintenance of the resting membrane potential in the nerve cell and the myocyte.
- Also responsible for the restoration of the resting membrane potential following depolarization.

Drugs
- Venom peptides can act on the voltage-gated K^+ channels and are being studied for cancer, autoimmune disease, neurological and cardiovascular disease.
- Retigabine and Flupirtine are known to mediate their actions by modulating the voltage-gated K^+ channels.

- **Ligand-gated ion channels**
- Ligand-gated ion channels are activated when a ligand binds to the ligand-binding region of the receptor:
  – Ligand-gated ion channels are not very selective in the ions they allow to pass, e.g. the nicotinic ACh receptors allow both K^+ and Na^+ ions.
  – Functions

    Neurotransmitters like acetylcholine, GABA and glutamate stimulate ligand-gated ion channels to bring about synaptic transmission in the central and peripheral nervous system.

    Nicotinic receptors are present on the neurons and the muscles and bring about synaptic transmission as well as muscle contraction.

    HCN channels (hyperpolarization-activated cyclic nucleotide-gated ion channels) in the heart produce the slow depolarization seen in phase 4 nodal action potentials. The bradycardia drug ivabradine, used in the management of angina and heart failure, acts by modulating these channels.

    CNG channels (cyclic nucleotide-gated ion channels) are responsible for vision.

    IP_3 receptors in the endoplasmic reticulum are responsible for the release of intracellular calcium from the stores into the cytoplasm.

    SUR1 (sulphonylurea receptor 1) is responsible for mediating the insulin-releasing action of sulphonylureas.
  – Clinical use

    Drugs like rocuronium act by inhibiting the nicotinic neuromuscular receptors. The nicotinic receptors have four subunits. While the ACh binding subunit is similar in both types, the other three subunits are different allowing receptors selectivity in the drugs used (non-depolarizing skeletal muscles relaxants and the ganglion blockers).

9.7.5 Transient Receptor Potential Channels

- The transient receptor potential channels (TRP channels) are cationic channels involved in various physiological and pathological sensory transmissions.
- They modulate pain perception, mechanosensation, temperature sensation and sensation mediated by menthol and capsaicin.

- Genetic mutations in the TRP channels are seen in channelopathies, pain syndromes, urinary tract disorders and skeletal muscle dysplasias.
- Agonists and antagonists at the TRP receptors are being developed to treat various diseases.

9.7.6 Receptors Linked to Enzymes

- **Receptor tyrosine kinases**
  - Receptor tyrosine kinases are single polypeptide chain (e.g. insulin receptor) with a cysteine-rich extracellular domain, short transmembrane spanning domain and an intracellular part containing the tyrosine kinase enzyme.
  - The receptors are monomers when they are inactive (except, for insulin). They dimerize when activated and cross-phosphorylation of kinase domains on the tyrosine residues.
  - The phosphorylated tyrosine residues can directly activate enzymes (like PKC gamma) or attract adaptor proteins that result in the activation of Ras pathways and others (e.g. Ras-MAPK pathway).
  - Functions
    Mediates the action of insulin.
    Growth signal transduction (e.g. EGF, PDGF, NGF and VEGF).
    Mediates the action of ephrins – ephrins are responsible for neuronal angiogenesis and axonal migration.
- **JAK-STAT pathway**
  - JAK-STAT pathway alters nuclear transcription directly compared to the other tyrosine kinases.
  - There is no intrinsic activity of the tyrosine kinase.
  - There is a special tyrosine kinase called JAK (Janus kinase) which on activation phosphorylates STATs (signal transducer and activator of transcription proteins) which move into the nucleus and alter transcription factors.
  - JAK-STAT pathway is responsible for gamma-interferon action, growth hormone and prolactin action.
- **Receptor serine-threonine kinases**
  - They are similar to receptor tyrosine kinases in that they have a serine-threonine kinase domain at the catalytic site.
  - There are two types (type I and type II), and they function similar to receptor tyrosine kinases.
  - A common example of a ligand binding to these receptors is TGF-beta.
  - The effector mechanism involves the activation of a gene regulator called Smad.
- **Toll-like receptors**
  - **Structure**
    Composed of a large extracellular ligand-binding area, transmembrane domain and a cytoplasmic region called TIR domain.
    The transmembrane domain spans the cell membrane ten times.

  - **Function**
    Common ligands on these receptors are the peptides, peptidoglycans and lipids derived from fragmentation of various pathogens like bacteria and viruses.
    The first step in activation is the dimerization of the receptor. Activation results in recruitment of adaptor proteins.
    Various other proteins are recruited in turn, and the response is mediated.
    The NF-kb response is mediated by the toll-like receptor activation.
- **TNF-alpha receptors**
  - The structure is similar to the toll-like receptors. The cytoplasmic domain of the TNF-alpha receptors is termed as the death domain.
  - The death domain binds to adaptor proteins which in turn activate various other intermediary proteins in signal transduction to the nucleus.
  - Composed of TNF receptor 1 and TNF receptor 2.
  - Activation of the receptor causes trimerization.
  - Currently, drugs do not alter the signal transduction but interfere with ligand binding and regulate TNF-alpha action e.g. infliximab, adalimumab and etanercept.

9.7.7 Receptors That Activate cGMP

- Receptors
  - Natriuretic peptide receptors
  - Soluble guanylyl cyclase
- Downstream effects are mediated by PKG, cGMP-gated ion channels and cGMP-modulated PDEs.
- **Natriuretic peptide receptors**
  - There are three natriuretic peptides:
  - **ANP (atrial natriuretic peptide)**
    Stored in atrial cells within granules.
    Released in response to hypervolemia or increased intravascular pressure caused by pressor hormones or drugs.
    Functions to decrease blood pressure.
  - **BNP (brain natriuretic peptide)**
    Released from the ventricular tissue in the heart. It is not stored in granules.
    The name is due to the fact that it was first extracted from a pig's brain.
    Released in response to volume overload conditions.
    Functions to decrease blood pressure and reduce cardiac fibrosis.
    Nesiritide is an agonist of BNP used in refractory congestive heart failure.
    Sacubitril is a prodrug (sacubitrilat is the active form) that inhibits neprilysin (an enzyme that is responsible for the degradation of ANP and BNP).
  - **CNP (C-type natriuretic peptide)**
    Released from the brain, heart and endothelial cells. It is not stored in granules.

Functions to stimulate growth in long bones and modulates electrolyte balance and vascular tone.

– **Receptors**

NPR-A

- The NPR-A binds to both the ANP and BNP.
- Expressed in the kidneys, heart, vascular tissue, lungs and adipose tissue.
- Has guanylyl cyclase domain that gets activated on ligand binding.
- NPR-A knockout mice are hypertensive with hypertrophied hearts.

NPR-B

- The NPR-B binds to CNP. It is also a ligand-activated guanylyl cyclase.
- Expressed in the heart and long bones in mice.
- NPR-B knockout mice exhibit dwarfism.

NPR-C

- The NPR-C receptors do not have any guanylyl cyclase activity.
- Function as clearance receptors. Bind to excess levels of the natriuretic peptides and clears them from circulation regulating their function.

- **NO synthase and soluble guanylyl cyclase**
  - Nitric oxide is produced by the action of nitric oxide synthase (NOS) locally. NOS converts L-arginine into L-citrulline and NO.
  - Three isoforms of NOS:
    eNOS – endothelial NOS
    nNOS – neuronal NOS
    iNOS – inflammatory NOS
  - eNOS and nNOS are inducible by high levels of calcium, whereas the iNOS is induced by inflammatory mediators.
  - sGC (soluble guanylyl cyclase) is the molecular target of NO.
  - NO binds to the protoporphyrin-IX heme-containing domain of sGC.
  - sGC results in an increase in the cellular concentration of cGMP. The further downstream events are:
    Inhibition of IP_3-Ca^{2+} pathway.
    Inhibition of Ca^{2+} entry by phosphorylating voltage-gated Ca^{2+} channels.
    Phosphorylation of phospholamban resulting in Ca^{2+} reuptake into the intracellular stores.
    Phosphorylation of Ca^{2+} activated K^+ channels that hyperpolarize the cell membranes.

9.7.8 Nuclear Hormone Receptors and Transcription Factors

- Nuclear receptors are receptors that can regulate the expression of genes by modulating the transcription factors.
- They are involved in mediating hormonal responses, e.g. thyroid hormones, sex steroids and glucocorticoids.

- Actions mediated by nuclear receptors are generally slower than GPCRs or ion channels.
- Examples include retinoid X receptor (RXR), liver X receptor (LXR), farnesoid X receptor (FXR) and the peroxisome proliferator-activated receptor (PPAR)-alpha, beta and gamma.
- Location
  - Some of these receptors (e.g. glucocorticoid receptor) are present in the cytoplasm in the inactivated state. On activation, they translocate to the nucleus and alter gene transcription.
  - Receptors like LXR are already present in the nucleus. The ligand that acts on them has to enter the nucleus to produce the necessary action.
- Structure
  - Generally, nuclear receptors have four domains in a single polypeptide chain:
    Activation region (AF-1): The region that is responsible for altering the transcription process in the nucleus. They are regulated by various processes like phosphorylation. Present towards the N-terminal of the polypeptide chain.
    DNA-binding region: The region that is responsible for binding to the DNA through zinc fingers.
    Hinge region: This region basically connects the polypeptide with the ligand-binding region. However, this may also be involved in binding to the DNA along with the DNA-binding region.
    Ligand-binding domain (LBD): The region responsible for binding to the hormonal or drug ligand. It also contains sites where coactivators and corepressors can bind and modulate action through the receptor.
  - The receptor that is bound to the DNA exists as dimers. They are either homodimers (receptor for steroids) or heterodimers (receptors for lipids).
  - The nuclear receptors bind to specific regions in the DNA called the hormone response element (HRE). The HRE is specific for each type of nuclear receptor. The HRE contains repeat sequences of DNA.
  - For a nuclear receptor to modulate transcription, four conditions must be met:
    Appropriate ligand must be bound.
    Receptor must be bound to HRE.
    More coactivators must bind to the receptors.
    Less corepressor must bind to the receptors.
  - Coactivators recruit the enzyme histone acetylase which opens up the DNA for transcription, while the corepressors recruit histone deacetylase which keeps the DNA tightly packed and inhibits transcription. Hence, the ratio of coactivators and corepressors must be favourable to activate transcription.

9.8 Apoptosis and Autophagy Pathways

- Apoptosis is a series of reactions that result in the cell being phagocytized and killed without disturbing the surrounding.

- Pharmacological intervention is required to restore a dysregulated apoptotic pathway as many diseases can be associated with dysregulated apoptosis. For example, resistance to some of the chemotherapeutic drugs is associated with reduced apoptosis.
- Caspases are specialized cytoplasmic cysteine-aspartic proteases that are involved in the apoptosis pathway. They are inactive in a normal cell.
- Hallmark features in a cell undergoing apoptosis are:
  - Cell rounding
  - Shrinking of cytoplasm
  - Nuclear condensation
  - Presentation of phosphatidylserine on the outer surface

9.8.1 Apoptotic Signaling Pathways

- **External apoptosis signaling**
  - Activated by ligands like TNF, Fas (Apo-1) or TRAIL.
  - Receptors for these ligands have an intracellular death domain and do have intrinsic enzymatic activity. Activation results in recruitment of adaptor proteins.
  - Adaptor proteins recruit RIP1 and caspase 8.
  - Results in activation of caspase 8.
  - Caspase 8 activates caspase 3.
  - Final stages of apoptosis are activated (caspase 6 and 7).
- **Internal apoptosis signaling**
  - Activated by damaged DNA, misfolded proteins and reduction in the cell survival factors.
  - The first step in internal apoptosis signaling is the increase in the levels of p53 expression.
  - p53 activation arrests the cell cycle at a checkpoint until the DNA damage is repaired.
  - If DNA damage is extensive or cannot be repaired for other reasons, the proapoptotic proteins of the Bcl-2 family are activated.
  - Bcl-2 has proapoptotic proteins (Bax, Bak and Bad) and anti-apoptotic proteins (Bcl-2, Bcl-W and Bcl-X). They balance each other where there are no apoptotic signals in the cell.
  - Bax translocates into the mitochondria and results in the release of cytochrome c and SMAC.
  - SMAC binds to IAPs (inhibitors of apoptosis proteins) and inactivates it resulting in activation of apoptotic signals.
  - Cytochrome c binds to Apaf-1 in the cytoplasm and activates caspase 9.
  - Activated caspase 9 results in the activation of caspase 3.
  - Caspase 3 is the common point where the external apoptosis signaling and the internal apoptosis signaling join.

9.8.2 Autophagy

- Autophagy is a process of cellular recycling of certain subcellular contents and organelles to provide for energy under condition of stress or starvation.
- The cellular contents and organelles are covered by a double-layered autosome which fuses with a lysosome to accomplish the digestive process.
- This process has been found to play a pathogenic role in:
  - Neurodegenerative diseases like Alzheimer's and Huntington's disease
  - Certain infections like *Salmonella typhi* and *Mycobacterium*
  - Tumour suppression in brain cancer while tumour survival in breast, ovary, prostate cancer
- Regulators of autophagy and potential drug targets.
- AuTophaGy genes (ATGs) – genes responsible for mediating the process of autophagy.
- PI3K-PKB-mTOR pathway (activated mTOR proteins inhibit autophagy).
- Bcl-2 anti-apoptotic proteins also inhibit autophagy.
- Ubiquitination regulates and compliments autophagy.
- p53 interacts with ATGs to regulate autophagy.

9.9 Receptor Regulation

All receptors are regulated by feedback from their own signaling activity. Change in the ability of the receptors to be stimulated over a period of time is called receptor regulation and affects the efficacy of drugs used clinically.

9.9.1 Receptor Desensitization

- Reduction in the receptor response to a drug when the drug stimulates the receptor repeatedly is called *receptor desensitization*. It is also referred to as *adaptation*, *refractoriness* or *downregulation*.
- There are two types of desensitization that are observed clinically:
  - **Tachyphylaxis**
    A rapid development of the desensitization response to drug administration is called *tachyphylaxis*. Examples are reduced response to ephedrine used in an experimental strip of vascular tissue or reduction in the bronchodilatory response to β_2 adrenergic agonists when used in asthma.
  - **Tolerance**
    Development of the desensitization response to drug administration repeatedly over a longer period of time is called tolerance.
- Mechanisms of receptor desensitization
  - Reduction in the number of receptors synthesized
  - Inaccessibility of receptors to agonists
  - Internalization of receptors and degradation

- In the case of GPCR, phosphorylation of the receptors occurs. This is followed by binding of arrestins which can result in uncoupling of G-proteins from the receptors and reduction in the cAMP downstream signaling.

9.9.2 Receptor Supersensitivity

- When receptors are chronically under-stimulated, the opposite of receptor desensitization can happen.
- Often seen when drugs are withdrawn after a long period of time, e.g. withdrawal of β blockers after long-term administration.
- The phenomenon can also be seen after denervation.

9.10 Receptor Dysfunction

Alteration in receptor function or expression can result in disease and/or altered responses to drugs.

Examples:

- Congenital deficiency of vitamin D receptors and android receptors.
- Autoimmune antibodies against nicotinic receptor in myasthenia gravis.
- Interference with insulin receptor functions in some types of insulin-resistant diabetes.
- Tumours caused by mutation of growth factors or tumour suppressor genes.

9.11 Drugs That Modify Genes and Their Expression

Genetic diseases that do not cause mutations in proteins which do not take part in any ligand-binding or signal transduction pathway could not be easily treated by drugs. Recently drugs have been designed that can be used as gene therapies in some form or the other.

9.11.1 Overcoming the Nonsense Mutations

A successful case of such treatment is ataluren (Translarna) which is used in the management of nonsense mutation Duchenne muscular dystrophy (nmDMD). It helps the ribosomes override the premature stop codon and continue the full translation of the complete dystrophin protein.

Other diseases for which gene therapy is developed include cystic fibrosis and aniridia (refer to Chap. 23)

9.11.2 Antisense Oligonucleotides

- Antisense oligonucleotides (ASOs) block DNA expression by preventing mRNA translation by binding to it.
- They are complementary to the gene that causes the disease and need to be silenced.
- Examples:
  - Fomivirsen – used in the treatment of CMV retinitis.
  - Mipomersen – used in the treatment of familial hypercholesterolemia.

9.11.3 Small/Short Interfering RNA

- RNA-induced silencing complex (RISC) physiologically regulates gene expression.
- The short interfering RNA (siRNA) binds to RISC and results in the destruction of copies of mRNA.
- The action of siRNA can last from days to weeks as multiple copies of mRNA are lost, while siRNA can stay for a longer period of time.
- Various pharmacotherapies have been developed where siRNA is given as such (naked siRNA) or within suitable delivery systems which includes certain viruses, liposomes and nanoparticle carriers.

9.11.4 Clustered Regularly Interspaced Short Palindromic Repeats Genome Editing System

- Clustered regularly interspaced short palindromic repeats (CRISPR) uses RNA guidance to edit the transcriptional activity on a single gene.
- A sgRNA (single-guide RNA) is needed along with a catalytically inactive Cas9 enzyme.
- The sgRNA targets specific sequences of DNA that require modulation. The Cas9 enzyme binds to the DNA without the sgRNA and causes steric hindrance that can cause inhibition of DNA transcription or prevention of elongation.

9.12 Systems Integrating Multiple Signals

- Various biological processes are tightly regulated by multiple stimuli and factors.
- For example, the arterial tone is maintained by angiotensin II and norepinephrine released from sympathetic nerve endings through the same pathway.
- AngII binding to AT_1R results in activation of Gq-PLC-IP_3-Ca^{2+} pathway. The released calcium stimulates calmodulin which in turn actuates the myosin light

chain kinase. This results in contraction of the smooth muscle and increases the arterial tone.

- Norepinephrine also increases the total peripheral resistance by acting on α_1 receptors which activate the Gq-PLC-IP_3-Ca^{2+}.
- Smooth muscle relaxation is mediated by nitric oxide, BNP and sympathomimetic ligands acting on the β_2 receptors.
- NO is released due to the activation of eNOS by the Gq-PLC-IP_3-Ca^{2+} pathway. NO results in activation of sGC and increased formation of cGMP which eventually activates PKG and reduction in the intracellular concentrations of calcium.
- The BNP binding to NPRs also results in increase in the guanylyl cyclase activity and increase in cGMP.
- In addition to the dynamic response of altering the arterial tone, ligands like PDGF, AngII, NE and various prostaglandins alter the gene expression in the vascular smooth muscle cells.

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Drug Transporters

10

Rekha Priyadarshini

Abstract

Drug transporters are membrane proteins. They play a critical role in cellular homeostasis by influx or efflux of nutrients, ions, cellular waste, and environmental toxins. Drug transporters are also involved in the regulation of drug distribution and their bioavailability, transport of compounds across the blood-brain barrier, and hematopoietic stem cell protection from toxins. Functionally, they are classified as influx and efflux transporters. Based on their origin, transporters are classified into two major superfamilies, namely, the *ABC (ATP-binding cassette)* and the *SLC (solute carrier)* transporters. Clinically important families in ABC superfamily are ABCB, ABCC, and ABCG. All the members of this family are primary active transporters. Important families of SLC superfamily are OAT, OCT, OCTN, OATP, and MATE. Members of this superfamily are either facilitated transporters, secondary active transporters, or tertiary active transporters. All uptake transporters belong to SLC family and efflux transporters belong to ABC family with an exception of MATE export transporter which belongs to SLC family. Transporters expressed in the small intestine, the liver, and the kidney are of particular importance for drug disposition and drug-drug interactions. The P-glycoprotein (coded by the *ABCB1* gene), also known as the MDR1 or ABCB1 transporter, gained much importance because of its propensity to cause multidrug resistance; it is one of the best studied export transporters.

Keywords

Drug transporter · Membrane protein · ABCB1 transporter · P-glycoprotein · SLC transporter

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10.1 Introduction

Transporters are membrane proteins. They play a critical role in cellular homeostasis. Around 2000 genes code for these transporters.

- **Physiological Role**
  - Influx or efflux of nutrients, ions, cellular waste, and environmental toxins
  - Regulation of drug distribution and their bioavailability
  - Transport of compounds across the blood-brain barrier
  - Hematopoietic stem cell protection from toxins

10.2 Mechanism of Drug Transport

Transport of the drugs across biological membranes is the initial step involved in all pharmacokinetic processes.

- Mechanisms of membrane transport
  - Passive transport
    Simple diffusion
    Filtration
    Facilitated diffusion
  - Active transport
    Primary active transport
    Secondary active transport
      - Symport
      - Antiport

10.3 Passive Transport

Movement of solutes or drugs follows concentration gradient – down the electrochemical potential gradient (higher concentration to lower concentration). No energy is needed (Fig. 10.1).

10.3.1 Simple Diffusion

- Solutes or drugs diffuse by dissolving in the lipoidal matrix of the membrane.

10.3.2 Filtration

- Solutes or drugs diffuse through paracellular spaces or aqueous pores in the membrane.

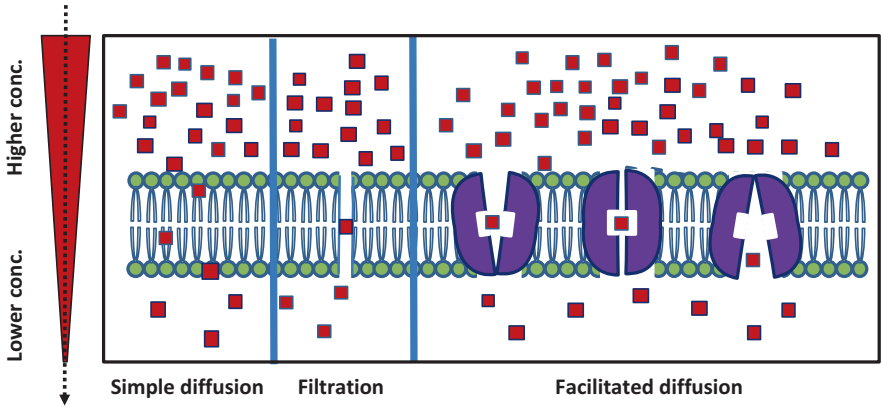

Fig. 10.1 Passive transport

10.3.3 Facilitated Diffusion

- Solutes or drugs diffuse through membrane transporters (transporter-mediated membrane transport).

10.3.4 Factors Determining the Rate of Transport in Passive Transport

- Lipid solubility – the greater the lipid solubility, the faster the transport, as they get concentrated in the membrane and diffuse quickly.
- Particle size – the smaller the size, the more easily they pass through pores and paracellular spaces of the membrane.
- Concentration gradient – the greater the difference in concentration across the membrane, the faster the diffusion of drugs.
- Ionization pH – unionized forms cross the membrane easily as they are lipid soluble. Strong acids and alkalis rapidly ionize irrespective of the medium and become lipid insoluble. Majority of the drugs are weak electrolytes, and their ionization is pH dependent. Thus, weakly acidic drugs ionize more at alkaline pH and become lipid insoluble and vice versa.

10.4 Active Transport

- Movement of solute or drugs against the concentration gradient (lower concentration to higher concentration).
- Requires energy – by ATP hydrolysis or by downhill movement of another solute.

- Active transport is divided into primary and secondary active transports based on the driving energy source.

10.4.1 Primary Active Transport

- Driving energy source – ATP hydrolysis.
- Membrane transporters contain ATPase activity in their structures.
- For example, ABC (ATP-binding cassette) transporter superfamily, Na^+,K^+-ATPase (Fig. 10.2).

10.4.2 Secondary Active Transport

- Driving energy source – movement of another solute down its electrochemical potential gradient.
- It is divided into symport or antiport based on the direction of movement of solute in relation to another solute which is providing the energy source.
- For example, SLC (solute carrier) transporter superfamily – the organic anion transporting polypeptide (OATP) and the organic cation transporter (OCT).
- **Symport**
  - Movement of both the solutes in the same direction. It is also called as *cotransport*. For example, Na^+/Ca^{++} exchanger.
- **Antiport**
  - Movement of both the solutes in the opposite direction. It is also called as *exchange transport*. For example, Na^+-glucose transporter (SGLT1) (Fig. 10.3).

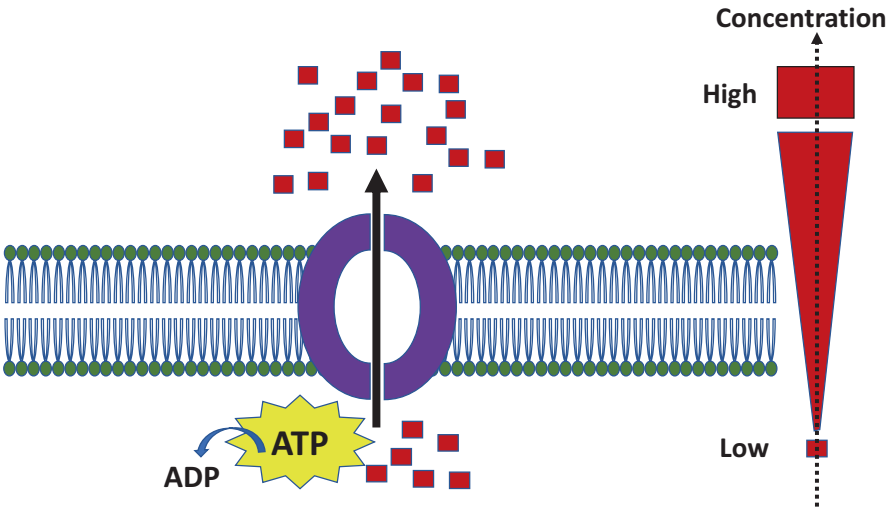

Fig. 10.2 Primary active transport

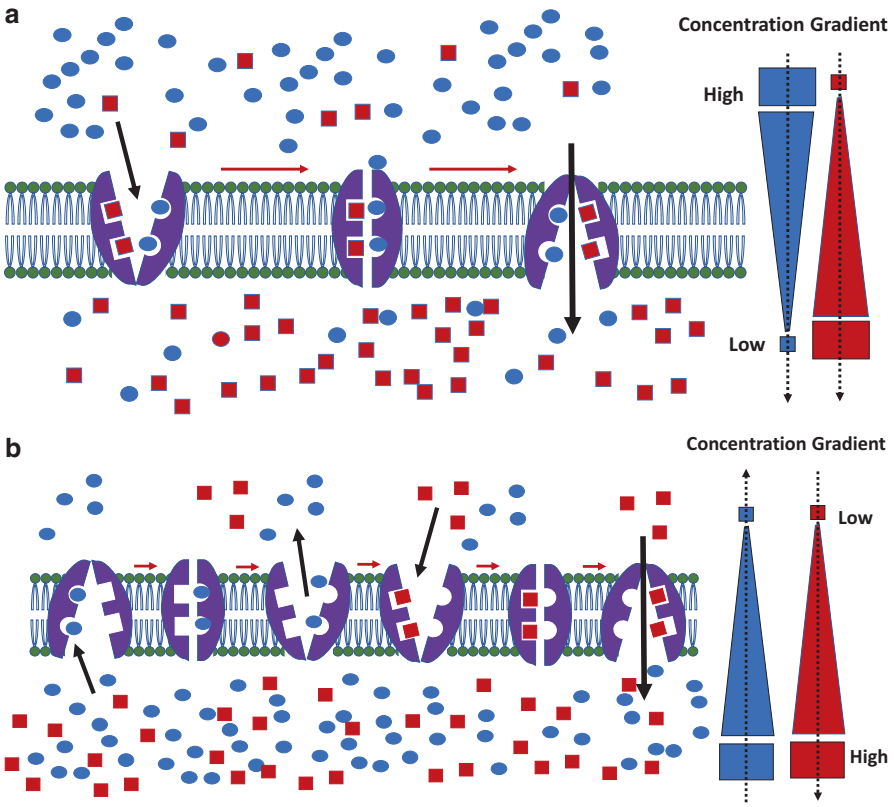

Fig. 10.3 (**a**) Secondary active transport – symport. (**b**) Secondary active transport – antiport

10.5 Classification

10.5.1 Functional Classification

- Functionally, transporters are classified into two major categories; they are uptake transporters (mediate drugs and metabolites into the cell) and export transporters (mediate drugs and metabolites outside the cell).
  - **Uptake (Influx) Transporters**
    Organic anion transporting polypeptide family (OATP) [SLC family, SLC21/SLCO]
    Organic anion transporter family (OAT) [SLC family, SLC22]
    Organic cation transporter family (OCT) [SLC family, SLC22]
    Organic cation/carnitine transporter family (OCTN) [SLC family, SLC22]
  - **Efflux Transporters**
    Multidrug resistance/transporters of antigen presentation family (MDR/TAP) [ABC family, ABCB]
    Multidrug resistance protein (MRP) [ABC family, ABCC]
    Breast cancer resistance protein (BCRP) [ABC family, ABCG]
    Multidrug and toxin extrusion protein family (MATE) [SLC family, SLC47]

- **Vectorial Transport**
  - Unidirectional transport of compounds (ions, molecules or drugs) across an epithelium of cell membrane.
  - A nonuniform distribution of transporter proteins on the two faces of the epithelium of plasma membrane makes way for the vectorial transport.
  - It plays an important role in the absorption of bile and nutrients from the intestine and excretion of drugs from the blood through hepatobiliary and urinary route and removal of drugs from the brain through brain endothelial cells and epithelial cells of choroid plexus of the brain.
  - Either ABC (mediate only unidirectional efflux) alone or both ABC and SLC (mediate both influx and efflux) transporters are necessary for vectorial transport of substances.

    ABC transporters alone are sufficient for vectorial transport of lipophilic compounds as these compounds have sufficient membrane permeability.

    Coordination between influx and efflux transporters is needed for vectorial transport of hydrophilic organic compounds.

10.5.2 Structural Classification

Based on their origin, transporters are classified into two major superfamilies. They are ABC (ATP-binding cassette) and SLC (solute carrier) transporters (Fig. 10.4).

- **ABC Superfamily**
  - The ABC superfamily contains 49 studied ABC genes organized in 7 families (A to G).
  - Members of these families have a pivotal role in cellular processes like multidrug resistance of cancer cells.
  - Clinically important families are ABCB, ABCC, and ABCG.
  - All members of this family are primary active transporters.
- **SLC Superfamily**
  - The SLC superfamily includes 400 genes classified into 65 families.
  - These transporters play a vital role in the transport of diverse organic molecules (include both charged and uncharged), inorganic ions, and the gases (ammonia).

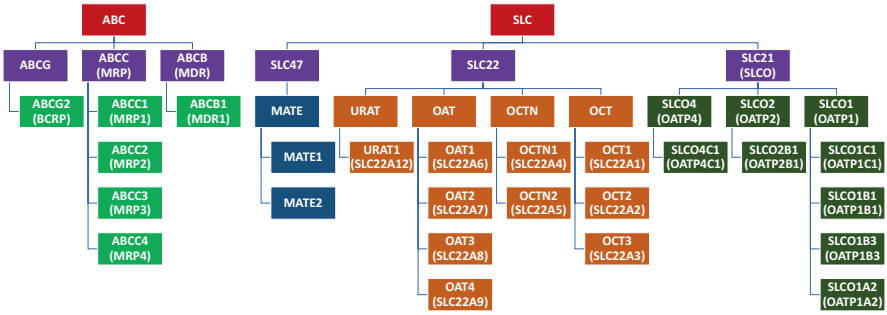

Fig. 10.4 Classification of ABC and SLC drug transporters

- Important families of SLC superfamily are OATs, OCTs, OCTNs, OATPs, and MATEs.
- Members of this superfamily are either facilitated transporters or secondary active transporters.
- All uptake transporters belong to SLC family, and efflux transporters belong to ABC family with an exception of MATE export transporter which belongs to SLC family.

10.6 Structure and Function of Transporters

10.6.1 ATP-Binding Cassette (ABC) Superfamily

- One of the largest and ubiquitous gene superfamilies.
- Transmembrane domains (TMD) and nucleotide-binding domains (NBD) are the common features of these transporters.
- Transporters consist of multiple subunits with one or few transmembrane domains (TMD) and one or few nucleotide-binding domains (NBD) which is an essential cytoplasmic factor with ATPase activity.
  - The TMDs are more variable reflecting the chemical diversity of substrates and comprising of several hydrophobic alpha helices in the membrane lipid bilayer. They involve in substrate recognition and their translocation across the membrane by undergoing a conformational change.
  - The cytoplasmic NBD or ATP-binding cassette (ABC) domain contains highly conserved motifs (Walker A and Walker B motifs, the ABC signature motif, the H loop, and the Q loop), the sites for ATP binding and hydrolysis.
- The core unit of ABC transporter comprises of four domains, two NBDs which are joined together to form a conserved fold and two TMDs ("full transporters"). However, some of the ABC transporters possess either two NBDs, two TMDs [homodimers, BCRP (ABCG2)], or one each of NBD and TMD [heterodimers, ABCG5, and ABCG8] – called as the "half transporters" (Fig. 10.5).

10.6.2 SLC Transporter Superfamily

- This superfamily consists of members with highly diverse structures with a variety of membrane folds, unlike ABC superfamily.
- These are typical integral membrane proteins with various number of hydrophobic transmembrane alpha helices linked to each other by intra- and extracellular hydrophilic loops.
- The two structural folds predicted to be most common in these transporters are major facilitator superfamily (MFS) and the leucine transporter (LeuT)-like folds. Both the folds are structurally dissimilar.
  - MFS fold – it is the most common one, and it contains 12 transmembrane (TM) helices arranged in two pseudo repeats of six TMs each and both facing each other. It is present in various human transporter families like glucose

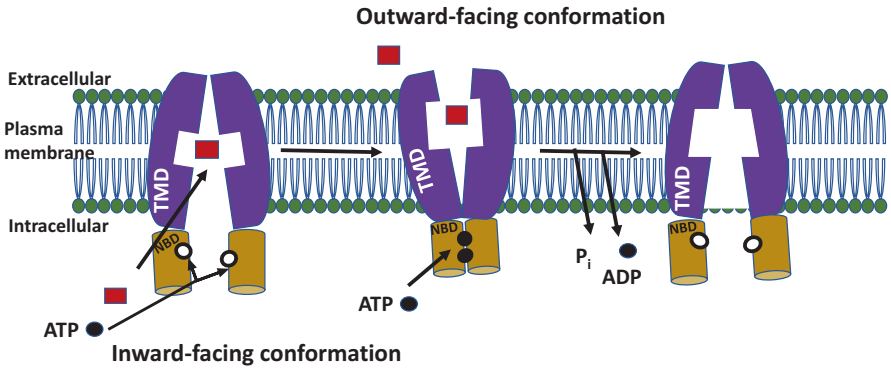

Fig. 10.5 ABC transport mechanism model. Inward-facing conformation permits the binding of substrate and ATP. The conformation gets converted to outward-facing (transporters open to outside) with binding of two ATP molecules and, ultimately, resulting in the release of substrates to the extracellular medium. Hydrolysis of NBD-bound ATP to phosphate and ADP changes the outward conformation to inward conformation. NBD, nucleotide-binding domains and TMD, transmembrane domains

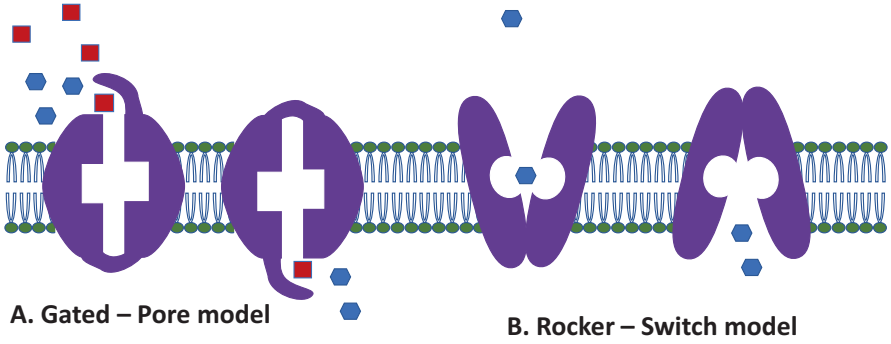

Fig. 10.6 SLC transport mechanism models. (**a**) Gated pore model represents the alternate opening and closing of gates thereby facilitating movement of substrates from one side to the other side (extracellular to intracellular or vice versa) of the plasma membrane. For example, sodium-dependent glucose cotransporter (SGLT). (**b**) Rocker switch represents the binding of substrate to the open configuration of transporter triggers the inward configuration and release the substrate to the other side. For example, facilitated glucose transporter (GLUT2)

transporters, GLUTs/SLC2 and the proton oligopeptide cotransporter family, SLC15.

– LeuT-like fold – it contains ten TMs arranged into two inverted pseudo repeats with five TMs each. It has two domains, i.e., "bundle domain" and "scaffold domain." The first two TMs in both pseudo repeats are tilted and form a bundle domain, and the other three TMs in both the pseudo repeats form a "scaffold domain." It is present in SLC5 and SLC6 family.

- These transporters function as either monomers, homo-, or hetero-oligomers depending on the SLC type (Fig. 10.6).

10.7 Tissue Distribution of Transporters

- Transporters are distributed all over the body. Transporters expressed in the small intestine, the liver, and the kidney are of special significance as they are involved in drug disposition and drug-drug interactions. Other areas where transporters are gaining clinical importance are the brain, testes, and placenta (organs with barrier functions).
- These transporters play a major role in pharmacokinetics, i.e., absorption, distribution, metabolism, and elimination of drugs.
- Three major groups are intestinal, hepatic, and renal transporters.

10.7.1 Intestinal Transporters

- Absorption of nutrients, endogenous compounds, and drugs from the intestine takes place by various transporters expressed in the brush-border membranes and also on the basolateral membrane of the intestinal epithelium.
- Intestinal transporters are gaining much importance as they influence the bioavailability of drugs when administered orally.
- Several transporters are identified, but few are involved in drug absorption (Fig. 10.7).

10.7.2 Hepatic Transporters

- Transporter systems play a significant role in pharmacokinetics in addition to the drug-metabolizing enzymes.
- Hepatic transporters (both uptake and efflux transporters) have substantial impact on modulating drug metabolism and pharmacological efficacy.
- Uptake transporters are expressed in the basolateral (sinusoidal) membrane of hepatocytes, and efflux transporters are expressed in both bile canalicular membrane and sinusoidal membrane of hepatocytes.
- Among all, the pharmacologically important drug transporters are OATPs, OATs, OCTs, MRPs, MDR1, BCRP, and MATE (Fig. 10.8).

10.7.3 Renal Transporters

- Transporters expressed in renal tubules are involved in excretion of endogenous compounds when concentrations are excess and removal of xenobiotics and toxins from the blood and reabsorption of essential compounds.
- Both efflux and influx transporters are expressed in basolateral membrane (facing toward the blood side) and apical membrane (facing toward the tubular lumen) of renal tubules (Fig. 10.9).

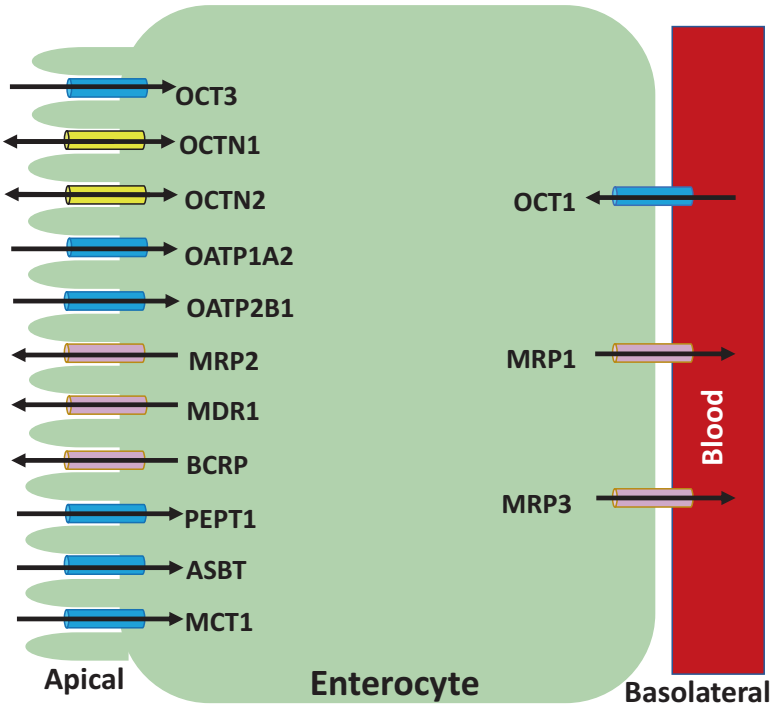

Fig. 10.7 Intestinal transporters. Uptake transporters (blue), efflux transporters (pink), and transporters with both efflux and uptake function (yellow). OCT, organic cation transporter; OCTN, organic cation/carnitine transporter; OATP, organic anion transporting polypeptide; MRP, multidrug resistance-associated protein; MDR, multidrug resistance protein; BCRP, breast cancer resistance protein; PEPT, peptide transporter protein; ASBT, apical sodium-dependent bile acid transporter; and MCT, monocarboxylate transporter protein

10.8 Pharmacological Role

10.8.1 ABC Superfamily

ABCB, ABCC, and ABCG are pharmacologically important families of ABC.

- **ABCB, MDR (Multidrug Resistance), or TAP (Transporters of Antigen Presentation) Family**
  - Totally 11 transporters belong to this family. Out of these, ABCB1, ABCB2, ABCB3, ABCB4, and ABCB11 are the important ones.
    ABCB1 (MDR1 or P-gp) is the drug export pump.
    ABCB2 and ABCB3 (TAP1 and TAP2) are peptide transporters.
    ABCB4 (MDR3) is a phospholipid translocator.
    ABCB11 (BSEP) is the bile salt export pump.

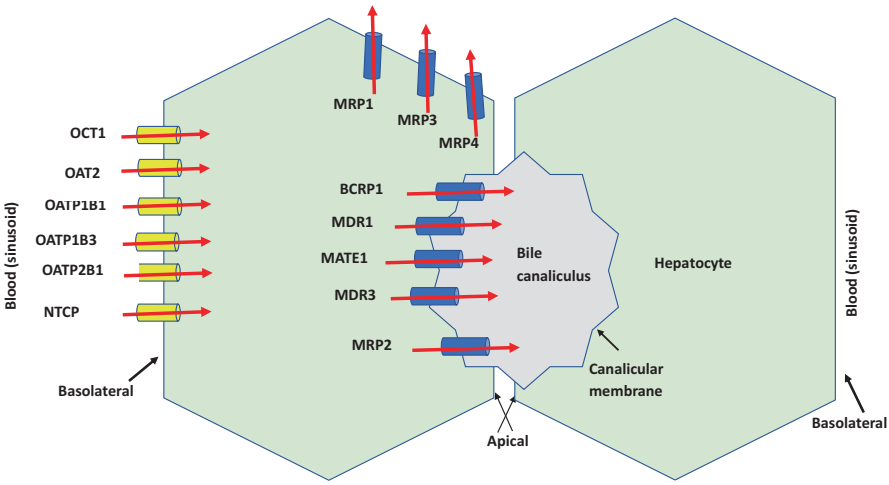

Fig. 10.8 Hepatic transporters. Uptake transporters (yellow) and efflux transporters (blue). OCT, organic cation transporter; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; MRP, multidrug resistance-associated protein; BCRP, breast cancer resistance protein; MDR, multidrug resistance protein; MATE, multidrug and toxin extrusion transporter; and NTCP, sodium taurocholate co-transporting polypeptide

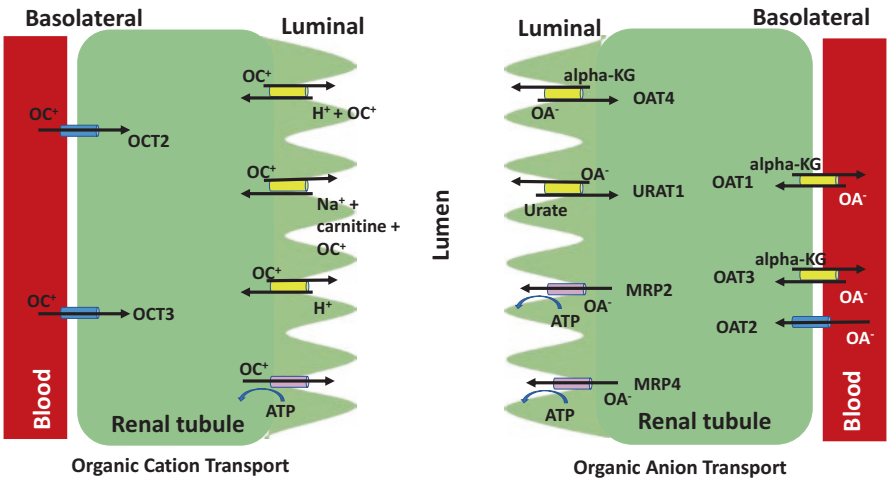

Fig. 10.9 Renal transporters. Uptake transporters (blue), efflux transporters (pink), and transporters with both efflux and uptake function (yellow). OCT, organic cation transporter; OCTN, organic cation/carnitine transporter; MATE, multidrug and toxin extrusion transporter; MDR, multidrug resistance protein; OAT, organic anion transporter; and URAT1, uric acid transporter

– **MDR1, ABCB1, or P-gp**
  MDR1 (coded by the *ABCB1* gene) is an export transporter which mediates movement of drugs from intracellular space to extracellular space contributing to the limitation of drug action thereby protecting tissues from xenobiotics.
  It is also known as the P-glycoprotein (P-gp).
  It gained much importance because of its propensity to cause multidrug resistance.
  One of the best studied export transporters among ABC superfamily members as it has wide substrate spectrum.
  Function:
    • Plays a role in absorption, disposition, and excretion and also in clinically important drug-drug interactions
    • Reduces the bioavailability of drugs
    • Mediates the transport of substrates to the bile and urine
    • Protects from the entry of toxic compounds and drugs into the central nervous system
  Transport mechanism: primary active transport (Box 10.1).

Points of clinical relevance:
ABCB1 variant (c.3435T >C) is the most studied genetic polymorphism.
Functional genetic polymorphisms of these genes and their expression have association with differential response to chemotherapeutic agents and prognosis of numerous tumor types.
Failure of anticonvulsant therapy due to P-gp expression at the blood-brain barrier.
P-gp limits CNS entry of fexofenadine (a third-generation antihistamine) thereby reducing its CNS side effect, namely, sedation.

• **Multidrug Resistance-Associated Proteins (MRP/ABCC) Family**
  – There are 12 proteins in this MRP/ABCC family. They are nine MRPs (MRP1–MRP9), one cystic fibrosis transmembrane conductance regulator protein (CFTR), and two sulfonylurea proteins (SUR1 and SUR2).

Box 10.1: Characteristics of MDR1 (ABCB1) Transporter

Transporter protein (gene)	Location	Substrates		Inhibitors
		Endogenous	Exogenous[a]	
MDR1, ABCB1, or P-gp *(ABCB1)*	Intestinal enterocytes, kidney proximal tubule, hepatocytes (canalicular), brain endothelia	Steroids, lipids, bilirubin, and bile acids	Digoxin, loperamide, berberine, irinotecan, doxorubicin, vinblastine, paclitaxel, and fexofenadine	Cyclosporine, quinidine, tariquidar, verapamil, and grapefruit juice

[a]Not an exhaustive list

– Genes coding for MRPs (MRP1–MRP9) are *ABCC1–ABCC6* and *ABCC10–ABCC12*, and the genes coding for CFTR, SUR1, and SUR2 are *ABCC7*, *ABCC8*, and *ABCC9*, respectively.
– MRP1 (ABCC1), MRP2 (ABCC2), and MRP4 (ABCC4) have been most widely studied in the context of drug response and toxicity. MRP2 (ABCC2) is extensively considered for its involvement in drug disposition.
– Function: bile salt enterohepatic circulation, leukotrienes transport, and detoxification and efflux of structurally diverse endogenous and xenobiotic organic anionic, some neutral or cationic compounds, and their metabolites into bile and urine.
– Transport mechanism: cotransport or symport mechanism with GSH (Box 10.2).

Box 10.2: Characteristics of MRP (ABCC) Transporters

Transporter protein (gene)	Location	Substrates		Inhibitors
		Endogenous	Exogenous	
MRP1 *(ABCC1)*	Kidney, lung, testis, cardiac and skeletal muscle, and placenta	Conjugates of glutathione, glucuronide and sulfate, bile salts, estradiol, and organic anions	Anticancer drugs (methotrexate, vinca alkaloids, anthracyclines), and antiviral drugs	MK-571, LTC4, sulfinpyrazone, benzbromarone, and probenecid
MRP2 *(ABCC2)*	Hepatocyte (canalicular membrane) and renal proximal tubule (apical membrane of endothelial cells), apical membrane of the small intestine, gall bladder, and placenta	Conjugates of glutathione, glucuronide and sulfate, bile salts, estradiol, and organic anions	Anticancer drugs (methotrexate, vinca alkaloids, anthracyclines, melphalan), statins, and antiviral drugs	MK-571, cyclosporine, probenecid, indomethacin, LTC4, furosemide, and grapefruit juice
MRP4 *(ABCC4)*	Hepatocytes (basolateral membrane), choroid plexus (epithelium), renal proximal tubule (apical membrane), and brain (capillary endothelium)	Cyclic nucleotides, glutathione conjugates, eicosanoids, urate, folate, bile acids, and conjugated steroids	Anticancer drugs (6-mercaptopurine, methotrexate), antiviral drugs (adefovir, tenofovir), diuretics (furosemide, trichlormethiazide), and cephalosporins (ceftizoxime, cefazolin)	MK-571

Points of clinical relevance:

ABCC2 (MRP2) gene mutations cause Dubin-Johnson syndrome, which presents as hyperbilirubinemia due to impaired transport of conjugated bilirubin into bile.

ABCC4 polymorphisms are associated with side effects and poor survival in methotrexate-treated childhood acute lymphoblastic leukemia patients.

- **Breast Cancer Resistance Protein (BCRP/ABCG2)**
  - BCRP is a "half-ABC transporter." It is also known as the mitoxantrone resistance protein (MXR).
  - Function:
    Intestinal absorption and secretion of both neutral and negatively charged molecules into bile and urine.
    Protection of the fetus and brain by acting as a barrier from toxins.
    Play a major role in multidrug resistance.
    Secretion of vitamins, such as riboflavin, biotin, and vitamin K, into breast milk.
  - Transport mechanism: primary active transport (Box 10.3).

Box 10.3: Characteristics of BCRP (ABCG2) Transporter

Transporter protein (gene)	Location	Substrates		Inhibitors
		Endogenous	Exogenous	
BCRP *(ABCG2)*	Intestine (luminal membrane of enterocytes), liver (canalicular membrane), kidney, organs with barrier functions (brain, testis, placenta), and mammary glands	Porphyrins, estrogen sulfate, and uric acid	Anticancer agents (anthracyclines, imatinib, topotecan, methotrexate), prazosin, pantoprazole, antibiotics (nitrofurantoin, fluoroquinolones), and statins	Cyclosporine, omeprazole, pantoprazole saquinavir, and tacrolimus

Points of clinical relevance:

Association of genetic polymorphisms of *ABCG2* with increased exposure or increased risk of toxicity to cancer chemotherapeutic drugs.

Association of poor prognosis with chemotherapy and expression of BCRP in cancer cells.

Genetic polymorphisms of the transporter protein are found to influence the disposition of xanthine oxidase inhibitors and uric acid and, hence, have been associated in the management of gout.

10.8.2 SLC Superfamily

- **Organic Anion Transporting Polypeptides (OATP)**
  - All 11 human OATP members of OATP family are grouped into 6 OATP subfamilies.
  - OATP1A2 (SLCO1A2), OATP1B1 (SLCO1B1), OATP1B3 (SLCO1B3), and OATP2B1 (SLCO2B1) are the most extensively studied members among human OATPs.
  - Function:
    Transport of thyroid hormones, organic anions, neutral and cationic compounds.
    Drug disposition and hepatic drug uptake.
  - Transport mechanism:
    Neutral anion exchangers (exchanged anion is bicarbonate).
    OATP1B1 and OATP1B3 are electrogenic transporters, but pH variations also influence their activity.
    - This property is pharmacologically helpful in the entry of cytotoxic drugs into tumors as tumor environment is acidic.
  - Transporters have wide substrate specificity for amphipathic molecules and transport mainly large hydrophobic organic anions (Box 10.4).

Points of clinical relevance:

Important drug-drug interactions associated with OATPs are cerivastatin-gemfibrozil and atorvastatin-rifampin.

Polymorphisms of *SLCO1B1* (loss -of- function polymorphism, c.521T>C) associated with failure of statin therapy (reduced hepatic uptake of statins) and toxic effects of statins and methotrexate (increased plasma exposure leading to systemic toxicity).

OATP2B1 is related to statin-associated muscle toxicity as it plays a significant role for statin transport into muscle tissue.

Complete deficiency of OATP1B1 and OATP1B3 causes Rotor syndrome due to interruption of the reuptake of conjugated bilirubin into the liver.

Box 10.4: Characteristics of OATP (SLCO) Transporters

Transporter protein (gene)	Location	Substrates		Inhibitors
		Endogenous	Exogenous	
OATP1A2 ***(SLCO1A2)***	Liver (sinusoidal membrane), intestine (apical membrane), kidney (basolateral membrane), and majority in the brain (blood-brain barrier)	Bile acid derivatives, bilirubin and its conjugates, and estrone sulfate	Statins (pitavastatin), fexofenadine, methotrexate, imatinib, erythromycin, fluoroquinolones, protease inhibitors, and beta-blockers	Rifampin, bromosulfophthalein
OATP1B1 (***SLCO1B1***)	Both in the kidney (basolateral membrane) and liver but predominantly on hepatocytes	Bile acid derivatives, bilirubin conjugates, and thyroxine	Statins (pitavastatin, pravastatin), rifampin, repaglinide, methotrexate, and enalapril	Rifampin
OATP1B3 ***(SLCO1B3)***	Liver (sinusoidal membrane)	Paraaminohippuric acid (PAH) and estrone sulfate (higher affinity)	Statins (pitavastatin, pravastatin), rifampin, digoxin methotrexate fexofenadine, enalapril, erythromycin, and taxanes	Rifampin
OATP2B1 ***(SLCO2B1)***	Ubiquitous	Estrone sulfate and PAH	Statins (pitavastatin), amiodarone, and methotrexate	Rifampin, bromosulfophthalein

- **Organic Cation Transporters (OCT)**
  - Organic cation transporters are uptake transporters belonging to SLC22A family.
  - There are three recognized OCTs; they are OCT1 (SCL22A1), OCT2 (SCL22A2), and OCT3 (SCL22A3).
  - Function:

    Exclusively transport positively charged substrates with low molecular weight.

    Controls extracellular neurotransmitter concentrations in several areas of the brain.

    Helpful in both the renal elimination and the intestinal absorption of metformin.

– Transport mechanism:
  Small organic cations – sodium- and proton-independent passive facilitated transport (movement follows electrochemical gradient).
  Charged substrates – electrogenic, sodium-dependent, and bidirectional (Box 10.5).

Points of clinical relevance:
  Loss-of-function polymorphisms of OCT1 gene *(SLC22A1)* are associated with altered response to metformin therapy and also associated with increased plasma concentrations of antiemetic drugs tropisetron and ondansetron (reduced hepatic uptake ➔ ↓ metabolic inactivation of drugs by the liver).

Box 10.5: Characteristics of OCT Transporters

Transporter protein (gene)	Location	Substrates		Inhibitors
		Endogenous	Exogenous	
OCT1 (*SCL22A1*)	Sinusoidal membrane of hepatocytes (predominantly) and luminal membrane of lung epithelia	Choline, acetylcholine, thiamine, monoamine neurotransmitters and MPP+ (1-methyl-4-phenylpyridinium), and TEA (tetraethylammonium)	Metformin, oxaliplatin, irinotecan, paclitaxel, imatinib, lamivudine, acyclovir, and cimetidine	Rifampin, bromosulfophthalein, amitriptyline, and verapamil
OCT2 (*SCL22A2*)	Basolateral membranes of renal tubules (predominantly), hepatocytes, and luminal membrane of lungs epithelia	Bile acid derivatives, bilirubin conjugates, creatinine, thyroxine, acetylcholine, monoamine neurotransmitters and MPP+ (1-methyl-4-phenylpyridinium), and TEA	Metformin, oxaliplatin, cisplatin, procainamide, beta-blockers, H2 blockers (cimetidine), memantine, amantadine, zalcitabine, and lamivudine	Rifampin
OCT3 (*SCL22A3*)	Ubiquitous but highly expressed in the brain, intestinal epithelium, and hepatocytes and in proximal renal tubules	Estrone sulfate (higher affinity), paraaminohippuric acid (PAH), creatinine, carnitine, choline, acetylcholine, monoamine neurotransmitters, and MPP+	Metformin, oxaliplatin, lamivudine, lidocaine, quinidine, quinine, d-amphetamine, memantine, amantadine, ketamine, citalopram, desipramine, and imipramine	Rifampin

Coadministration of metformin with verapamil, amitriptyline, or rifampicin causes altered metformin response as they interact with OCT transporters.

- **Organic Carnitine/Cation Transporter (OCTNs)**
  - OCTNs are the new cation uptake transporters belonging to SLC22A family.
  - OCTN1 (SLC22A4) and OCTN2 (SLC22A5) are two recognized transporters well studied in OCTN family.
  - Function:
    OCTNs involve in hydrophilic organic cation flux. Exclusively transport positively charged substrates with high molecular weight.
    Majorly involved in carnitine transport like the transport of carnitine into breast milk which is the source for β-oxidation of fatty acids in neonates.
  - Transport mechanism:
    These are bidirectional transporters (both influx and efflux).
    They function as facilitated transporter and proton-cation or cation-cation exchangers depending on the sodium and pH gradient. Transport mode can be changed according to the substrate.
    They function as Na^+ cotransporters for carnitine reuptake (mainly OCTN2) (Box 10.6).

Box 10.6: Characteristics of OCTN Transporters

Transporter protein (gene)	Location	Substrates		Inhibitors
		Endogenous	Exogenous	
OCTN1 (*SLC22A4*)	Expressed ubiquitously	Ergothioneine, carnitine, and acetylcholine	Gabapentin, pregabalin, quinidine, quinine, verapamil, ipratropium, tiotropium, and oxaliplatin	Cimetidine, pyrilamine, verapamil
OCTN2 (*SLC22A5*)	Highly expressed in the intestine and kidney (proximal convoluted tubules)	Carnitine and choline	Verapamil, quinidine, levofloxacin, cephaloridine, spironolactone, imatinib, etoposide, ipratropium, valproic acid, and tiotropium	Pyrilamine, tetraethylammonium (TEA)

Points of clinical relevance:

Mutations of OCTN2 transporter lead to insufficient renal reabsorption of carnitine resulting in primary systemic carnitine deficiency manifesting as cardiomyopathy and progressive skeletal weakness.

L503F variant of OCTN1 is associated with familial and sporadic inflammatory bowel disease.

- **Organic Anion Transporter (OAT) Family**
  - These are anion exchangers belonging to the SLC22 family.
  - There are four transporters under this OAT family, namely, OAT1 (SLC22A6), OAT2 (SLC22A7), OAT3 (SLC22A8), and OAT4 (SLC22A11).
  - Function:

    Remove weakly acidic compounds (xenobiotics) from the body.

    Transport both hydrophilic and hydrophobic anions with two carboxylate groups and also some cations and neutral compounds.

    Hepatocytes – uptake of organic anions into the cells and efflux of glutamate into sinusoids.

    Renal tubules – secretion of urate and anionic drug metabolites into urine.

    Brain – efflux of neurotransmitter and anionic drug metabolites.
  - Transport mechanism:

    These are tertiary-active transporters and are saturable.

    They depend on the energy gradient generated by the secondary active transport of alpha-ketoglutarate (alpha-KG) by Na^+ dicarboxylate cotransporter (NaDC3) which in turn relies on the energy gradient from the primary active transporter Na^+-K^+-ATPase (Fig. 10.10).

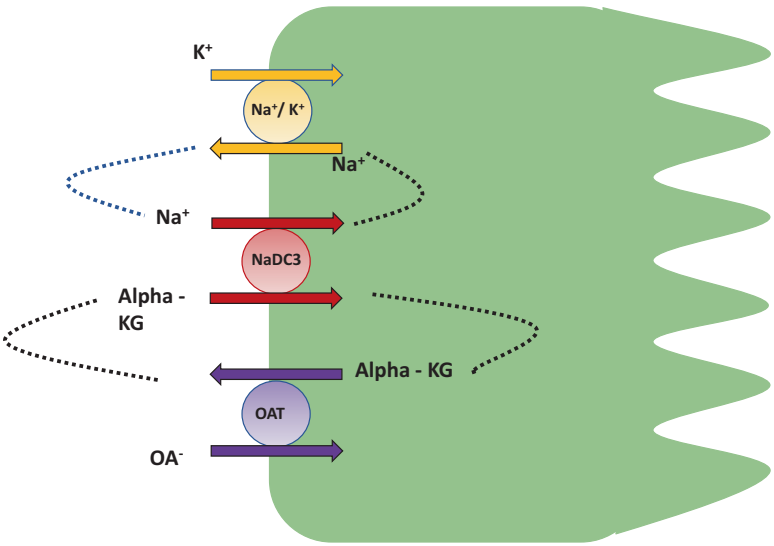

Fig. 10.10 Tertiary active transport. (Refer text for explanation)

They act as bidirectional transporters (both influx and efflux) for the transport of guanine nucleotides.

OAT4 is both efflux and influx transporter.

– Substrates to these transporters are structurally diverse. They transport exclusively low molecular weight organic anions (Box 10.7).

Points of clinical relevance:

Coadministration of anionic drugs with other anionic compounds will have undesirable consequences.

Box 10.7: Characteristics of OAT Transporters

Transporter protein (gene)	Location	Substrates		Inhibitors
		Endogenous	Exogenous	
OAT1 ***(SLC22A6)***	Basolateral membrane of proximal renal tubules (predominant) and choroid plexus of the brain, skeletal muscle, and placenta	Paraaminohippuric acid (PAH), PGE_2, and urate	Methotrexate, cimetidine, olmesartan, furosemide, penicillins, acyclovir, cidofovir, tenofovir, and zidovudine	Probenecid, mycophenolate, glyburide, cimetidine, diclofenac, statins, and novobiocin
OAT2 ***(SLC22A7)***	Both in the kidney (basolateral membrane) and liver but predominantly on hepatocytes	Nucleotides, neurotransmitters, PGE_2, PGF_2, estrogen-3-sulfate, dehydroepiandrosterone sulfate, and alpha-ketoglutarate	Zidovudine, paclitaxel, cimetidine, methotrexate, salicylate, bumetanide, erythromycin, tetracycline, 5-fluorouracil, and allopurinol	Cimetidine, cetirizine, testosterone, quinidine Diclofenac, Methotrexate Cefadroxil and cefoperazone
OAT3 ***(SLC22A8)*** It has two variants. Shorter one involves in transport process	Predominantly on the kidney (basolateral membrane) and some on the brain (choroid plexus), skeletal muscle, and developing bone	PAH and estrone sulfate (higher affinity)	Pravastatin, rosuvastatin, cimetidine, 6-mercaptopurine, methotrexate, olmesartan, topotecan, benzylpenicillin, valacyclovir, and zidovudine	Statins, probenecid, and novobiocin
OAT4 ***(SLC22A11)***	Placenta and kidney (luminal membrane of the proximal tubule)	Estrone sulfate, dehydroepiandrosterone sulfate, and para-aminohippuric acid (PAH)	Zidovudine, tetracycline, bumetanide, torsemide, and methotrexate	

Probenecid and penicillin coadministration may result in increased penicillin concentration.
- Probenecid inhibits penicillin secretion into renal tubules, thereby, preventing its excretion.

NSAIDs and methotrexate interaction
- OAT transport inhibition by NSAIDs may lead on to very high concentrations of methotrexate.

Probenecid and antiviral drugs coadministration can prevent nephrotoxicity of antiviral drugs.
- Probenecid decreases the renal clearance of antiviral drugs.

No polymorphisms of OATs are significantly associated with drug disposition.

- **Multidrug and Toxin Extrusion (MATE) Transporters**
  - MATE transporters are efflux transporters belonging to the SLC47 family.
  - Two recognized MATE transporters are MATE1 (SLC47A1) and MATE2-K (SLC47A2).
  - Function:
    Export endogenous hydrophilic organic cations or cationic drug substrates into the bile and urine.
  - Transport mechanism:
    Organic cation-proton exchangers.
  - MATE1 and MATE2 have almost similar inhibitors and substrate specificities (Box 10.8).

Box 10.8: Characteristics of MATE Transporters

Transporter protein (gene)	Location	Substrates		Inhibitors
		Endogenous	Exogenous	
MATE1 ***(SLC47A1)***	Proximal convoluted tubules (apical membrane), hepatocytes (canalicular membrane), and skeletal muscle	Guanidine, thiamine, *N*-methyl nicotinamide (NMN), and creatinine	Metformin, fexofenadine, topotecan, and some anionic drugs like acyclovir and ganciclovir	Cimetidine, quinidine, procainamide, tyrosine kinase inhibitors, and pyrimethamine
			Cephalexin, cephradine, and paraquat	
MATE2-K ***(SLC47A2)***	Proximal convoluted tubules (apical membrane)		Metformin, fexofenadine, topotecan, and some anionic drugs like acyclovir and ganciclovir	

Points of clinical relevance:

Genetic polymorphisms of MATE transporters are associated with altered response to metformin.

MATE1-OCT1 genotype interaction – presence of both *SLC47A1* (MATE1) polymorphism and *SLC22A1* (OCT1) polymorphism results in higher levels of metformin-mediated HbA_{1c} levels.

Cimetidine inhibits fexofenadine transport.

- Cimetidine is a MATE transporter inhibitor thereby inhibiting fexofenadine transport.

Coadministration of cimetidine with metformin may result in increased metformin concentration.

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Adverse Effects and Pharmacovigilance

11

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Abstract

No drug can be testified to be entirely free of undesired effects. These undesired or unintended effects of drug administration are broadly called adverse effects. When the concept of causality is factored in, the term adverse drug reaction is used. This causality assessment can be done in several ways, Naranjo scale and WHO-UMC scale being the most common ones. *Pharmacovigilance* is a growing domain that concerns the detection, assessment, understanding and prevention of drug-related adverse effects. On a similar line, *hemovigilance* is the set of activities that govern adverse effects related to transfusion chains. When the medical materials and devices are involved, it is called *materiovigilance*, and when cosmetic products are concerned, the *cosmetovigilance* comes in to play. *Addictovigilance* is an ill-defined category of events that cover drugs of addictive or recreational misuse. This chapter covers the broad types of vigilance *(medicovigilance)* and their relevance in the Indian scenario.

Keywords

Pharmacovigilance · Adverse effects · Hemovigilance · Materiovigilance · Cosmetovigilance · Addictovigilance

11.1 Adverse Effects

- *Adverse effects* are 'undesirable or unintended consequences of drug administration'. As is evident from the definition, it is a very broad terminology and includes not just serious and fatal consequences, but also the trivial ones.

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- The term *adverse drug reaction (ADR)* is defined as 'a noxious or unwanted change that is suspected to be due to a drug (when used at a dose normally used in humans for prophylaxis, diagnosis or therapy of disease, or for modification of physiological function), which requires treatment or decrease in dose or indicates caution with the future use of the same drug'. This definition is more specific than that of "adverse effects", and excludes trivial events, overdose and poisonings, thus channelizing better and focused analysis of adverse events.
- While causality has to be proven to call an event an ADR, another generalized category is that of *adverse drug events*, which are defined as 'untoward medical occurrences that present during treatment with a medication, but need not necessarily have any causal relationship with the medication'.

11.2 Classification of Adverse Effects

11.2.1 Based on Predictability (Box 11.1)

A major share (approximately 80%) of adverse events belong to type A. Since these are potentially avoidable and predictable, a proper understanding of adverse events is essential.

Box 11.1: Classification of Adverse Effects Based on the Likelihood of Occurrence

Type of adverse effect	Features	Example
A – augmented	Common	Bleeding with warfarin usage
	Dose-related	
	Exaggerated pharmacological action of a drug	
	Predictable	
	Low fatality	
B – bizarre	Uncommon	Malignant hyperthermia with general anaesthetic usage
	Non-dose-related	
	Not related to the pharmacological action of the drug	
	Unpredictable	
	High fatality	
C – chronic	Uncommon	HPA axis suppression with corticosteroids
	Dose-related and time-related	
	Depends on the total cumulative dose of the drug	

(continued)

Box 11.1 (continued)

Type of adverse effect	Features	Example
D – delayed	Uncommon	Carcinogenesis, teratogenesis
	Usually dose-related and time-related	
	Presence of time lag between use of drug and emergence of effect	
E – end of use	Uncommon	Withdrawal symptoms seen with opiated or benzodiazepines
	Usually soon after the withdrawal of a drug	
F – failure	Common	Resistance to antimicrobial therapies; OCP failure when used with enzyme inducers
	Dose-related	
	Mostly associated with drug-drug interactions	

11.2.2 Based on Severity (Box 11.2)

Box 11.2: Classification of Adverse Effects Based on the Severity of Reaction

Minor or trivial	No therapy, antidote or prolongation of hospitalization is mandated in these cases
Moderate	Mandates specific therapy or change in existing therapy or prolongs hospitalization by at least 24 h
Severe	Potentially life-threatening or causes permanent damage or requires intensive medical therapy
Lethal or fatal	Results in death of the patients, either directly or indirectly

11.2.3 Based on Type (Box 11.3)

Box 11.3: Classification of Adverse Effects Based on the Characteristic Features

Side effects	Unwanted but unavoidable effects of drug administration at therapeutic doses
	Non-serious and can be predicted
	For example, glyceryl trinitrate acts by vasodilation, which helps in its antianginal action, but, at the same time, also causes headache and postural hypotension

(continued)

Box 11.3 (continued)

Secondary effects	Indirect consequences of the primary action of drug administration
	For example, tetracyclines cause suppression of not just the pathogenic flora but also the protective flora, thus leading to superinfection
Toxic effects	Expansion of pharmacological action due to prolonged usage or overdose
	For example, respiratory failure with overdose of morphine
Intolerance	Opposite of 'tolerance'
	Lower threshold of patients to the action of a drug, i.e. 'manifestation of toxic effects of a drug even at therapeutic doses'. In simple terms, 'increased sensitivity to a drug'
	E.g., A single dose of triflupromazine may cause muscular dystonia in children
Idiosyncrasy	Genetically determined abnormal reaction to a drug
	For example, barbiturates may cause CNS excitement and mental confusion in some individuals with a particular genotype
Allergy or hypersensitivity	Immunological reaction unrelated to the pharmacodynamic properties of a drug
	Commonly occurs with even minute doses of a drug
	Occurs only in a few individuals and thus cannot be generalized or replicated in the common population
	Prior exposure or sensitization is needed (can be incidental or accidental)
	Positive de-challenge and re-challenge are characteristic. Can be of four types: type 1 (anaphylaxis); type 2 (cytolytic); type 3 (immune complex); and type 4 (delayed or cell-mediated)
	For example, drugs commonly implicated in allergic reactions include penicillins, cephalosporins, allopurinol and local anaesthetics
Photosensitivity	Cutaneous manifestation resulting from drug-induced sensitization of skin to UV rays
	Can be of two types:
	a) Phototoxicity (more common; UV-B rays are involved; shorter wavelengths at 290–320 nm are responsible)
	b) Photoallergy (less common; UV-A rays are involved; longer wavelengths at 320–400 nm are responsible)
	For example, tetracyclines may cause phototoxicity, while sulphonylureas may cause photoallergic reactions
Dependence	A state in which patients prioritize intake of drugs for personal satisfaction over other basic needs, often with preserved insight of the known health hazards
	Conventionally divided into two ill-defined categories: psychological (in simple terms, compulsion of the mind to be dependent on the drug) and physical (compulsion of the body to be dependent on the drug; discontinuation results in withdrawal symptoms; also equated with 'addiction'). Physical dependence is characterized by the phenomenon of 'reinforcement', which results in the drug-seeking behaviour. The process of psychological dependence occurs due to 'neuroadaptation'

(continued)

Box 11.3 (continued)

	For example, opioids and alcohol produce physical and psychological dependence, whereas cocaine and amphetamines produce only psychological dependence
	A milder phenomenon is drug habituation, wherein there is less intensive involvement with the drug
	For example, habituation with tea or coffee
Withdrawal	Sudden interruption of therapy with certain drugs resulting in adverse consequences, which are essentially manifestations of worsening status of the underlying condition
	Cessation of therapy with these drugs should be gradual
	For example, clonidine cessation might result in sudden severe hypertension, restlessness and sympathetic hyperactivity
Teratogenicity	Potential of a drug to cause foetal abnormalities when administered to the carrying mother
	Teratogenic drugs can affect the growing foetus at three phases of development:
	(a) Fertilization and implantation (till 17 days of conception)
	(b) Organogenesis, which is the most critical phase (18–55 days)
	(c) Growth and development (56th day onwards)
	Historically, drugs have been classified into five categories by the US-FDA based on their risk in pregnancy (recently, this risk categorization has been scrapped by the US-FDA. Instead, the drug manufacturers are mandated to list all data relating to the use of the drug during pregnancy and lactation on the drug label, thus enabling the treating clinicians to make an informed decision based on risk-benefit analysis):
	(a) Category A – no risk, e.g. thyroxine, magnesium sulphate
	(b) Category B – no evidence of risk in humans, e.g. penicillin V, amoxicillin, paracetamol
	(c) Category C – risk cannot be ruled out, e.g. morphine, codeine, corticosteroids, adrenaline
	(d) Category D – benefit may outweigh potential risk, e.g. aspirin, phenytoin, carbamazepine, sodium valproate
	(e) Category X – contraindicated, e.g. oestrogens, isotretinoin, thalidomide
	Common teratogens and the anomalies caused by their use during pregnancy are listed below:
	Thalidomide – phocomelia
	Sodium valproate – neural tube defects
	Phenytoin – cleft lip and palate
	Lithium – foetal goitre
	Alcohol – foetal alcohol syndrome, low IQ, growth retardation
	Aspirin, indomethacin – premature closure of ductus arteriosus
	Warfarin – foetal warfarin syndrome
	Stilboestrol – vaginal carcinoma

(continued)

Box 11.3 (continued)

Mutagenicity and carcinogenicity	Potential of a drug to cause genetic mutations and cancers, respectively
	For example, radioisotopes, anticancer drugs, oestrogen
Iatrogenicity	Phenomenon of physician-induced diseases
	Functional disturbances caused by drugs
	Might persist even after cessation of drug therapy
	For example, parkinsonism caused by antipsychotics, SLE caused by sulphonamides or hydralazine

11.3 Causality Assessment

Causality assessment refers to the analysis of the strength of relationship between the drug exposure and the occurrence of adverse reactions. Causality assessment is broadly based on the following parameters:

- **Temporal association:** Time-based association of the adverse effect with the time of drug administration (i.e., adverse effect has to happen following the administration of the drug).
- **Prior documentation:** Earlier documented evidence of the drug causing the same adverse effect.
- **De-challenge:** Subsidence of the adverse effect on cessation of therapy.
- **Re-challenge:** Recurrence of the adverse effect with reintroduction of therapy, post-subsidence of the first adverse effect. Re-challenge is the hallmark of a positive association of a drug to its adverse reaction. However, it may not be ethical to do a re-challenge in most cases, since it might be life-threatening or troublesome to the patient.

11.3.1 Causality Assessment Scales

The two common scales used for causality assessment are the Naranjo scale and the World Health Organization-Uppsala Monitoring Centre (WHO-UMC) scale.

- **Naranjo Probability Scale**

This simple scoring scale was devised by Naranjo and colleagues in the year 1981 (Box 11.4).

Box 11.4: Naranjo Probability Scale

Question	Yes	No	Don't know
Are there previous documented reports of this reaction?	+1	0	0
Did the adverse reaction appear after the suspect drug was administered?	+2	−1	0
Did the adverse reaction improve when the drug was discontinued or when a specific antagonist was administered?	+1	0	0
Did the adverse reaction reappear when the drug was readministered?	+2	−1	0
Are there alternative causes (other than the drug) that could have solely caused the adverse reaction?	−1	+2	0
Did the adverse reaction reappear when a placebo was given?	−1	+1	0
Was the drug detected in blood (or other fluids) in a concentration known to be toxic?	+1	0	0
Was the adverse reaction more severe when the dose was increased or less severe when the dose was decreased?	+1	0	0
Did the patient have a similar reaction to the same or similar drugs in any previous exposure?	+1	0	0
Was the adverse reaction confirmed by objective evidence?	+1	0	0

Cumulative scores and their interpretation:
>9 = Definite
5–8 = Probable
1–4 = Possible
0 = Doubtful

- **WHO-UMC Scale** (Box 11.5)

11.3.2 Other Causality Scales and Methods

- Kramer scoring
- Begaud method
- Venulet algorithm
- Karch and Lasagna scale
- Mashford criteria
- Gallagher method
- Jones scale
- Hutchinson method

Box 11.5: WHO-UMC Scale

Causality term	Criteria
Certain	Event or laboratory test abnormality, with plausible time relationship to drug intake
	Cannot be explained by disease or other drugs
	Response to withdrawal plausible (pharmacologically or pathologically)
	Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon)
	Re-challenge satisfactory, if necessary
Probable or likely	Event or laboratory test abnormality, with reasonable time relationship to drug intake
	Unlikely to be attributed to disease or other drugs
	Response to withdrawal clinically reasonable
	Re-challenge not required
Possible	Event or laboratory test abnormality, with a reasonable time relationship to drug intake
	Could also be explained by disease or other drugs
	Information on drug withdrawal may be lacking or unclear
Unlikely	Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible)
	Disease or other drugs provide plausible explanations
Conditional or unclassified	Event or laboratory test abnormality
	More data for proper assessment needed
	Additional data under examination
Unassessable or unclassifiable	Report suggesting an adverse reaction
	Cannot be judged because information is insufficient or contradictory
	Data cannot be supplemented or verified

11.4 Pharmacovigilance

Pharmacovigilance, as per WHO (2002), is defined as 'the science and activities relating to detection, assessment, understanding and prevention of adverse effects or other drug-related problems'.

11.4.1 Aims of Pharmacovigilance

- Pharmacovigilance aims at educating healthcare providers (HCPs) regarding the various adverse effects and their clinical implications.

- The main aim includes the reduction of risk of drug-related harm to patients and the general community.
- It also aims at rationalizing the use of medications.

11.4.2 Pharmacovigilance Activities

- Voluntary reporting of adverse drug reactions
- Post-marketing surveillance (PMS)
- Prescription monitoring
- Anecdotal case reports, case series and research studies
- Dissemination of adverse drug reaction data
- Regulatory changes including changes in labelling, withdrawal or recall of marketed products

11.4.3 Pharmacovigilance in India

- The Pharmacovigilance Programme of India (PvPI) is a government initiative (Central Drugs Standard Control Organization – CDSCO – under the aegis of Ministry of Health and Family Welfare), started in the year 2010.
- AIIMS, New Delhi, was initially the National Coordination Centre (NCC), which was later shifted to Indian Pharmacopoeia Commission (IPC), Ghaziabad, in 2011. The NCC functions under the observation of a steering committee. IPC-NCC has been designated as the WHO Collaborating Centre for Pharmacovigilance in Public Health Programmes and Regulatory Services in October 2017.
- Medical colleges and hospitals across the country act as adverse drug reaction monitoring centres (AMCs). At present, there are 250 AMCs in the country. These AMCs collect relevant Individual case safety reports (ICSRs). The ICSR data are then entered into the digital database called VigiFlow. The global counterpart of this database is called VigiBase.
- There are four designated zonal centres under PvPI, named as 'Regional Resource Centres'.
  - **East:** Institute of Post Graduate Medical Education and Research (IPGMER), Kolkata
  - **West:** King Edward Memorial (KEM) Hospital, Mumbai
  - **North:** Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh
  - **South:** JSS Medical College & Hospital, Mysore
- Apart from ICSRs, marketing authorization holders (MAHs) are also required to submit post-marketing surveillance (PMS) or periodic safety update reports (PSURs) after licensure of their pharmaceutical product. PSURs are mandated every 6 months for the first 2 years of approval and then annually for the next 2 years. The total duration of PSUR requirement may be extended by the licencing authorities, in the interest of public health.

- PvPI, so far, has generated 5 India-specific signals and 71 alerts. Also, 24 cases of package insert changes have been recommended to the CDSCO.
  - *Signals* are information reported on possible causal relationships between adverse events and drugs, when such relationships are previously unknown or incompletely documented, as defined by WHO.

11.4.4 Software Related to Pharmacovigilance

- **VigiFlow:** A web-based ICSR management system used by national centres and maintained by the Uppsala Monitoring Centre (UMC), Sweden
- **VigiBase:** WHO global database of all its member nations
- **VigiMine:** A statistical filter attachment to VigiBase
- **VigiMed:** A web-based forum with access to safety data across different countries
- **VigiSearch:** A search tool for easy location of data within VigiBase
- **VigiLyze**: A search-cum-analysis tool that works across different data from member countries

11.4.5 Other Miscellaneous Terminologies in Pharmacovigilance

- **Data-mining** is a process of data analysis and generation of useful information like signal generation and benefit-risk analysis.
- **Suspected Unexpected Serious Adverse Drug Reactions (SUSAR)** are serious adverse drug reactions whose nature, severity or frequency is not identified in the safety information columns of the investigator's brochure or drug label.
- **Medical Dictionary for Regulatory Activities (MedDRA)** is a set of clinically validated international medical terms used throughout the regulatory process, from pre-marketing to post-marketing.
- **EUDRAGENE** is a European collaboration of a collection of DNA samples as a resource for studying genes that influence serious adverse drug reactions.

11.5 Hemovigilance

- The term 'hemovigilance' is formed from two words 'hema' and 'vigilans'. While 'hema' is derived from Greek and means 'blood', 'vigilans' is derived from Latin and means 'watchful'.
- During the 1980s and 1990s, many haemophilia patients contracted HIV and hepatitis C infections when they were transfused blood and factor concentrates. The need for safe transfusion of blood and blood products was felt then. France was the first country to set up a national hemovigilance system way back in 1994.

- Currently, the International Haemovigilance Network (IHN) functions on a global scale.
- IHN aims at developing and maintaining a joint structure dealing with hemovigilance in transfusion medicine and safety of blood components throughout the globe.
- Hemovigilance and pharmacovigilance are different entities. Hemovigilance deals with blood and blood products like erythrocyte concentrates, whole blood, fresh frozen plasma and thrombocyte concentrates. Pharmacovigilance, on the other hand, is responsible for fractionated products like immunoglobulins, albumin and clotting factor concentrates in addition to medicinal products.
- The ultimate aim of hemovigilance is to improve the safety and reduce adverse effects associated with blood transfusion.

11.5.1 Definition of Hemovigilance

Set of procedures monitoring the entire transfusion chain (from the collection of blood from the donor to transfusion to recipients followed by follow-up), which gathers and assesses the information on undesirable or unexpected effects associated with the therapeutic use of blood products.

11.5.2 Components of Hemovigilance System

Hemovigilance, as mentioned above, deals with each and every step involved in the transfusion process. It stretches from donor to recipient or in other words from 'vein to vein'.

If hemovigilance has to fulfil its aim, it needs collaboration of four key players in a coordinated manner (Fig. 11.1). The key players are as follows.

- **Industries:** They manufacture and supply disposables, equipment and reagents to blood banks and hospitals. Post-marketing surveillance of these firms forms a robust tool for collecting and compiling data linked either directly or indirectly to blood transfusion.
- **Blood banks:** They use disposables, equipment and reagents manufactured by companies. They are involved in the manufacture of all types of labile blood components.
- **Hospitals (physicians and paramedics):** Since the focus is predominantly on patients, surveillance of this segment is of utmost importance. As hospitals are the predominant sites of blood transfusion, they are in an ideal position to report errors, events, accidents, reactions and side effects.
- **Authorities:** They play an important role in budgeting, legislating, inspecting, accrediting and surveilling either by delegating or directly.

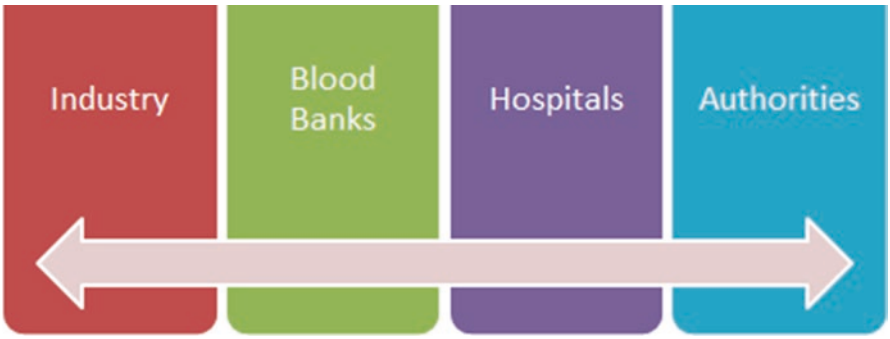

Fig. 11.1 Stakeholders of the hemovigilance system

11.5.3 Donor Hemovigilance

- Hemovigilance aims to improve the safety of the recipient as well as the donor. Collection of blood from the donor is one of the initial steps in the transfusion chain. It is also one of the most frequently performed procedures. Blood banks and hospitals should document the complications or adverse events seen in donors, the actions taken and the final outcome.
- The most common complication seen in donors is a vasovagal reaction. The symptoms are dizziness, nausea, vomiting, weakness, sweating, hypotension and bradycardia. In order to diagnose vasovagal reactions, the last two signs are mandatory. Around 10% of these reactions occur once the donor has left the centre and, hence, are dangerous. Delayed vasovagal reactions are more common in women and in individuals with low blood volume.
- The second most common complication is haematoma, characterized by pain, swelling and bruising at the site of puncture.
- Hemovigilance data have shown that donors who experience complications have a tendency to avoid donating in the future. Therefore, it is important to avoid such complications.

11.5.4 Recipient Hemovigilance

- The most common complication in recipients is volume overload. Volume overload may be due to the following reasons:
  - Prescribing error
  - Incorrect haemoglobin levels due to diluted blood sample which resulted in transfusion
  - Wrong decision-making
- The severity of a reaction is graded using a scale that is internationally accepted.
  - The International Society of Blood Transfusion (ISBT) has laid down certain criteria for imputability or likelihood of transfusion reactions.

- The transfusion-related adverse reactions are subclassified as acute (occurring within 24 h) or delayed (occurring after 24 h).
- Transfusion-related adverse reactions can also be subdivided into infectious and non-infectious reactions based on pathogenesis. Non-infectious reactions include febrile non-haemolytic transfusion reactions (FNHTR), acute haemolytic transfusion reactions (AHTR), transfusion-associated acute lung injury (TRALI), anaphylactic reactions, hyperkalaemia and hypotensive reactions. Infectious reactions are due to contaminated blood containing hepatitis B, hepatitis C, HIV and malarial parasites.
- The most common reactions are FNHTR followed by allergic reactions.

11.5.5 Strengths of Hemovigilance

- Unlike two decades ago, we now have transparency. Based on available data, error-prone areas can be worked on and corrected. Hemovigilance systems ultimately contribute to transfusion safety.

11.5.6 Limitations of Hemovigilance

- Hemovigilance does not capture data of patients who failed to receive blood or blood products and the harms associated with it. The reasons may be non-availability of blood products or failure on the part of treating physician to order blood.
- Current vigilance system is good at detecting complications occurring immediately post-transfusion, but the same is not the case with delayed reactions.
- In addition, reactions associated with repeated transfusions are poorly captured.

11.5.7 Hemovigilance in India

- The National Haemovigilance Program of India was implemented during the 12th 5-year plan in December 2012. It functions as a part of the PvPI (Fig. 11.2).
- A software called ‘Haemo-vigil’ collects and analyses data pertaining to hemovigilance across the country.
- The main objective of reporting is to determine risks and hazards and collect information regarding the faulty areas in the system. This helps in reducing the risk of injury to patients in the future.
- In order to record and report adverse reactions, the Haemovigilance Program of India (HvPI) has prepared a Transfusion Reaction Reporting Form (TRRF). TRRF contains patient information, details on blood products used, details of the adverse reactions, relevant tests required to be done as well as the imputability assessment. Medical schools across the country are encouraged to record the

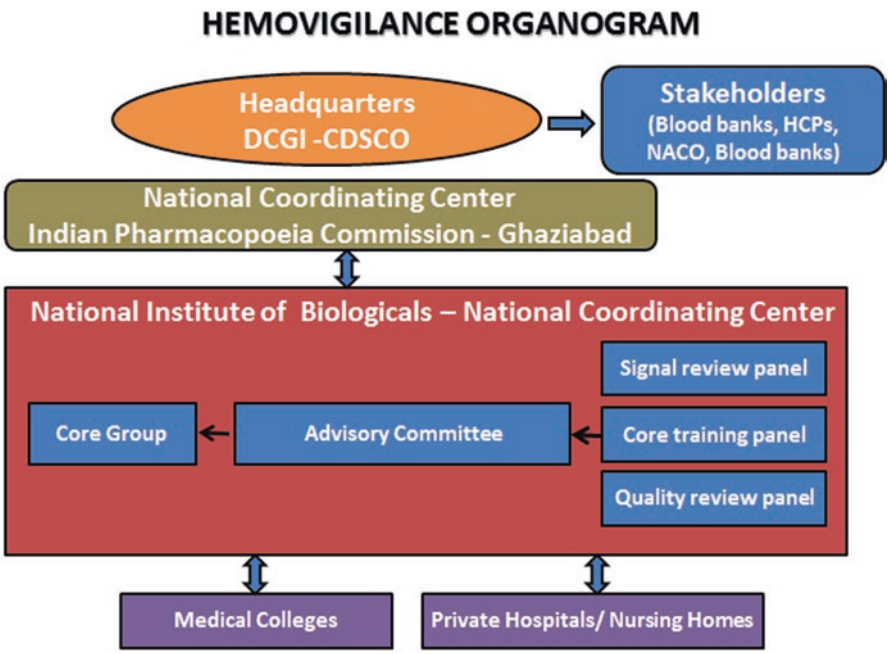

Fig. 11.2 Hierarchy of the hemovigilance system in India

adverse transfusion reactions in the TRRF and upload them using the Haemovigil software. The data from the software are then collated and assessed. Based on the trends, necessary interventions are proposed to improve patient safety.

- Since there was under-reporting of adverse reactions in donors, the National Blood Donor Vigilance Programme (NBDVP) was initiated in June 2015. Adverse Donor Reaction Reporting Form (ADRRF), a one-page document is now available to collect data on complications/reactions seen in donors.
- Both TRRF and ADRRF are freely available in the HvPI website.

11.6 Materiovigilance

- Medical devices form an important part of the healthcare system. They play a key role in diagnosis, monitoring, prevention and therapy of diseases.
- Medical devices extend from equipment for local use (like cutting tissues or covering wounds) to sophisticated diagnostic devices and computerized equipments. Examples for devices include instruments like commonly used clinical thermometers to sophisticated ventilators used in intensive care units.
- Thanks to the progress in the field of information technology and electronics over the last few decades, there are numerous devices available throughout the world with diverse applications because of the varied technologies involved.

- With this increase in the number of devices, the number of untoward or undesirable events related to their use has also increased. Examples for medical device-induced adverse events are burns caused by automated external defibrillator devices, malfunctioning infusion pumps and inaccurate glucometers.

11.6.1 Materiovigilance Programmes: Global Perspective

- A study conducted for a period of 35 months in San Francisco showed that out of 517 sudden deaths, 11 were due to malfunctioning cardiac implantable electronic devices. This shows that device-induced adverse events cannot be ignored and have to be cautiously observed and reported as well.
- The United States Food and Drug Administration (US-FDA) has a Medical Device Reporting (MDR) programme for monitoring adverse events related to the use of devices. It is mandatory for health facilities using devices, manufacturers and importers to report such events. This is called "*mandatory MDR*".
- There is another category termed as "*voluntary MDR*" where doctors, patients, consumers and caregivers are encouraged to voluntarily report such adverse events.
- Therapeutic Goods Administration (TGA) in Australia, Commission de Materiovigilance in France and Medicines and Healthcare Products Regulatory Agency (MHRA) in the United Kingdom are the other popular national surveillance programs.

11.6.2 Classification of Medical Devices

Based on the indications for use and extent of risk associated with the use of the medical devices, various regulatory authorities have classified medical devices as follows,

- US-FDA has classified medical devices into three classes based on the level of control required to ensure the safety or effectiveness of the device:
  - Class I (lowest risk to the patient)
  - Class II (intermediate risk to the patient)
  - Class III (highest risk to the patient)
- Similarly, TGA (Australia) has classified devices into five classes.
- MHRA (UK) has three groups of devices.

11.6.3 Materiovigilance Programme of India (MvPI)

MvPI was approved in February 2015 by the Ministry of Health and Family Welfare and subsequently launched by the DCGI in July 2015 in Ghaziabad at Indian Pharmacopoeia Commission (Box 11.6).

Box 11.6: Structure of the Materiovigilance Programme of India

National collaborating centre	Technical support partner	Regulator
Sree Chitra Tirunal Institute for Medical Sciences & Technology (SCTIMST), Kerala	National Health System Resource Centre (NHSRC), New Delhi	Central Drugs Standard Control Organization (CDSCO), New Delhi

11.6.4 Purpose of MvPI

- Monitoring medical device-associated adverse events.
- Creating awareness among healthcare professionals about the importance of capturing and reporting adverse events.
- To check the risk-to-benefit ratio associated with the use of these devices.
- To generate evidence-based data with respect to the safety of these devices.
- Medical device-associated adverse events are grouped based on severity into the following types:
  - Death – of the patient or the user of the device or any other person.
  - Serious injury – to the patient or the user of the device or any other person.
  - No death or serious injury, but is likely to cause grievous injury to the patient or the user of the device or any other person if the adverse event recurs.
- The reporting of these adverse events is done through Medical Device Adverse Event Reporting Form, which is freely available. Clinicians, clinical engineers, biomedical engineers, pharmacists, nurses, hospital technology managers and technicians can report medical device-associated adverse events. In addition, device-specific reporting can be done by the manufacturers of the device. A toll-free number is also available which provides assistance for reporting.

11.6.5 Benefits of Materiovigilance

- Prevention of complications and injuries.
- Upgrading the design and efficiency of devices.
- Preventing future adverse events by implementing corrective actions.

11.6.6 Current Status and Future Perspectives of Materiovigilance

Medical devices have been in use for decades for diagnosis and management of diseases. With the advances in science and technology, numerous sophisticated devices have come in use currently. Even though the devices are intended to do good to the patients, there are a few untoward events. Materiovigilance detects and

investigates the adverse events related to the use of medical devices. It aims at preventing these adverse events from recurring.

11.7 Cosmetovigilance

The term *cosmetovigilance* was first used in French literature in the year 1997 and hence can be claimed as a very recent health concept. Cosmetovigilance is defined as the ongoing and systematic monitoring of the safety of cosmetics in terms of human health. It is a public health surveillance on cosmetic products with a public health objective.

11.7.1 Scope of Cosmetovigilance

- The scope of cosmetovigilance is limited to cosmetic products. Cosmetics are preparations or substances intended for usage in contact with external human surfaces, teeth or mucous membranes. These products are commonly used for cleaning, perfuming, transfiguring or protecting the areas of contact.
- In India, cosmetics are regulated as per the Drugs and Cosmetics Act, 1940, and Rules, 1945. Indian cosmetics are subdivided into four categories as follows:
  - Skin products (with ten subcategories)
  - Hair and scalp products (with four subcategories)
  - Nail and cuticle products
  - Products for oral hygiene

11.7.2 Establishing Causality in Cosmetovigilance

As with other measures of vigilance, cosmetovigilance causality is defined as the analysis of the relation between a cause (the alleged cosmetic product) and an effect (the alleged manifestation or reaction). The common scales of causality used for cosmetovigilance are listed in the Box 11.7.

Box 11.7: Cosmetovigilance Causality Assessment Scales

Scale	Levels of causality
AFSSAPS* scale (sometimes called ANSM* scale)	Five levels: very likely, likely, not clearly attributable, unlikely, excluded
**Abbreviations of French health agencies*	
Colipa scale	Three levels: questionable, likely, very likely
Product life cycle management (PLM) call approach scale	Six levels: irrelevant, not enough information, unlikely, possible, probable, certain

11.7.3 Need for Cosmetovigilance in India

- As with drugs, the emergence of adverse effects with cosmetic usage is quite common. As we are aware, the population in our country is massive, and so is the market of cosmetic products. India is the fourth largest consumer of cosmetics in the Asia-Pacific region after Japan, China and South Korea.
- The most common reactions reported in India include contact dermatitis and dermatoses. As opposed to the Western countries, reactions to traditional products are quite common in India, as seen in cases of kumkum and kajal dermatitis.

11.7.4 Current Status and Future Perspectives of Cosmetovigilance

- The process of cosmetovigilance is evolving and coming up as a strong regulatory science to protect public health and beauty. Germany and Sweden are two nations with a formal cosmetovigilance system in place.
- Proper implementation of cosmetovigilance can help in controlling or preventing the use of potentially hazardous constituents in cosmetic products, ultimately enhancing the confidence on these products and also protecting the general population.

11.8 Addictovigilance

Addictovigilance is the systematic monitoring dedicated to the survey of medicinal or illicit psychoactive substance use disorders. It is a reporting system for health professionals or individuals of adverse events occurring with a drug or cases of dependence, abuse and misuse of psychoactive drugs. In simple terms, it is the 'surveillance of addiction or dependence'.

11.8.1 Data Sources for Addictovigilance

- There exist several challenges in addictovigilance as opposed to general pharmacovigilance. Since the behaviour of addiction and the users are often hidden, it is extremely challenging to generate signals from this restricted out-of-radar subset. Under-reporting is a very common phenomenon, and hence, there is a huge chance of missing a signal.
- Addictovigilance mostly relies on collecting indicators like forged prescriptions to pick up data sources. Spontaneous reporting is a minority. The key data sources include:
  - Symptoms
  - Deaths

  - New high-risk behaviours
  - New psychoactive substances
  - Forged prescriptions
  - Internet
- Very few countries like France have a full-fledged addictovigilance system in place. Various yearly or half-yearly pharmacoepidemiological surveys are conducted in France for data generation and signal identification. OPPIDUM, OPEMA, OSIAP, ASOS, DRAMES and DTA are key surveys done for different data sources.

In conclusion, we propose a new umbrella term encompassing the above discussed vigilances, namely, pharmacovigilance, hemovigilance, materiovigilance, cosmetovigilance and addictovigilance – the '*medicovigilance*'.

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Drug Interactions

12

Heta Shah and Neel Jayesh Shah

Abstract

Drug interaction is the alteration of the effect of one drug due to the concomitant or prior presence of another drug. Drug interactions represent a major preventable cause of adverse drug interactions. Factors which predispose to drug interactions include increasing age, polypharmacy, liver disease, kidney disease, and environmental and food interactions. In vitro interactions occur rarely when the drugs are mixed in a solution before administration intravenously. The two types of in vivo interactions are pharmacokinetic and pharmacodynamic interactions. Alteration of gastric pH, gastrointestinal motility, gut flora, and chelation represent interactions which can happen at the level of drug absorption stage. At the level of distribution, one drug can displace another from protein binding sites. The induction and inhibition of liver enzymes by one drug that metabolizes other drugs are one of the major reasons behind drug interactions. At the level of drug excretion, a drug can affect tubular secretion and reabsorption of other drugs. These interactions can be further classified as additive, synergistic, or antagonistic. In many instances, we have successfully employed drug interactions for our benefit.

Keywords

Drug interactions · Drug-drug interactions · Pharmacokinetic interactions · Pharmacodynamic interactions

12.1 Introduction

Drug interaction is defined as alteration of the effects of one drug (i.e., *the object drug*) by concomitant administration or prior presence of another drug (i.e., *the precipitant drug*). Drug interaction is considered to be one of the major causes of

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adverse drug reactions. An adverse drug interaction can cause a loss of efficacy or increased toxicity of a drug due to the simultaneous presence of two or more substances in the body.

The main aim of this chapter is to apply clinical knowledge to patient care by early detection, assessment, and prevention of drug interactions. The drug for which the effect is altered (increased or decreased) is called as the *object drug*, whereas the drug that provokes the interaction is named as the *precipitant drug*. At times, the object drug can provoke the interaction of the precipitant drug; hence, these designations are not fixed.

- Certain factors which predispose to drug interactions are as follows:
  - Age and sex
  - Pharmacogenomics
  - Polypharmacy (prescription/OTC)
  - Co-morbidities like liver and kidney diseases (drug-disease interactions)
  - Environment (drug-chemical or drug-herb interactions)
  - Food and beverages (drug-food or drug-alcohol interaction)
- **Adverse drug interaction**
  - *In vitro*

    Penicillins and aminoglycoside antibiotics inactivate each other.

    Suxamethonium can cause precipitation of thiopentone when mixed together.

    Calcium and ceftriaxone mixed in the same intravenous solution can cause precipitates. Hence, ceftriaxone should not be mixed in Ringer's lactate. Neonatal deaths brought this reaction to light, and afterward warnings were issued to spread awareness of this interaction.
  - *In vivo*

    Drug interactions occur due to either pharmacokinetic reasons or pharmacodynamic reasons.

12.2 Mechanisms of Drug Interactions

Based on the mechanistic approach, drug interactions are mainly classified as pharmacokinetic and pharmacodynamic.

12.2.1 Pharmacokinetic Interactions

Pharmacokinetic interactions are those in which precipitant drug alters object drug's kinetics including absorption, distribution, metabolism, and excretion.

Altered GI absorption

Drug interactions modifying the absorption pattern mainly occur in the gastrointestinal tract. Interactions affecting absorption usually require the simultaneous

presence of object and precipitant drug in the stomach. By separating the administration time, by at least 2 hours, this type of interaction can be prevented.

- **Alteration of pH**
  - Depending on drug salt (either weak acid or base), pH of the GI tract may influence the site of absorption as well as the extent of absorption. Unionized drugs (more lipid-soluble drugs) are absorbed readily from the GI tract compared to ionized drugs.
  - Generally, acidic drugs are easily absorbed from the upper GI tract (lower pH region). Acidity lowering drugs including ranitidine, omeprazole, and antacids raise the GI pH which may delay or inhibit the absorption of acidic drugs. For example, antacids may delay absorption of phenobarbital so the hypnotic effect may not be observed in estimated time.
  - Other acidic drugs whose absorption would be delayed include azole antifungals, antineoplastic tyrosine kinase inhibitors like bosutinib, and antiretrovirals like atazanavir. Needless to say, the cost of these drugs is high, and drug interaction leading to reduced efficacy cannot be afforded.
  - Bisacodyl, a stimulant purgative, is available in enteric-coated form as it causes severe GI irritation; if it is given within an hour of administering antacids, the coating may dissolve in alkaline pH, and bisacodyl may cause severe GI irritation due to alkalosis.
- **Complexation and chelation**
  - Tetracycline can combine with heavy metal ions such as calcium, magnesium, aluminum, or iron and may form poorly soluble salts, ultimately delivering a poor response. Certain ion-exchange resins can bind to drugs and may reduce absorption. Cholestyramine, an anionic resin, is used to sequester bile salts so that this water-soluble complex can be easily excreted from the body, but it can bind to other drugs also like digoxin and warfarin.
- **Changes in GI motility**
  - Certain drugs can increase or decrease the rate of passage through the GI tract which ultimately affects the amount of drug absorbed.
  - Increased absorption can occur when the drug is available at the site of absorption for a prolonged period of time and vice-versa. For example, metoclopramide given with paracetamol in the treatment of migraine can increase the GI motility resulting in lesser time for paracetamol to reach the small intestine (absorption site) and, hence, producing a quick analgesic effect.
- **Inhibition of GI flora**
  - The large intestine has a large number of bacteria. Certain drugs like antibiotics alter the microbial flora of bowel.
  - Broad-spectrum antibiotics like tetracyclines and certain cephalosporins inhibit large intestinal bacteria which are involved in vitamin K synthesis. In their presence, the anticoagulant activity of warfarin is enhanced.
- **Malabsorption states**
  - Some drugs like laxatives, neomycin, and cholestyramine decrease absorption of vitamins and nutrients from the GI tract by causing malabsorption.

- **Drug-induced mucosal damage**
  - Drugs that damage the GI mucosa may reduce the absorption of some drugs. Certain cancer chemotherapy agents like cyclophosphamide, vincristine, and procarbazine reduce GI absorption of most oral drugs.

Alteration in distribution

- Interaction relating to the distribution of drugs depends on plasma protein binding.
- Several drugs are bound to plasma proteins reversibly.
- Examples of highly protein-bound drugs are phenytoin (90%), tolbutamide (96%), and warfarin (99%).

- **Displacement from protein-binding site**
  - When two protein-binding drugs are administered simultaneously, they may compete for same binding sites, and the drug that has a greater affinity for the binding site will displace the other drug.
  - Phenylbutazone and warfarin both are highly protein-bound drugs. As phenylbutazone has greater affinity to protein, it displaces warfarin from the binding site. Consequently, a higher concentration of free warfarin leads to warfarin toxicity manifesting as hemorrhage.
- **Volume of distribution**
  - Elderly have a lesser volume of distribution; hence, the concentration of drugs like propranolol may be higher compared to the young.

Altered metabolism

Some of the most important drug interactions occur through metabolic induction or inhibition.

- **Increased metabolism**
  - When precipitant drug induces (increases) the enzymes that increase the metabolism of the object drug, the therapeutic activity is reduced. If the responsible drug (precipitant drug) for enzyme induction is discontinued, the process reverses. Some drugs increase their own metabolism, e.g., carbamazepine.
  - Oral contraceptives in the presence of antiepileptic phenytoin cause contraceptive failure. Almost all antiepileptic drugs induce CYP3A4 enzymes causing a decrease in the levels of estrogen and progesterone which lead to contraceptive failure.
  - The metabolism of theophylline and phenytoin is mutually increased by each other. So if administered together, serum drug concentration may decrease, and lack of seizure control or accentuation of pulmonary symptoms may occur.
  - Certain pesticides like DDT also increase hepatic microsomal activity. Individuals exposed to such chemicals have higher enzymatic activity; therefore, drug concentration may change in that condition.

  - Even smoking increases the metabolism of certain drugs like chlorpromazine, diazepam, and chlordiazepoxide.
  - Chronic alcoholism leads to enzyme induction, and warfarin and phenytoin levels are reduced. Contrastingly, in acute alcoholism, barbiturates toxicity is observed because of enzyme inhibition.
- **Decreased metabolism**
  - Enzyme inhibition is one of the common mechanisms of drug interaction. This mechanism involves direct competition for binding sites between the object drug and the precipitant drug.
  - The onset of action of this type of drug interaction is rapid as compared to enzyme induction. The object drug reaches steady-state concentration faster. This interaction usually produces an increase in serum drug concentration, which leads to augmentation of therapeutic as well as toxic effects.
  - Omeprazole inhibits oxidative metabolism of diazepam, which increases the benzodiazepine concentrations and its effect. Isoniazid inhibits phenytoin metabolism during concomitant administration resulting in phenytoin toxicity. This is mainly observed in slow acetylators.
  - Alcohol-disulfiram interaction: disulfiram inhibits oxidation of acetaldehyde by blocking the enzyme aldehyde dehydrogenase resulting in accumulation of acetaldehyde. This will lead to disulfiram reactions like palpitation, flushing, dyspnea, and hypotension.
- **Altering first-pass metabolism**
  - Certain drugs are metabolized during their initial (first) pass through the GI tract and liver. For example, only 10 % of orally administered propranolol reaches systemic circulation due to first-pass metabolism. The first pass and hepatic blood flow reduce with age and are likely to be the reason for higher concentrations of propranolol-like drugs in the elderly.

Alteration of renal excretion

By concomitant administration of one drug, the renal excretion of another drug can be increased or decreased. Lipid-soluble drugs are metabolized by liver to water-soluble drug metabolites for renal excretion. Mechanisms which alter renal excretion are changes in urinary pH, active tubular secretion, and glomerular filtration rate.

- **Active tubular secretion**
  - The process of active tubular secretion takes place in the proximal renal tubule. From the systemic circulation, some drugs are transported inside the lumen by a protein transporter. Each transporter has different affinities for anions, cations, and drugs.
  - Drugs that use similar transport system interact by competitive inhibition which decreases the tubular secretion of the object drug.
  - Probenecid impairs the tubular secretion of methotrexate, which increases its serum concentration three to four times causing toxicity. This effect is used in a beneficial way when probenecid inhibits tubular secretion of penicillin and increases the serum penicillin level causing prolongation of activity.

- **Tubular reabsorption**
  - Many drugs are excreted by the glomeruli only to be reabsorbed from renal tubules by passive diffusion which depends on concentration and lipid solubility of drugs across the sides of the membrane.
  - Lithium is mainly excreted by the kidney, but in the presence of thiazide diuretics which prevent sodium reabsorption, increased tubular reabsorption of lithium occurs resulting in lithium toxicity. Thiazide diuretics increase lithium concentrations by 25 to 40%.
- **Changes in urinary pH**
  - Weakly acidic drugs are reabsorbed from acidic urine, whereas basic drugs get excreted since they are in the ionized state in acidic urine.
  - Sodium citrate is an alkalinizing agent used in the treatment of urinary tract infection. It increases excretion of acidic drugs like penicillin, cephalosporins, and thiazide diuretics, whereas it decreases excretion of basic drugs like ranitidine and amiloride.
  - Sodium bicarbonate can be used concurrently during sulfonamide treatment to prevent crystalluria.

12.2.2 Pharmacodynamic Interaction

Pharmacodynamic interactions are those in which precipitant drug alters the mechanism of action of the object drug without altering the serum concentration of object drug.

- *Additive effect*
  - When the object drug and the precipitant drug both produce a similar pharmacological effect, it is called as an *additive effect*.
  - For the treatment of severe pain, a combination of different class of pain relievers is used which prolong the pain-relieving activity. Sometimes adverse effects can get added, e.g., a combination of spironolactone and ACE inhibitors produces hyperkalemia.
- *Synergistic effect*
  - Synergism is one of the common mechanism by which interaction occurs. Concurrent administration of propranolol and verapamil has synergistic cardiovascular effects. Both the drugs produce negative inotropic and chronotropic effects and are used to manage angina. However, the chances of serious cardiovascular outcomes are there.
  - Other classical example includes the use of sulfamethoxazole and trimethoprim where two bacteriostatic drugs act at two different steps in a common pathway *(the sequential blockade)*, thus producing a synergistic bactericidal effect.

- *Antagonistic effect*
  - It is an interaction between two or more drugs that have opposite effects on the body. Patients who are taking levodopa (an antiparkinsonian drug) along with metoclopramide (a dopamine antagonist) will have an exaggeration of parkinsonian symptoms.
  - Nevertheless, antagonistic interactions are sometimes used to treat toxicities. For example, atropine is used as an antidote for organophosphorus poisoning, and naloxone is used to treat opioid overdose.
- *Altered transport system and effects at receptor site*
  - These drug interactions show interference with the physiologic transport system, restricting the access of certain drugs into cells.
  - Phenothiazines like chlorpromazine and tricyclic antidepressants like amitriptyline inhibit neuronal uptake of guanethidine, preventing the antihypertensive effect of guanethidine.

12.3 Outcomes of Drug Interaction (Box 12.1)

- Harmful (increased toxicity or decreased efficacy)
- No clinical significance
- Biological interference with laboratory test which may lead to misdiagnosis
- Beneficial (increased activity, e.g., penicillins or cephalosporins with probenecid)

12.4 Reducing Risk of Drug Interaction

- Identify the risk factor.
- Understanding the mechanism of interaction by which drugs interact.
- Awareness of high-risk patients likely to develop drug interactions.
- Closely monitoring the patient during concurrent administration of drugs.
- Drugs having a narrow therapeutic index should be closely reviewed (e.g., digoxin, phenytoin, theophylline, and lithium).

Box 12.1: Drugs or Classes of Drugs Requiring Monitoring for Drug Interaction

Antiarrhythmics	Anticonvulsants	Aminoglycoside antibiotics
Antidiabetics (Sulfonylureas)	Cytotoxic drugs	Monoamine oxidase inhibitors
Lithium	Heparin	Tricyclic antidepressants
Theophylline	Warfarin	Selective serotonin reuptake inhibitors
Verapamil	Digoxin	Selective norepinephrine reuptake inhibitors

- Personalized therapy approach should be followed in high-risk patients (e.g., pregnancy, malignancy, and elderly).
- Obtain a thorough drug history regarding over-the-counter drugs, herbal supplements, and nutritional products which can unexpectedly produce drug interactions.
- Educating patients about monitoring and reporting unexpected drug reactions during concomitant drug therapy.

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Structure-Activity Relationships

13

Mageshwaran Lakshmanan

Abstract

In-depth analysis of the structure of the drug and its subsequent modification yield drugs with high affinity and increased receptor specificity with an improved pharmacokinetic profile. Modification of the parent molecule of catecholamines, called β-phenyl-ethyl-amine, provided orally active adrenergic bronchodilators, long-acting β_2-adrenergic agonists, COMT-resistant catecholamines, and isomerism-based increased potency in adrenergic agonists. Similarly, alteration of the cyclo-pentano-perhydro-phenanthrene ring of steroid molecules delivered androgen, progestins, and estrogen with low first-pass metabolism, long-acting injectable steroids, and corticosteroids with negligible mineralocorticoid activity. In addition to this, manipulation of the structure of morphine resulted in a plethora of "opioid agonists and antagonists" that are used in clinics for various conditions. Minor alteration of "spacer" in the structure of antihistaminic molecules resulted in "nonsedative" antihistaminics. Therefore, SAR plays a vital role in the drug development process which ultimately determines the "success" or "failure" of the drug.

Keywords

SAR · Drug structure alteration · Phenylethylamine · Functional group · Pharmacophore

13.1 Introduction

Structure-activity relationship (SAR) studies the association between the three-dimensional structure of a molecule with its biological, pharmacological, and chemical activity on the receptors. Often, the new parent molecules developed to treat particular disease express low affinity, less specificity, and high toxicity. In order to

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improve affinity and specificity, knowledge about the SAR of the parent molecule is important. Majority of drugs with better efficacy and safety used clinically are being developed by meticulous analysis of SAR of the parent molecule and modification of its structure.

13.2 Implications of Structure-Activity Relationship

- Determination of chemical group (pharmacophore) responsible for affinity of the molecule to its receptor. By altering the structure of pharmacophore, the affinity of the molecule to the desired receptor can be increased (achieving high affinity resulting in better potency).
- Modification of chemical group responsible for interactions with unwanted receptors thereby improving the specificity of drug (lesser incidences of side effects).
- Slight alteration in chemical structure of parent molecule can improve the pharmacokinetic properties of the drug which would benefit by ease of administration, long duration of action, better tolerability for patients in hepatic and renal co-morbid conditions, and others.
- Several antagonists of particular receptors were developed by minor alterations in the natural agonist after the detailed SAR analysis of natural ligands.
- With the aid of SAR analysis, development of agonists with improved pharmacokinetic and pharmacodynamics profile is possible with modifications in their structure.

The SAR of catecholamines, corticosteroids, opioids, antihistaminic drugs, and antipsychotic drugs is reviewed in this chapter. In each section, for ease of understanding, readers will be introduced to the structure of parent molecule initially followed by importance of each group of molecules (e.g., amino, alkyl, aryl, and others) in the structure with the advantages gained by altering these structures.

13.3 SAR of Catecholamines

13.3.1 Parent Molecule of Catecholamines

- The chemical structure which is common to norepinephrine and epinephrine is β-phenyl-ethyl-amine. As the name indicates, β-phenyl-ethyl-amine has phenyl molecule at one pole which is linked with amine group at the other end by a two carbon chain (ethyl-bridge).
- The carbon atom which is near to amine end is termed as α-carbon, and another one in the ethyl-bridge is called as β-carbon. Various adrenergic agonists were developed by the alteration of this β-phenyl-ethyl-amine, and hence it is considered as the parent molecule for catecholamines (Fig. 13.1).

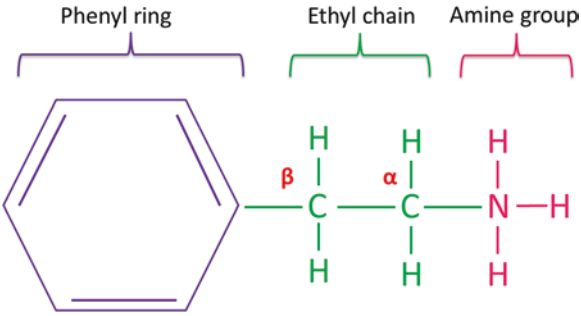


Fig. 13.1 The basic β-phenyl-ethyl-amine structure

13.3.2 Targets for Structural Alterations

The structure of β-phenyl-ethyl-amine permits the modification in the following five ways:

- **Substitution of Alkyl or Aryl Molecule in the Amino Group**
  - As a rule of thumb, when the size of substituted alkyl in the amine of β-phenyl-ethyl-amine increases, the activity on the alpha-adrenergic receptor decreases, and activity on the beta-adrenergic receptor increases. For example, norepinephrine, dopamine, and metaraminol have only "H" in the amine and have higher alpha-adrenergic activity, while isoproterenol, metaproterenol, and prenalterol have -CH $(CH_3)_2$ substitution in the amine and have higher selectivity for beta-adrenergic receptors.
  - Substituting the tertiary butyl [–$C(CH_3)_3$] molecule in the amine of β-phenyl-ethyl-amine increases the selectivity for β_2-adrenergic receptors. For example, isoproterenol with -$CH(CH_3)_2$ substitution is a nonselective beta-adrenergic agonist, while colterol with –$C(CH_3)_3$ makes it a selective β_2-adrenergic agonist.
  - Substitution of aryl group in the amine of β-phenyl-ethyl-amine increases the lipophilic nature of the molecule and hence can cross placenta and blood-brain barrier. For example, ritodrine used for tocolysis in preterm labor can cross placenta and cause fetal tachycardia. Benzphetamine used as an anorectic can readily cross GIT and blood-brain barrier.
- **Substitution of Molecules (Hydroxy, Alkyl, or Aryl Groups) in the Benzene Ring**
  - Substitution of –OH molecule in the third carbon of benzene ring in β-phenyl-ethyl-amine is essential for alpha-adrenergic activity. For example, phenylephrine and metaraminol have only 3-OH substitution and are more alpha selective.
  - Substitution of –OH molecule in the fourth carbon of benzene ring in β-phenyl-ethyl-amine provides beta-adrenergic activity. For example, epinephrine, dopamine, and norepinephrine have both 3-OH and 4-OH substitution and are α+β agonists.

  - Molecules with 3-OH and 4-OH substitution in benzene ring are readily acted upon by catechol-*O*-methyltransferase (COMT) and hence have poor oral bioavailability. However, instead of 4-OH, when hydroxyl group is added to fifth carbon, then molecule becomes resistant to COMT and hence has good oral bioavailability. For example, epinephrine, dopamine, dobutamine, and norepinephrine are readily metabolized in GIT by COMT, while metaproterenol, terbutaline, and methoxamine are GIT-COMT resistant with good oral bioavailability.
  - Addition of hydroxyl group in third and fifth position with larger amino substituent improves the selectivity for β_2-adrenergic receptors.
  - When no hydroxyl group is substituted, the molecule becomes more lipophilic and can cross blood-brain barrier readily and exerts CNS actions. For example, amphetamine, methamphetamine, and phenylpropanolamine have no hydroxyl substitutions and exert more CNS action when compared to epinephrine and norepinephrine upon administration.
- **Substitution of Molecules in the Alpha Carbon Atom in Ethyl-Bridge**
  - Addition of methyl group (-CH_3) in the alpha carbon makes the molecule more selective for alpha-adrenergic receptors. For example, metaraminol and methoxamine have –CH_3 group in alpha carbon atom with alpha-adrenergic selective actions.
  - Addition of ethyl group (-CH_2-CH_3) in the alpha carbon makes the molecule more selective for beta-adrenergic receptors. For example, ethylnorepinephrine and isoetharine have -CH_2-CH_3 group in the alpha carbon with pronounced beta-adrenergic receptor action.
  - Substitution of any small molecule in the alpha carbon atom decreases the action of monoamine oxidase (MAO) upon the molecule, and hence the duration of action will be increased. For example, ephedrine, amphetamine, and methamphetamine produce longer pharmacological actions than norepinephrine or dopamine.
  - Diastereoisomer can be achieved by substitution in alpha carbon atom. In general, upon alpha carbon substitution, dextrorotatory compounds are made more potent than the levorotatory. For example, d-amphetamine is more potent than the l-amphetamine.
- **Substitution of Molecules in the Beta Carbon Atom in the Ethyl-Bridge**
  - Substitution of molecule on the beta carbon also produces diastereoisomer. However, in the case of beta carbon substitution, the levorotatory compounds produced are more potent than the dextrorotatory compounds. For example, l-epinephrine is more potent than the d-epinephrine.
  - Adrenergic molecules with hydroxyl group in the beta carbon have direct sympathomimetic activity, and molecules with no hydroxyl group in the beta carbon act as indirect sympathomimetics. For example, tyramine, amphetamine, and methamphetamine have no hydroxyl group and act as indirect sympathomimetics.
  - On the other hand, hydroxyl substitution in the beta carbon lowers lipid solubility, and hence molecules without hydroxyl group can exert CNS action

better than those with hydroxyl group. For example, the CNS action of amphetamine (no hydroxyl group in beta carbon) is better than norepinephrine due to its high lipid solubility.

- **Separation of Alpha and Beta Carbon Chain**
  - For good adrenergic agonistic action, the benzene ring should be separated from the amine group with two carbon chain. Any alteration with the length of the chain by introduction of new group in the bridge will result in loss of adrenergic activity and may convert the molecules into antagonists. Thus, all the beta-adrenergic antagonists usually have additional groups like –O–CH_2– (propranolol, nadolol, and pindolol) or –CH2– (Labetalol) between the aryl and beta carbon atom.

The summary of SAR of catecholamines is illustrated in Fig. 13.2.

13.4 SAR of Steroids

13.4.1 Parent Molecule of Steroids

The steroid family consists of corticosteroids, norsteroids, estrogens, androgens, progestins, neurosteroids (DHEA), and secosteroids (vitamin D). All the molecules share a common structure called "cyclo-pentano-perhydro-phenanthrene" (sterane) ring which is nothing but a 17 carbon structure arranged in four fused rings. First three rings are six-member cyclohexanes (named as A, B, and C), and the fourth ring is a five-member cyclopentane (Fig. 13.3). All the derivatives of steroids available now were produced by chemical alteration of this cyclo-pentano-perhydro-phenanthrene ring, and hence it is considered as a parent or core molecule of the steroids.

13.4.2 SAR of Corticosteroids

- The corticosteroids have 21 carbon skeleton structures in which additional 2 methyl and 1 –CO–CH_2OH chain are attached to the cyclo-pentano-perhydro-phenanthrene ring.
- In the ring A of cyclo-pentano-perhydro-phenanthrene molecule, the double bond between the fourth and fifth carbon atom and keto group (-C=O-) in the third carbon atom is absolutely essential for binding with corticosteroid receptors.
- For the basic glucocorticoid activity, the 21 ring structure should have:
  - One β-hydroxyl group in the 11th carbon in the ring C (always essential)
  - One α-hydroxyl group in the 17th carbon in the ring C (not essential always)
  - One hydroxyl group in the 21st carbon in the ring D (not essential always for glucocorticoid but absolute requirement for mineralocorticoid action)
- Introduction of double bond between the first and second carbon in the ring A increases glucocorticoid activity and results in slower metabolism (e.g., prednisolone and prednisone).

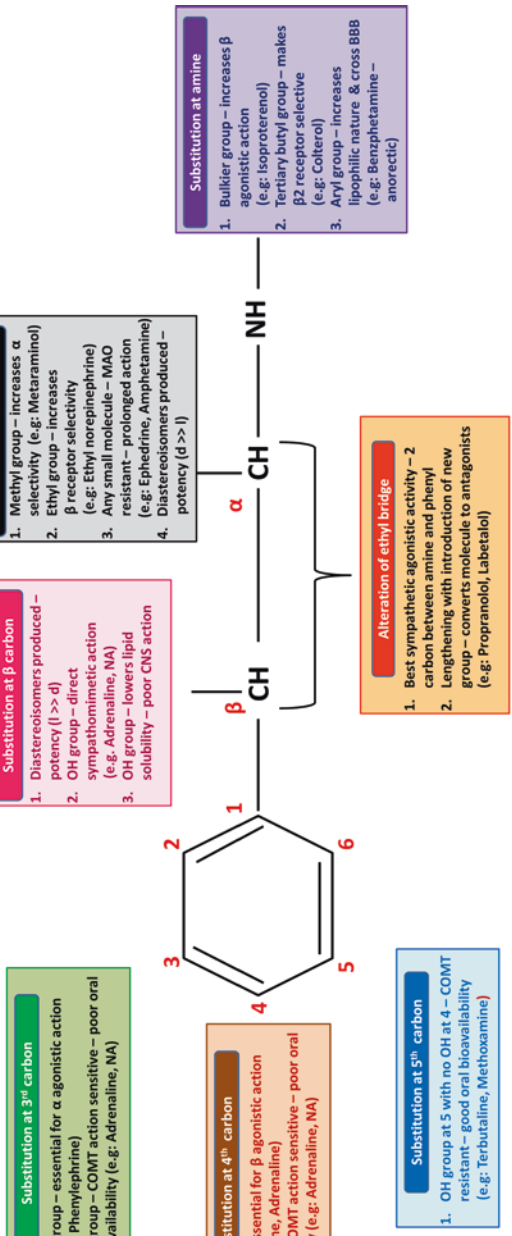


Fig. 13.2 Structure-activity relationships of catecholamines

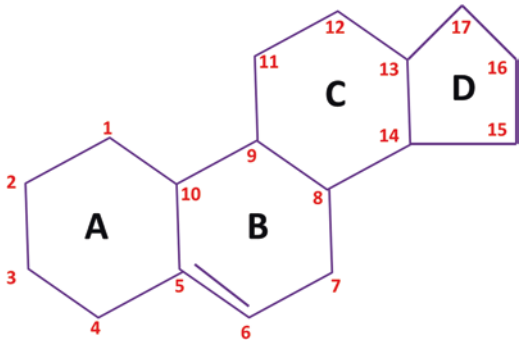


Fig. 13.3 Structure of cyclo-pentano-perhydro-phenanthrene ring

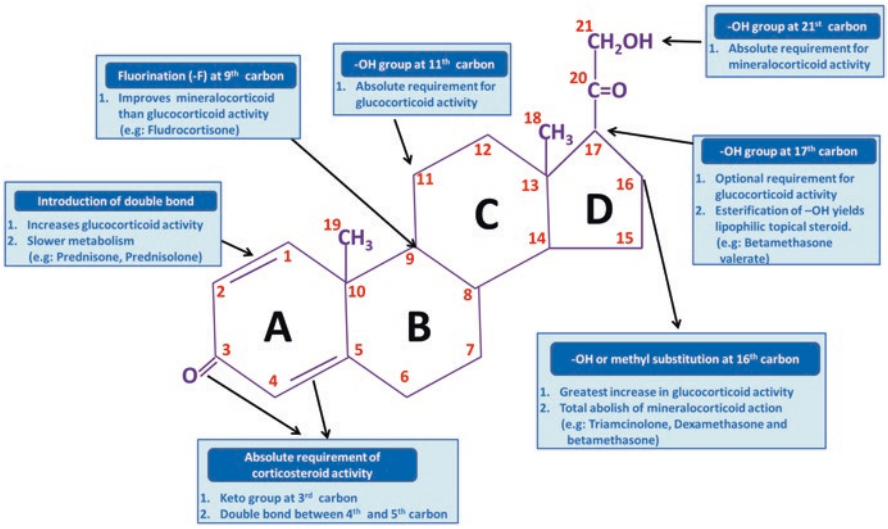


Fig. 13.4 Structure-activity relationships of corticosteroids

- Substitution of hydroxyl or methyl group in 16th carbon in the ring D completely eliminates the mineralocorticoid activity (e.g., triamcinolone, dexamethasone, and betamethasone).
- α-Fluorination of the ninth carbon in the ring B improves both glucocorticoid and mineralocorticoid activity. However, the increment of mineralocorticoid activity is higher than the glucocorticoid (i.e., 125 times compared to 10 times in fludrocortisone).
- The hydroxyl groups in the 17th and 21st carbon can be esterified with valerate and propionate to produce corticosteroids with good lipophilic nature and used topically (e.g., betamethasone dipropionate, betamethasone valerate).

The summary of SAR of corticosteroids is illustrated in Fig. 13.4.

13.5 SAR of Estrogens

- The parent molecule of "estradiol" is an 18 carbon structure with one additional methyl group attached to the cyclo-pentano-perhydro-phenanthrene ring.
- Presence of hydroxyl group at third carbon in the ring is the most important criteria required for estrogenic activity. It enables the effective binding of the molecule to both ERα and ERβ receptors (e.g., estradiol). Surprisingly, the compounds like anole, anethole, diethylstilbestrol, bisphenol-A, and genistein which possess 3-OH with no sterane ring structure can also exert potent estrogenic activity.
- Any alkyl substitution in the ring A will lead to the impairment in the estrogenic activity. Moreover, unsaturation of double bond in ring B will also lead to decrease in estrogenic activity.
- Ethinyl substitution in the 17th carbon will produce estrogenic derivative with good oral bioavailability and potency (e.g., ethinyl estradiol).
- Attachment of a large alkyl-amide group at the 7α-position in the ring B will make the molecule as an antagonist to estrogen receptor (e.g., fulvestrant).

13.6 SAR of Progestins

- One group of progestins called as pregnanes contain all 21 carbons like corticosteroids, while the other groups called as 19-norcompounds lack C19, C20, and C21. The former group of molecules have narrow spectrum of activity affecting only progesterone receptor, while the latter group has additional androgenic and estrogenic activity.
- Presence of keto group at the carbon three of ring A in sterane structure is essential for progesteronergic activity.
- Attachment of esters at the sixth carbon in the ring B reduces first-pass metabolism and yields orally active progestins (e.g., medroxyprogesterone acetate, chlormadinone acetate, and megestrol acetate).
- Ethinyl substitution at the 17th carbon in the 19-norcompound groups also decreases first-pass metabolism and yields orally active compounds (e.g., norethindrone, norethindrone acetate, and ethynodiol diacetate).
- Potency will be increased when methyl group in the 13th carbon of 19-norcompounds are replaced with ethyl group. These molecules are called as gonanes (e.g., norgestrel, a gonane, is more potent than norethindrone).
- Bulky aminophenol substitution at 11β-position in the ring C in the 19-norcompounds will make the molecule as an antagonist to progesterone receptor (e.g., mifepristone). However, when the same molecule possesses additional acetoxy group in the 17th carbon, the molecule becomes agonist-antagonist to the progesterone receptor (e.g., ulipristal).

13.7 SAR of Androgens

- The parent molecule testosterone is a 19 carbon structure, i.e., two methyl substitution to sterane ring structure.
- Similar to progestins, androgens also require keto group at the third carbon of ring A for the molecule to bind to the androgen receptor. In addition, hydroxyl group at 17th carbon is also essential. Removal of these groups will drastically reduce the androgenic agonistic activity and convert the molecule to pro-androgens (e.g., in testosterone, conversion of 17-OH to keto group will produce androstenedione, and further conversion of 3 keto to 3 –OH will produce dehydroepiandrosterone).
- Removal of double bond in the ring A (aromatization) will completely abolish the androgenic activity and converts the molecule to estrogen (e.g., aromatization of androstenedione will produce estrone).
- When a double bond is formed between C4 and C5, the androgenic activity is increased to several folds. This is called 5-alpha reduction (e.g., testosterone to dihydrotestosterone).
- The activity of 5-alpha reductase enzyme on the androgenic molecule can be reduced by:
  - Formation of double bond between C1 and C2 (e.g., metandienone)
  - Attachment of chlorine or hydroxyl molecule at C4 (e.g., oxabolone)
  - Attachment of methyl group in the C7 position (e.g., tibolone)
- The potency of the androgenic molecule can be improved by the following ways:
  - Formation of double bonds between the C9 and C10 and between the C11 and C12 (e.g., metribolone and trenbolone)
  - Attachment of methyl group in the C7 position (e.g., tibolone)
- The high first-pass metabolism of androgens can be overcome by attaching the esters in the C17 position making the molecule highly lipophilic. When given orally, this is absorbed by GI lymphatics and bypasses liver metabolism (e.g., testosterone undecanoate). When administered intramuscularly by dissolving this lipophilic molecule in oil, the duration of action will be increased (e.g., testosterone enanthate and testosterone cypionate).
- Alkylation at C17 decreases first-pass hepatic metabolism, and hence many orally active androgenic agonists are produced (e.g., oxandrolone and methyltestosterone).
- Attachment of bulkier and long-extended group at C17 (e.g., dienogest) or substitution at C16 (e.g., metogest) will convert androgen into antiandrogens. The SAR of androgens is summarized in Fig. 13.5.

13.8 SAR of Opioids

13.8.1 SAR of Morphine Derivatives (Multi-cyclic Opioids)

- The morphine and its derivatives share a common structure with variable rings (bicyclic to pentacyclic), and the 4-phenyl-piperidine structure (as shown in Fig. 13.6) in it is the "pharmacophore."

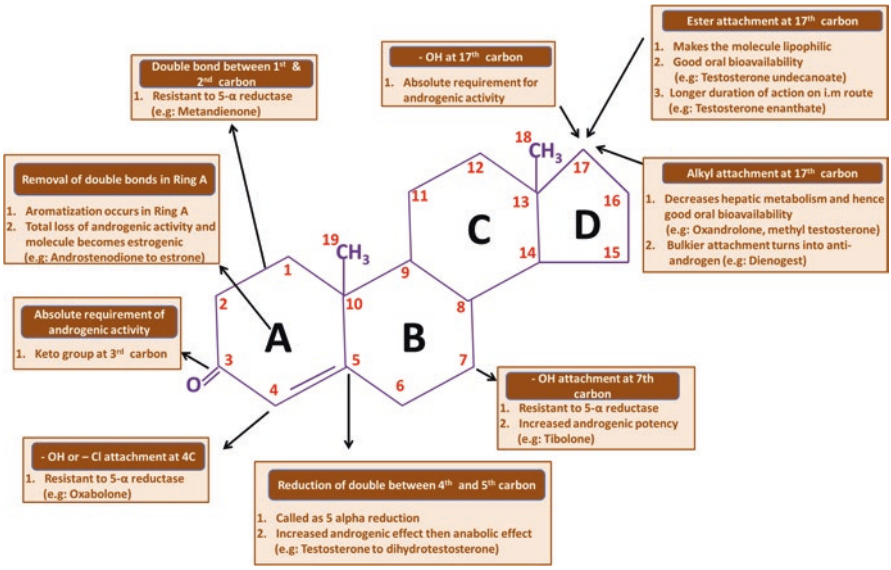
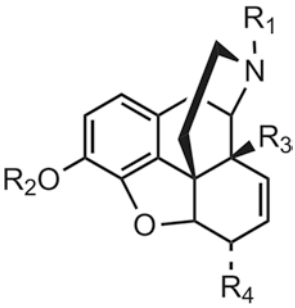


Fig. 13.5 Structure-activity relationships of androgens and its derivatives

Fig. 13.6 The pharmacophore (darkened lines) in the multi-cyclic opioids



- A protonated amino nitrogen in the structure is always required for the binding capacity to the opioid receptor. The protonated nitrogen in cationic conjugate form permits the binding of molecule to the aspartate residue in the opioid receptor.
- Addition of phenethyl group in the R1 significantly increases the μ receptor affinity and CNS distribution. However, the natural opioids commonly have methyl substitution in the R1 (e.g., morphine and codeine).
- Antagonism of μ receptor can be achieved by lengthening the R1 attachment from the nitrogen atom. Pure μ receptor antagonism is achieved by adding –OH group at 14 carbon and "cyclopropane" or "allyl" group in the R1 (e.g., naloxone and naltrexone).

- If the R1 substitution is dimethylallyl or cyclobutylmethyl group instead of allyl or cyclopropane, respectively, then the μ receptor antagonism is retained, but the molecule gains additional κ receptor agonism (e.g., pentazocine and nalbuphine).
- Presence of phenol group (-OH at third carbon) is an essential criterion for μ and κ receptor binding (e.g., morphine, hydromorphone). Same activity can be achieved by presence of methoxy-esters at the third carbon (e.g., codeine and oxycodone).
- Presence of –OH at third carbon (R2) makes the compound to be significantly metabolized and inactivated by glucuronic conjugation in GI tract, and hence, very low bioavailability is noted (e.g., morphine). However, addition of esters at third carbon provides significant resistance to metabolism with high bioavailability (e.g., codeine).
- Addition of –OH at the 14th carbon (R3) will increase the CNS penetration and agonism at μ and κ receptor, but the antitussive activity will be drastically decreased (e.g., morphine and codeine with no –OH at 14th carbon are excellent antitussive, while oxymorphone and oxycodone with –OH at 14th carbon are poor antitussives).
- Presence of –OH at sixth carbon will cause mast cell degranulation and results in allergy (e.g., morphine and codeine). When the keto group is substituted at sixth carbon instead of –OH group and double bond between seventh and eighth carbon is reduced, the analgesic activity will be increased to sixfold (e.g., morphine versus hydromorphone).

13.8.2 SAR of Fentanyl Derivatives (Flexible Opioids)

- Fentanyl derivative drugs are chemically called as “anilino-piperidines.” They are also called “flexible opioids” along with meperidine. The common pharmacophore of the flexible opioids is shown in Fig. 13.7.
- N-methyl or N-aralkyl addition in the R1 will always boost the μ receptor agonism.
- Aralkyl substitution at the R1 also promotes the rapid CNS penetration (e.g., sufentanil).

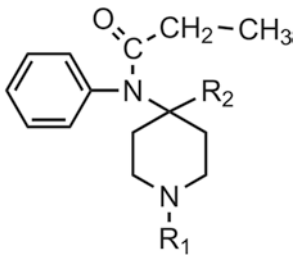


Fig. 13.7 The pharmacophore (anilino-piperidine) of flexible opioids

- Presence of ester moiety at R2 also promotes the CNS penetration by increasing the lipophilicity and produces high affinity binding at the μ receptor (e.g., alfentanil and sufentanil).

13.8.3 SAR of Endogenous Opioid Peptides

- The first five amino acid residues of almost all of the endogenous opioid peptides are either leu-enkephalin or met-enkephalin. Enkephalin is a combination of tyrosine–glycine-glycine-phenylalanine.
- The first amino acid residue is tyrosine, and the phenolic hydroxyl group of the tyrosine linked to the nitrogen is essential for the opioid receptor binding activity.
- In addition to this, the phenyl group of the phenylalanine residue also plays equal role in opioid receptor binding.
- Conversion of carboxy group present in the rear end of the amino acid chain into –OH or amide will create peptides that are resistant to carboxy peptidase metabolism.
- The peptide can be made resistant to peptidase by introduction of bulky groups in the fourth amino acid (i.e., phenylalanine).
- The glycine residue in the second place can be replaced with D-alanine in order to achieve resistance to peptidases.
- The affinity for the κ receptors can be achieved by incorporation of basic amino acid (arginine) into C terminus chain (e.g., dynorphins).

13.9 SAR of H_1 Antihistamines

- The basic pharmacophore structure of the H_1 receptor antagonists can be represented as two aromatic groups (Ar1 & Ar2) separated from a tertiary aliphatic amine by a short chain (X or spacer) of nitrogen or carbon or oxygen.
- The spacer is a nitrogen atom in the ethylenediamine series (e.g., phenbenzamine).
- In the case of ethanolamine series, the spacer will be a carbon atom linked to the oxygen molecule and then to the tertiary amine. This structure possesses additional antimuscarinic activity (e.g., diphenhydramine and doxylamine).
- When the alkyl group substituted in the two carbon of the spacer has higher size, then the antihistaminic activity is decreased dramatically, and anticholinergic activity is increased.
- When the heteroatom (N or O) in the spacer is replaced with carbon itself, then another series of compounds are produced called as *alklyamines*. These compounds, in general, possess long duration of action with moderate CNS penetration (e.g., pheniramine and chlorpheneramine).

- A closely related molecular entity called *piperazines* also possesses significant antihistaminic action due to the presence of aryl groups separated from tertiary amine with a spacer (e.g., cyclizine, hydroxyzine, cetirizine, and buclizine).
- When the two aromatic groups present in the antihistaminics are connected to each other with heteroatoms like N or S, then it produces another series of compounds called *tricyclic antihistaminics* (e.g., promethazine and cyproheptadine).
- Addition of bulkier and long-chain molecules to the alkyl group in the tertiary amine produces antihistaminics with less lipophilicity and less BBB entry called *second-generation antihistaminics* (e.g., loratadine and fexofenadine).

13.10 SAR of Antipsychotic Drugs

- The parent molecule in the antipsychotic drug class "chlorpromazine" was derived from the antihistaminic drug promethazine after several modifications. Later many molecules were derived from chlorpromazine, and the phenothiazine class of drugs got evolved.
- Presence of halogen (chlorine) atom in the tricyclic ring is responsible for creating asymmetry in the molecule and exerts antipsychotic activity.
- Apart from this, another important requirement is the presence of three carbon separation between the two nitrogen atoms in the side chain. Presence of only two carbon atoms between the two nitrogen atoms results in lack of antipsychotic activity (e.g., chlorpromazine versus promethazine).
- Esters like enanthate and decanoate could be added to tricyclic ring in phenothiazine group and may result in long-acting antipsychotic drugs (e.g., fluphenazine enanthate).
- Butyrophenones, a derivative of meperidine molecule, show neuroleptic activity when a tertiary amino group is attached to the fourth carbon of the butyrophenone skeleton. The neuroleptic potency is reduced when the three carbon propyl chain is lengthened, branched, or shortened.
- Replacement of haloperidol butyrophenone side chain with keto function moiety results in production of diphenylbutyl piperidine neuroleptics. These compounds possess longer duration of action (e.g., pimozide and penfluridol).

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Part III

Special Topics in Pharmacology

Drug Information

14

Nishanthi Anandabaskar

Abstract

Drug information refers to "current, critically examined, relevant data about drugs and drug use in a given patient in a particular situation". Access to authentic information about drugs is an essential prerequisite for rational drug use. The various sources of drug information can be classified into primary, secondary and tertiary. Primary sources of drug information include unpublished studies, original articles published in reputed peer-reviewed journals reporting original research, ideas or opinions. Secondary sources of drug information refer to indexing and abstracting systems that organize and provide easy retrieval of primary resources. Tertiary sources of drug information summarize data from the primary literature and they include reference books, drug compendia, essential drugs list, treatment guidelines, drug formularies, drug bulletins and pharmacopoeias. Commercial sources of drug information refer to drug information from pharmaceutical companies or drug manufacturers with the main aim of promoting their drug and increasing the sales of their product. Computerized or electronic information systems and verbal information are the other sources of drug information. Drug information centres (DICs) also provide drug information (verbal and/or written) on request from the practicing physicians, pharmacists and other healthcare professionals, patients or the general public.

Keywords

Abstracting systems · Drug information centres · Electronic information

14.1 Introduction

- Drug information refers to "current, critically examined, relevant data about drugs and drug use in a given patient in a particular situation".

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- Access to drug information is an essential prerequisite for the rational use of drugs.
- The drug information is available verbally or in printed or electronic forms.

14.2 Sources of Drug Information

The various sources of drug information can be classified into primary, secondary and tertiary.

14.2.1 Primary Sources

- Primary sources of drug information include unpublished studies and original articles published in reputed peer-reviewed journals reporting original research, ideas or opinions.
- They form the basis for all other sources of information.
- They provide the most up-to-date information on that particular topic.
- However, the evaluation and interpretation of research articles is difficult and requires time and expertise.
- Well-conducted randomized controlled trials (RCTs) provide the most reliable source of information.
- Examples of primary literature sources are provided in Box 14.1.

14.2.2 Secondary Sources

- Secondary sources of drug information refer to indexing and abstracting systems that organize and provide easy retrieval of primary resources.
- They also include review articles and meta-analyses.
- Indexing systems include the article citation, which may or may not provide access to the abstract or the link to full text of the article.
- Abstracting systems provide both citation and abstract of the article. Many of the abstracting systems provide a link to the full-text article.

Box 14.1: Examples of Primary Literature Sources
Peer-reviewed journals that publish original articles, for example:

- *Annals of Internal Medicine*
- *British Medical Journal*
- *Journal of the American Medical Association*
- *The Lancet*
- *The New England Journal of Medicine*
- *Indian Journal of Pharmacology*
- *Journal of Pharmacology and Pharmacotherapeutics*

Box 14.2: Examples of Secondary Literature Sources

- Peer-reviewed journals that publish review articles like Journal Watch
- Electronic databases that can be used to search for primary literature and provide abstracts, for example:
  - Index Medicus
  - Medline (through PubMed, EBSCO, Ovid)
  - EMBASE
  - Micromedex CD-ROM
  - International Pharmaceutical Abstracts (IPA)
- Electronic databases that provide evidence-based evaluations, for example, Cochrane Library abstracts (which are free) and evaluations

- However, proper training is required for efficient use of these resources.
- Examples of secondary literature sources are provided in Box 14.2.

14.2.3 Tertiary Sources

Tertiary sources of drug information summarize data from the primary literature, and they include the following sources (Box 14.3):

- **Reference Books**
  - Reference books cover topics on general or clinical pharmacology or specialize in a particular field.
  - Some examples of standard reference books are enlisted below:
    Pharmacology textbooks like *Goodman and Gilman's The Pharmacological Basis of Therapeutics* and *Laurence and Bennett's Clinical Pharmacology*
    Martindale: The Complete Drug Reference
    Avery's Drug Treatment
    Meyler's Side Effects of Drugs
  - Choose the latest edition of the books for reference.
  - Textbooks need to be revised every 2–5 years to provide up-to-date drug information.
- **Drug Compendia**
  - Drug compendia are a source of drug information listing the drugs available in the market published by some countries.
  - The information contained in the drug compendia includes the following details about the drugs available in the market:
    Generic and brand names
    Chemical composition
    Therapeutic indications
    Side effects, contraindications and warnings
    Precautions and interactions
    Administration and dosage recommendations

Box 14.3: Examples of Tertiary Literature Sources

1. **Reference Books**
   - *Goodman and Gilman's The Pharmacological Basis of Therapeutics*
   - *Laurence and Bennett's Clinical Pharmacology*
   - *Martindale: The Complete Drug Reference*
   - *Avery's Drug Treatment*
   - *Meyler's Side Effects of Drugs*
2. **Drug Compendia**
   - Annual Physician's Desk Reference
   - Monthly Index of Medical Specialties
3. **National List or WHO Model List of Essential Drugs**
   - 20th WHO Essential Medicines List (EML), 2017
   - 6th WHO Essential Medicines List for Children (EMLc), 2017
   - National List of Essential Medicines (NLEM) 2015, India
   - Indian Academy of Paediatrics Essential Medicines List for children of India (IAP EMLc), 2011
4. **National, International, Institutional or WHO Treatment Guidelines**
   - Joint National Committee (JNC 8) guidelines for management of hypertension.
   - American Diabetes Association (ADA) standards of medical care in diabetes, 2018 guidelines.
   - The 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure (by the American College of Cardiology/American Heart Association and the Heart Failure Society of America).
   - The 2018 National technical guidelines on antiretroviral therapy by the National AIDS Control Organization (NACO), Government of India.
5. **Drug Formularies – National or Hospital Formulary**
   - *WHO Model Formulary*
   - *British National Formulary (BNF)*
   - *National Formulary of India*
6. **Drug Bulletins**
   - Drug and Therapeutics Bulletin (UK)
   - Medical Letter (USA)
   - Australian Prescriber (Australia)
7. **Pharmacopoeias**
   - British Pharmacopoeia
   - European Pharmacopoeia
   - United States Pharmacopeia
   - Martindale's The Extra Pharmacopoeia
   - Indian Pharmacopoeia

  - The information contained in the official drug label of the product and approved by the national regulatory authority is used for the preparation of drug compendia.
  - Examples of drug compendia are:
    Annual *Physician's Desk Reference* (provided free of cost to physicians in the United States)
    Monthly Index of Medical Specialties (commercially sponsored drug compendia)
- **National List or WHO Model List of Essential Drugs**
  - Essential medicines are defined by the WHO as the medicines that "satisfy the priority healthcare needs of the population".
  - Every country has a national list of essential drugs which is formulated based on their local needs. Nevertheless, if a country lacks the national list of essential drugs, the WHO model list can be referred to.
  - The drugs are listed with reference to the levels of healthcare, namely, primary (P), secondary (S) and tertiary (T).
  - It consists of a core list and a complementary list of essential drugs.
  - The core list consists of a list of minimum medicines needed for a basic healthcare system. It contains the most efficacious, safe and cost-effective medicines for priority health conditions.
  - The complementary list includes essential medicines for priority diseases which require specialized diagnostic or monitoring facilities and/or specialist medical care and/or specialist training.
  - Also, there is a separate WHO Model List of Essential Medicines for Children. The WHO essential medicines list is updated every 2 years.
  - The current versions (updated in March 2017) of the WHO list of essential medicines are as follows:
    20th WHO Essential Medicines List (EML)
    6th WHO Essential Medicines List for Children (EMLc)
  - The current versions of the national list of essential medicines in India are as follows:
    National List of Essential Medicines (NLEM) 2015, India
    Indian Academy of Paediatrics Essential Medicines List for children of India (IAP EMLc), 2011
- **National, International, Institutional or WHO Treatment Guidelines**
  - The treatment guidelines include the most essential therapeutic information for the prescriber like the drug(s) of choice, recommended dosage schedule, adverse effects, contraindications, alternative drugs and others.
  - The treatment guidelines for various diseases can be published by healthcare institutions, national and international organizations or professional societies.
  - World Health Organization has also published a multitude of treatment guidelines for various diseases.
  - Some examples of treatment guidelines are:

Joint National Committee (JNC 8) guidelines for the management of hypertension.

American Diabetes Association (ADA) standards of medical care in diabetes, 2018 guidelines.

The 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure (by the American College of Cardiology/American Heart Association and the Heart Failure Society of America).

The 2018 National technical guidelines on antiretroviral therapy by the National AIDS Control Organization (NACO), Government of India.

- **Drug Formularies – National or Hospital Formulary**
  - Drug formularies contain the list of approved and available drugs in a country, region, district or hospital.
  - Thus, a drug formulary can be national, regional or institutional.
  - Some of the examples of a formulary are:

    WHO model formulary – could be used by countries to develop their own national formulary.

    British National Formulary (BNF) – free to UK prescribers, includes information on cost, which is not often included in other compendia.

    National Formulary of India (recent edition was released in 2016) – prepared by the Indian Pharmacopoeia Commission for use by medical practitioners in India.
- **Drug Bulletins**
  - Drug bulletins provide information on the relative merits and demerits of new drugs.
  - They are published every week or quarterly. Thus, they aid in updating the knowledge of prescribers and promoting rational drug therapy.
  - The various sponsors of drug bulletins are – government bodies, professional agencies, pharmaceutical companies, university departments, philanthropic foundations and consumer organizations.
  - Nonindustry sponsored bulletins – provide unbiased evaluation of drugs and practical recommendations in comparison with other treatment alternatives.
  - Examples of drug bulletins are as follows:

    Drug and Therapeutics Bulletin (UK)

    Medical Letter (USA)

    Australian Prescriber (Australia)
- **Pharmacopoeias**
  - Pharmacopoeia is a legally binding book which contains standards and quality specifications for medicines used in that country or region.
  - It contains the following list of appropriate tests:

    To confirm the identity and purity of the pharmaceutical product

    To ascertain the strength (or amount) of the active substance

    To assess its performance characteristic
  - It also enumerates various tests for assessing the quality of medicines like analytical methods, microbiological purity, dissolution testing and stability.

- It is prepared by a national or regional authority and updated periodically.
- International Pharmacopoeia issued by the World Health Organization provides international standards for assessment of the quality of pharmaceutical products, excipients and dosage forms. It is used as a reference document by the member states to develop their own pharmacopoeias.
- Some examples of pharmacopoeias are as follows:
  British Pharmacopoeia – issued by the United Kingdom
  European Pharmacopoeia – issued by the European Union
  United States Pharmacopeia – issued by the United States
  Indian Pharmacopoeia – issued by Indian Pharmacopoeia Commission (IPC), the standard-setting institution for drugs in India. The IPC is an autonomous body under the Ministry of Health and Family Welfare. Adherence to the drug standards as per the Indian Pharmacopoeia is mandated by its inclusion in the Drugs and Cosmetics Act, 1940, and Drugs and Cosmetics Rules, 1945.

14.3 Commercial Sources of Drug Information

- It refers to drug information from pharmaceutical companies or drug manufacturers.
- The information is provided through verbal, written and electronic sources.
- Majority of the physicians are exposed to advertisements and promotional materials of pharmaceutical companies by the following sources:
  - Medical representatives
  - Talks in professional meetings (by sponsoring scientific conferences and symposia)
  - Directly mailing the physicians
- The main aim of this commercial drug information is to promote their drug and increase the sales of their product.
- Thus, they tend to highlight the positive aspects of the drug and overlook the negative aspects.
- The information might not be authentic and could mislead the physicians leading to irrational prescribing practices.
- Physicians can use the following methods to verify the authenticity of information from the medical representatives or drug advertisements:
  - Comparing the drug information with other validated sources like registered drug information sheet, drug label, package insert of the drug, drug bulletins or therapeutic reviews
  - Concentrating on the side effects, contraindications and cost of the drugs
  - Searching the published literature for proof of the safety and efficacy of the drugs (the quality of the research can be evaluated from the quality of the journal publishing the research)
- Stringent regulations are required to control unethical drug promotion practices by the pharmaceutical industry.

- WHO has issued global guidelines for drug promotional activities by publishing the document on "Ethical Criteria for Medicinal Drug Promotion".

14.4 Electronic or Computerized Information

- Computerized drug information systems that record and maintain the information of patients are being developed.
- They have a formulary for each diagnosis which the physician can refer and choose from the list of indicated drugs including dosage schedule and quantity.
- They also alert about contraindications for drugs and drug interactions.
- The physicians can upload their own formulary in the system, but it requires frequent updating.
- Recently, mobile phone-based applications of drug information have been made available for subscription at low cost like the following applications:
  - Drug Essentials
  - Epocrates
  - Medscape
- If the World Wide Web is used for searching drug information, authentic and reliable sites should be referred. Some examples are as follows:
  - http://www.fda.gov/ – FDA
  - http://www.cdsco.nic.in/ – CDSCO
  - http://www.ncbi.nlm.nih.gov/pubmed – PubMed
  - http://www.uptodate.com/ – UpToDate
  - http://www.clinicalkey.com – ClinicalKey

14.5 Verbal Drug Information

- They are obtained from specialists (a clinical pharmacologist and/or a clinical pharmacist), professional colleagues, pharmacists or pharmacologists.
- They can be obtained by informal talks, meetings or through structured training programmes conducted by the specialists.
- The Drugs and Therapeutic Committee consisting of diverse specialists including clinical pharmacologists and clinical pharmacists, meet regularly to deliberate about various aspects of drug treatment, which are also sources of verbal information.

14.6 Drugs and Therapeutic Committees

- Drugs and Therapeutic Committees (DTCs) are a forum to bring together all stakeholders associated in decision-making about drug use to promote efficient and rational use of medicines.

- The various members of a DTC are as follows:
  - Clinicians from each major specialty
  - A clinical pharmacologist
  - A nurse
  - A pharmacist
  - An administrator
  - A clinical microbiologist
  - A member of the hospital records department
  - A dedicated and committed chairperson and secretary
- The DTCs may exist at any of the following levels of the healthcare system:
  - At district level (overseeing primary healthcare facilities)
  - In hospitals
  - At the national level
- Goal of DTCs
  - To provide the best possible cost-effective and quality of care to the patients by determining the availability, cost and usage of medicines
- Objectives of DTCs are:
  - To develop and implement an efficient and cost-effective formulary system which includes standard treatment protocols, a formulary list and formulary manual
  - To ensure the use of only efficacious, safe, cost-effective and good quality medicines
  - To ensure the best possible drug safety by monitoring, evaluating and preventing adverse drug reactions and medication errors
  - To develop and implement interventions to improve medicine use by prescribers, dispensers and patients

    To accomplish this objective, investigation and monitoring of medicine use is essential.
- Functions of DTCs are as follows:
  - Advisory committee provides advice to medical staff, nurses, administration, pharmacy and other departments and groups within the hospital, on all issues, policies and guidelines concerning the selection, distribution and use of medicines.
  - Development of drug policies.
  - Evaluating and selecting medicines for the formulary list.
  - Developing standard treatment guidelines.
  - Assessing medicine use to identify problems.
  - Conducting effective interventions to improve medicine use.
  - Managing adverse drug reactions.
  - Managing medication errors.
  - Information dissemination and transparency.

14.7 Drug Information Centre

- Drug information centres (DICs) provide "authentic, individualized, accurate, relevant and unbiased drug information" (Fig. 14.1).
- The drug information (verbal and/or written) is provided on request from the practicing physicians, pharmacists and other healthcare professionals, patients or general public.
- The activities of drug information services are usually undertaken by pharmacologists, clinical pharmacologists and pharmacists.
- The main aim of a DIC is to promote rational use of drugs.
- The DICs are usually established in hospitals and teaching institutions, and they are often linked to the Poison Information Centres. These centres are affiliated with the Department of Pharmacology, Clinical Pharmacology or Clinical Pharmacy.
- Requirements for the establishment of a DIC:
  - Organization and space
  - Resources for drug information – primary, secondary and tertiary sources
  - Adequate staff
  - Policies and procedures
  - Budget

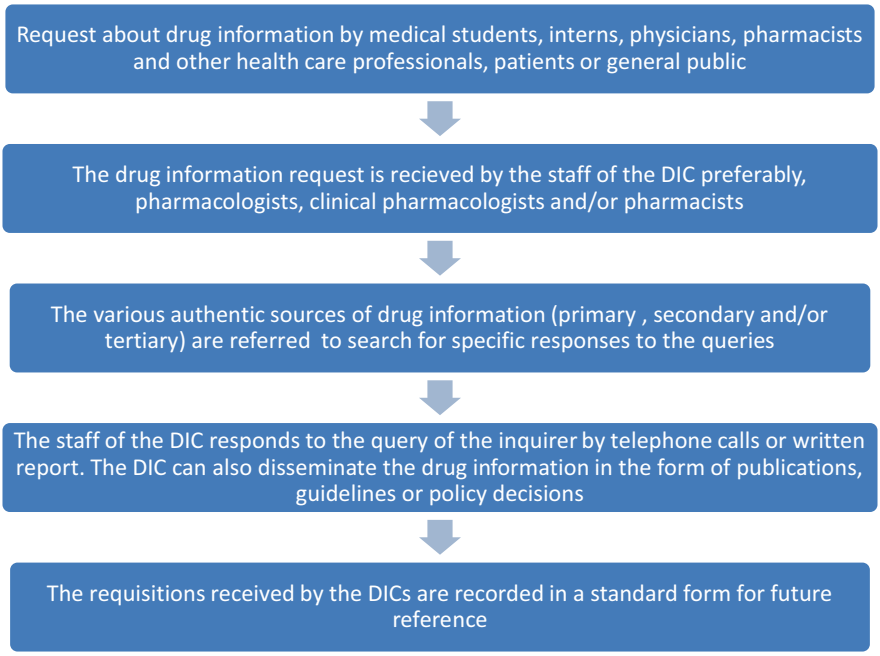

Fig. 14.1 Flow chart explaining the processes involved in the functioning of a drug information centre

- In 1962, the first DIC was opened at the University of Kentucky Medical Center, USA.
- In India, the first independent DIC was established by the Karnataka State Pharmacy Council at Bangalore, in 1997, and since then many DICs have been established all over India.
- DIC services are rendered in two types of approaches – reactive approach and proactive approach:
  - Reactive approach – the DIC answers to the queries of healthcare providers and the public regarding the safe and effective use of therapeutic and diagnostic pharmaceuticals. The nature of the information sought from DIC is usually as follows:
    Therapeutic uses or indications of drugs
    Adverse drug reactions and contraindications of drugs
    Drug use in pregnancy or lactation
    Drug-drug and drug-food interactions
    Pharmacokinetics or dosage of drugs
    Availability and cost of drugs
    Availability of generic drugs
    Drug profile
    Drug poisoning
  - Proactive approach – the DIC publishes and circulates regular updates on various topics as follows:
    Dosing guidance in organ impairment
    Interpretation of therapeutic drug monitoring (TDM) levels
    Possible drug-drug or drug-disease interactions
    Safety profile including the Food and Drug Administration (FDA) alert
    Adverse event linked to a drug
    Efficacy comparison
    Recent updates in treatment guidelines
    New drug approvals and local availability
    Drug use in any special situation
    Important study findings in reputed journals
- Unlike the hospital-based DICs which cater primarily to the healthcare personnel, some countries have community-based DIC which provides the following services:
  - Provide patient counselling regarding drug use
  - Conduct public awareness lectures
  - Publish articles in newspapers
  - Answer queries on phone except commenting on prescription
- The requisitions received by the DIC are recorded in a standard form which includes the following information:
  - Details of the inquirer
  - Questions asked and its urgency
  - Patient details relevant to the question
  - The time and mode of response

- Response provided
- Reference materials used for preparing the response
- Signature and name of the staff providing the response

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Pharmacogenetics, Pharmacogenomics, and Personalized Medicine

15

Gerard Marshall Raj

Abstract

The genetic heterogeneity existing in individuals can be responsible for the differential therapeutic and/or adverse outcomes to drugs observed across different patient groups. Though the terms pharmacogenetics and pharmacogenomics are often used interchangeably, there is a thin-yet-tough line of demarcation. *Pharmacogenetics* refers to how variations in a single gene (monogenetic variants) affect the response to a drug. Whereas, *pharmacogenomics* is a broader "multifactorial" term, which studies how the entire spectrum of genes (a larger number of variants or the whole genome) can influence drug response pertaining to both efficacy and safety. The science about who will benefit from a particular drug and who will not is referred to as *personalized medicine* – which deals about the clinical application of pharmacogenetics and pharmacogenomics. Owing to the misinterpretation that was surrounding the usage of the term personalized medicine, an alternative term precision medicine came into vogue. Treatment and preventive approaches that would be effective for patients based on their genetic, environmental, and lifestyle factors were put under the umbrella term of *precision medicine*. Nearly 90% of the human genetic polymorphisms are single-nucleotide polymorphisms (SNPs). These SNPs can occur in the coding or noncoding regions; the coding SNPs can be either synonymous (no change in the amino acid) or nonsynonymous (change in the amino acid). Pharmacogenetics can be involved at the level of both pharmacokinetics (variation in the drug metabolizing enzymes or transporters) and pharmacodynamics (variation in the drug targets). The pharmacogenetic studies range from the specific candidate gene approach, through haplotype analysis and to the broader genome-wide association studies (GWAS). The application of pharmacogenomics in real patient-life settings is supported by organizations like the Clinical Pharmacogenetics Implementation Consortium (CPIC) and open-source databases like the Pharmacogenomics Knowledgebase (PharmGKB).

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Pharmacogenetics · Pharmacogenomics · Single-nucleotide polymorphism (SNP) · Genetic variation · Precision medicine

15.1 Introduction

The interindividual differences in both beneficial and adverse response to drugs can be partially explained by the genetic variations. For example, out of two phenotypically similar patients started on the same drug, one may not respond optimally, whereas the other may display exaggerated response. On similar lines, a particular individual may end up in fatal adverse drug reactions, whereas another individual may tolerate the drug without even mild adverse effects (Fig. 15.1).

15.1.1 Pharmacogenetics: Definition

- Pharmacogenetics is the study of the genetic basis for variation in drug response and often implies large effects of a small number of DNA variants.
- Pharmacogenetics is the study of the genetic variations that affect the response to drugs both therapeutically and in terms of adverse effects.
- Pharmacogenetics applies genetic information to select a drug, its dose, and duration of treatment that will have the highest probability for achieving optimal therapeutic outcomes with the least potential for harm in a given patient.
- The term pharmacogenetics was coined by Friedrich Vogel (Heidelberg, Germany) in 1959. However, the concept of genetic factors underlying variable

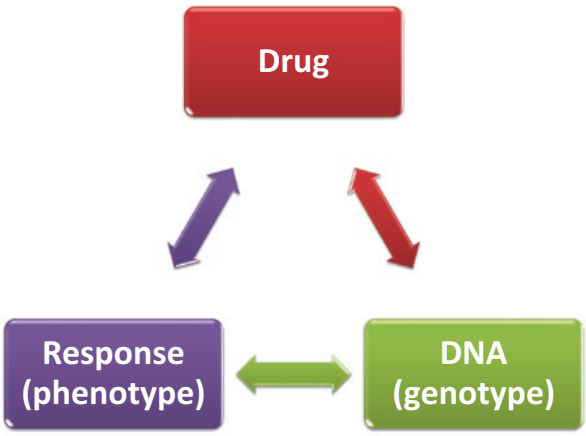

Fig. 15.1 Genotype-drug-phentotype interactions

response to drugs dates back to 1931 with Sir Archibald Garrod. Werner Kalow (Toronto, Canada) wrote the first textbook in the science of pharmacogenetics titled *Pharmacogenetics: Heredity and the Response to Drugs* in 1962; he is sometimes referred to as the "father of pharmacogenetics."

15.1.2 Pharmacogenomics: Definition

- Unlike pharmacogenetics, pharmacogenomics includes studies on larger numbers of variants, in an individual or across a population, to explain the genetic component of variable drug responses.
- Pharmacogenomics is the study of how a person's response to drugs is influenced by the genes. It is a conglomeration of two fields, namely, "pharmacology" *(the science of drugs)* and "genomics" *(the study of genes and their functions)* to develop effective and safe drugs based on the genetic makeup.
- In general, pharmacogenetics usually refers to how variations in one single gene (monogenetic variants) affect the response to a drug. On the other hand, pharmacogenomics is a broader "multifactorial" term, which studies how the entire spectrum of genes (the genome) can influence drug response with respect to its efficacy and safety.
- For example, if a study entails investigating the effect of the β_2-adrenergic receptor gene *(ADRB2)* on bronchodilation to salbutamol, it becomes a pharmacogenetic study, whereas in a pharmacogenomic study along with the *ADRB2* gene, other genes like the corticotropin-releasing hormone gene *(CRHR2)*, adenylyl cyclase gene *(ADCY9)*, and arginase genes *(ARG1* and *ARG2)* on salbutamol response and also the interactions between the genes are explored. As it is evident that multiple proteins play a decisive part in determining the final response to most drugs, currently the investigators are taking a more pharmacogenomic approach for elucidating genetic contributions to drug response.
- As per the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) – E15 Efficacy Guidelines:
  - Pharmacogenomics (PGx) is defined as "the study of variations of DNA and RNA characteristics as related to drug response."
  - Pharmacogenetics (PGt) is regarded as a subset of PGx and is defined as "the study of variations in DNA sequence as related to drug response."

15.1.3 Personalized Medicine: Definition

- *Personalized medicine* is the application of pharmacogenetics to understand how an individual can benefit from specific drugs; hence, the dictum "one size fits all" does not stand always true. Sometimes, also called as *stratified medicine*, it encompasses biomarker science and pharmacogenomics.

- Hence, personalized medicine appraises about who will benefit from a particular drug and who will not, and sometimes, even a certain group of patients may even be very intolerable to the same drug.
- Along with drugs, including biologicals, the domain of personalized medicine also includes diagnostic tests and the interlinkage between therapeutics and diagnostics.
- The interest in measuring the genetic determinants of drug response was rekindled with the advent of the Human Genome Project (www.ncbi.nlm.nih.gov) in the 1990s; the International HapMap Project (www.hapmap.org) and the 1000 Genomes Project (www.1000genomes.org) followed suit in catapulting personalized medicine to new arenas.
- The term personalized medicine can at times be misconstrued as the treatment and preventive modalities are developed for every individual uniquely. Therefore, the National Research Council (USA) had introduced the alternative term *precision medicine* – defined as the approaches which will be effective for patients based on genetic, environmental, and lifestyle factors.
- Precision medicine is defined by the Precision Medicine Initiative (PMI) as "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment and lifestyle for each person."
- In precision medicine, the prime motive is to cautiously consider the disparities between the individuals rather than the development of treatment policies for the so-called average person.
- The PMI, as a part of the National Institutes of Health (NIH), was proposed (President Barack Obama, January 2015) to accumulate robust scientific evidence so as to apply the concept of precision medicine in clinical practice. The program is now rechristened as the *All of Us* Research Program (a landmark longitudinal research study) – with the objective to collect data from more than one million volunteers living in the USA to accelerate research and improve health, thereby supporting the prevention and treatment of diseases. The researchers engaged shall aid in showing the path toward delivering precision medicine by considering individual differences in lifestyle, environment, and biology through this research resource platform.
- P4 medicine – standing for *predictive, personalized, preventive, and participatory medicine* is another alternative term ascribed.

15.2 Forms of Genetic Variations

15.2.1 Mutation

- Mutation is a *change* in the nucleotide sequence of a short region of the genome or DNA molecule. The term "variation" is preferred more nowadays.
- Mutations can be single point mutations wherein one nucleotide is replaced with another, also known as substitution mutation. Sometimes, insertion or deletion mutations also occur.

- Exogenous adverse exposure to mutagens or endogenous errors in DNA replication can result in mutation.
- Mutations occur in less than 1% of the populations.

15.2.2 Single-Nucleotide Polymorphisms (SNP)

- Polymorphisms are variations in the genetic sequence that occur in many individuals at a frequency of at least 1% (i.e., 1% or more). Hence, polymorphisms begin as mutations and as they become fixed in a given population with an adequate frequency, they are renamed as polymorphisms. These polymorphisms can be *cosmopolitan* (present in all ethnic groups) or *population-specific* (present only in a particular race, ethnicity, or ancestry).
- The simplest and commonest form of genetic polymorphisms in the human genome are *single-nucleotide polymorphisms* (SNPs) – pronounced as "snips." They are also referred to as *single-nucleotide variants* (SNVs). Almost 90% of the human genetic polymorphisms are SNPs.
- SNP refers to a single nitrogenous base mutation in DNA. There are two forms of nitrogenous base substitutions resulting in SNPs, as follows:
  - Transition
    Substitution between purines (A, G) or between pyrimidines (C, T), i.e., a purine is replaced with another purine or a pyrimidine is replaced with another pyrimidine, where A, G, C, and T stand for adenine, guanine, cytosine, and thymidine, respectively.
  - Transversion
    Substitution of a purine with a pyrimidine or vice versa.
- SNPs tend to occur at every 300 base pairs (i.e., ≈ 1 variant per 300 base pairs) along the 3 billion base pairs of the human genome; hence, roughly around 20 million SNPs. Functionally, SNPs can be classified as follows:
  - Coding SNPs (cSNPs):
    Occurs in the exonic region of the genome which codes for the polypeptide chain.
    Synonymous SNP (Silent SNP) – a single base change which does not result in the change of amino acid. For example, a SNP from CC**G** to CC**A** (G → A) may result in the generation of the same amino acid, namely, proline (as both the triplet codons, CCG and CCA, code for the same amino acid).
    Non-synonymous SNP – a single base change which results in the change of amino acid. For example, a SNP from C**C**G to C**A**G (C → A) may result in the generation of a different amino acid, namely, glutamine [CAG] rather than proline [CCG].
  - Noncoding SNPs can be any of the following:
    5'UTR or 3'UTR regulatory SNPs (rSNPs) – occur in the noncoding regulatory regions like the promoter or enhancer regions (UTR – untranslated region).
    Splice site SNPs (ssSNPs) – occur at the junction of exons and introns.

Intronic SNPs (iSNPs) – occur in the noncoding intronic region between the exons. Sometimes, called as random SNPs.

Anonymous SNPs (aSNPs) – occur in "junk" DNA.

- Apart from the SNPs, there are other categories of polymorphisms like insertion or deletion of multiple sequential nucleotides (called as *indels* or *insertion-deletion polymorphisms*); variable numbers of repeats, such as doublets or triplets *(tandem repeats)*; large-scale duplications or deletions in entire copies of genes or gene segments >1 kb in size (called as *copy number variations*, CNVs); *frameshift mutation*; *defective splicing*; *aberrant splice site;* and *premature stop codon polymorphisms*.

15.3 Pharmacogenetics on Drug Disposition and Therapeutic Response

- The in-depth understanding of pharmacokinetics and pharmacodynamics of particular drug is important in delineating those set of populations who respond *(responders)* and others who do not respond *(nonresponders)*.
- Any measurable or discernible genetic trait associated with a drug is referred to as a *pharmacogenetic trait*. Some traits are beneficial like a reduction in tumor size, and some are of adverse nature like an accentuated lowering of blood pressure.
- Majority of the drug response phenotypes are determined by the rare nonsense variants or missense variants in the noncoding regions.
- Table 15.1 summarizes the drugs with clinically significant pharmacogenetic effects.

15.3.1 Pharmacogenetics and Drug Metabolism

- Polymorphisms can occur in the genes coding for the drug-metabolizing enzymes like the cytochrome P450 (CYP) enzymes and other phase II enzymes including uridine diphosphate glucuronosyltransferase (UGT), thiopurine methyltransferase (TPMT), and dihydropyrimidine dehydrogenase (DPYD).
- Out of 57 CYP isoenzymes isolated in humans so far, 42 are found to be involved in the metabolism of exogenous xenobiotics, and 15 of these isoenzymes are associated with the metabolism of drugs. Clinically relevant CYP enzyme-related polymorphisms occur in the *CYP2D6*, *CYP2C19*, *CYP2C9*, *CYP2A6*, *CYP2B6*, and *CYP3A4/5* genes.
- Enzyme metabolizer capacities and potential effects are as follows:
  - Poor Metabolizers (PM)

    Lack of active enzyme gene alleles and, hence, individuals may suffer more adverse effects at usual doses. An *allele* is a sequence of nucleic acid bases at a given gene (chromosomal) locus; at each gene locus, there are two alleles – one from each parent (maternal and paternal).

Table 15.1 Drugs with clinically significant pharmacogenetic effects

S. No.	Drug	Gene	Phenotypic outcome	Recommendations
1.	Abacavir	*HLA-B*	*HLA-B*57:01* positive: Hypersensitivity reactions (severe, serious, and sometimes fatal)	*HLA-B*57:01* testing should be done before initiating treatment, and use of abacavir is contraindicated in *HLA-B*57:01* carriers
2.	Allopurinol	*HLA-B*	*HLA-B*58:01* positive: Severe cutaneous adverse reactions like Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)	*HLA-B*58:01* testing should be done before initiating treatment, and use of allopurinol is contraindicated in *HLA-B*58:01* carriers
3.	Amitriptyline	*CYP2D6*	Ultrarapid metabolizer: Increased probability of pharmacotherapy failure	Avoid use of amitriptyline due to potential lack of efficacy, and consider alternative drug not metabolized by CYP2D6
			Poor metabolizer: Increased probability of side effects	Avoid use of amitriptyline due to potential for side effects, and consider alternative drug not metabolized by CYP2D6
		CYP2C19	Ultrarapid and rapid metabolizer: Increased metabolism of amitriptyline to secondary amines and, hence, may affect response or side effects	Avoid amitriptyline use due to potential for sub-optimal response, and consider alternative drug not metabolized by CYP2C19. Consider using secondary amines like nortriptyline and desipramine
			Poor metabolizer: Decreased metabolism of amitriptyline to secondary amines and, hence, may affect response or side effects	Avoid amitriptyline use due to potential for suboptimal response, and consider alternative drug not metabolized by CYP2C19. Consider using secondary amines like nortriptyline and desipramine
4.	Atazanavir	*UGT1A1*	Poor metabolizer: Markedly decreased UGT1A1 activity and, hence, high likelihood of bilirubin-related discontinuation of atazanavir (as atazanavir inhibits UGT1A1)	Consider an alternative agent particularly where jaundice would be of concern to the patient

(continued)

Table 15.1 (continued)

S. No.	Drug	Gene	Phenotypic outcome	Recommendations
5.	Azathioprine	*TPMT*	Poor metabolizer: Extremely high concentrations of thioguanine metabolites; fatal toxicity possible without dose decrease. Greatly increased risk of thiopurine-related leukopenia, neutropenia, and myelosuppression	Consider alternative nonthiopurine immunosuppressant therapy for nonmalignant conditions. For malignancy, reduce both dose (10 times) and frequency (thrice weekly instead of daily) and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines
6.	Capecitabine	*DPYD*	Intermediate metabolizer: Decreased DPD activity (leukocyte DPD activity at 30–70% that of the normal population) and increased risk for severe or even fatal drug toxicity when treated with capecitabine like mucositis, diarrhea, neutropenia, and neurotoxicity	Reduce starting dose based on activity score followed by titration of dose based on toxicity or therapeutic drug monitoring
			Poor metabolizer: Complete DPD deficiency and increased risk for severe or even fatal drug toxicity when treated with capecitabine like mucositis, diarrhea, neutropenia, and neurotoxicity	Avoid use of capecitabine-based regimens
7.	Carbamazepine	*HLA-B*	*HLA-B*15:02* positive: Severe cutaneous adverse reactions like Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)	*HLA-B*15:02* testing should be done before initiating treatment, and use of carbamazepine is contraindicated in *HLA-B*15:02* carriers
8.	Citalopram	*CYP2C19*	Ultrarapid metabolizer: Increased metabolism and hence may result in lower plasma concentrations with increased probability of pharmacotherapy failure	Consider an alternative drug not predominantly metabolized by CYP2C19
			Poor metabolizer: Greatly reduced metabolism and, hence, may result in higher plasma concentrations with increased probability of side effects like QT prolongation	Consider a 50% reduction of recommended starting dose and titrate to response or select alternative drug not predominantly metabolized by CYP2C19
9.	Clomipramine	*CYP2D6*	Ultrarapid metabolizer: Increased probability of pharmacotherapy failure	Avoid use of clomipramine due to potential lack of efficacy, and consider alternative drug not metabolized by CYP2D6
			Poor metabolizer: Increased probability of side effects	Avoid use of clomipramine due to potential for side effects, and consider alternative drug not metabolized by CYP2D6

10.	Clopidogrel	*CYP2C19*	Heterozygous poor metabolizers: Diminished antiplatelet effect and so increased risk for adverse cardiovascular events	Adequate antiplatelet effects can be achieved by increasing the dose
			Homozygous poor metabolizers: Diminished antiplatelet effect and so increased risk for adverse cardiovascular events	Consider use of another platelet P2Y12 inhibitor like prasugrel and ticagrelor
11.	Codeine	*CYP2D6*	Ultrarapid metabolizer: Increased formation of morphine following codeine administration, leading to higher risk of toxicity including respiratory depression and eventually death	Avoid codeine use due to potential for toxicity, and go for alternatives that are not affected by this CYP2D6 phenotype like morphine and nonopioid analgesics
			Poor metabolizer: Greatly reduced morphine formation following codeine administration, leading to insufficient pain relief	Avoid codeine use due to lack of efficacy, and go for alternatives that are not affected by this CYP2D6 phenotype like morphine and nonopioid analgesics
12.	Dapsone	*G6PD*	Individuals with G6PD deficiency are more prone to hemolysis	To be used with caution
13.	Desflurane	*CACNA1S* *RYR1*	Malignant hyperthermia	Desflurane is relatively contraindicated, and it should not be used, except in extraordinary circumstances in which the benefits outweigh the risks
14.	Desipramine	*CYP2D6*	Ultrarapid metabolizer: Increased probability of pharmacotherapy failure	Avoid use of desipramine due to potential lack of efficacy, and consider alternative drug not metabolized by CYP2D6
			Poor metabolizer: Increased probability of side effects	Avoid use of desipramine due to potential for side effects, and consider alternative drug not metabolized by CYP2D6
15.	Doxepin	*CYP2D6*	Ultrarapid metabolizer: Increased probability of pharmacotherapy failure	Avoid use of doxepin due to potential lack of efficacy, and consider alternative drug not metabolized by CYP2D6
			Poor metabolizer: Increased probability of side effects	Avoid use of doxepin due to potential for side effects, and consider alternative drug not metabolized by CYP2D6
16.	Enflurane	*CACNA1S* *RYR1*	Malignant hyperthermia	Enflurane is relatively contraindicated, and it should not be used, except in extraordinary circumstances in which the benefits outweigh the risks

(continued)

Table 15.1 (continued)

S. No.	Drug	Gene	Phenotypic outcome	Recommendations
17.	Escitalopram	*CYP2C19*	Ultrarapid metabolizer: Increased metabolism and hence may result in lower plasma concentrations with increased probability of pharmacotherapy failure	Consider an alternative drug not predominantly metabolized by CYP2C19
			Poor metabolizer: Greatly reduced metabolism and, hence, may result in higher plasma concentrations with increased probability of side effects	Consider a 50% reduction of recommended starting dose and titrate to response or select alternative drug not predominantly metabolized by CYP2C19
18.	Fluorouracil	*DPYD*	Intermediate metabolizer: Decreased DPD activity (leukocyte DPD activity at 30–70% that of the normal population) and increased risk for severe or even fatal drug toxicity when treated with capecitabine like mucositis, diarrhea, neutropenia, and neurotoxicity	Reduce starting dose based on activity score followed by titration of dose based on toxicity or therapeutic drug monitoring
			Poor metabolizer: Complete DPD deficiency and increased risk for severe or even fatal drug toxicity when treated with capecitabine like mucositis, diarrhea, neutropenia, and neurotoxicity	Avoid use of fluorouracil-based regimens
19.	Fluvoxamine	*CYP2D6*	Poor metabolizer: Greatly reduced metabolism and, hence, may result in higher plasma concentrations and increased probability of side effects	Consider a 25–50% reduction of recommended starting dose and titrate to response or use an alternative drug not metabolized by CYP2D6
20.	Halothane	*CACNA1S* *RYR1*	Malignant hyperthermia	Halothane is relatively contraindicated, and it should not be used, except in extraordinary circumstances in which the benefits outweigh the risks
21.	Imipramine	*CYP2D6*	Ultrarapid metabolizer: Increased probability of pharmacotherapy failure	Avoid use of imipramine due to potential lack of efficacy, and consider alternative drug not metabolized by CYP2D6
			Poor metabolizer: Increased probability of side effects	Avoid use of imipramine due to potential for side effects, and consider alternative drug not metabolized by CYP2D6
22.	Irinotecan	*UGT1A1*	Homozygous for *UGT1A1*28* allele (*UGT1A1* 7/7 genotype): Reduced UGT1A1 activity and, hence, may result in increased concentrations of irinotecan and risk for neutropenia and more severe diarrhea	Reduce the starting dose by at least one level

23.	Ivacaftor	*CFTR*	Homozygous or heterozygous for *G551D-CFTR* (e.g., G551D/G551D, G551D/F508del): Significant improvement in lung function, weight, risk of pulmonary exacerbation, and reduction in sweat chloride concentrations through enhanced CFTR channel activity	Ivacaftor can be used according to the product label
			Noncarrier of *G551D-CFTR* (e.g., F508del/R553X): Not studied clinically. Likelihood of response not known	Ivacaftor is not recommended
			Homozygous for *F508del-CFTR* (e.g., F508del/F508del): Unlikely to respond to treatment	Ivacaftor is not recommended
24.	Mercaptopurine	*TPMT*	Poor metabolizer: Extremely high concentrations of thioguanine metabolites; fatal toxicity possible without dose decrease. Greatly increased risk of thiopurine-related leukopenia, neutropenia, and myelosuppression	For malignancy, reduce both dose (10 times) and frequency (thrice weekly instead of daily) and adjust doses of mercaptopurine based on degree of myelosuppression and disease-specific guidelines. Consider alternative nonthiopurine immunosuppressant therapy for nonmalignant conditions
25.	Methoxyflurane	*CACNA1S* *RYR1*	Malignant hyperthermia	Methoxyflurane is relatively contraindicated, and it should not be used, except in extraordinary circumstances in which the benefits outweigh the risks
26.	Nortriptyline	*CYP2D6*	Ultrarapid metabolizer: Increased probability of pharmacotherapy failure	Avoid use of nortriptyline due to potential lack of efficacy, and consider alternative drug not metabolized by CYP2D6
			Poor metabolizer: Increased probability of side effects	Avoid use of nortriptyline due to potential for side effects, and consider alternative drug not metabolized by CYP2D6
27.	Ondansetron	*CYP2D6*	Ultrarapid metabolizer: Increased metabolism to less active compounds and is associated with decreased response to vomiting	Use alternative drug not predominantly metabolized by CYP2D6 (like granisetron)
28.	Oxcarbazepine	*HLA-B*	*HLA-B*15:02* positive: Severe cutaneous adverse reactions like Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)	*HLA-B*15:02* testing should be done before initiating treatment, and use of oxcarbazepine is contraindicated in *HLA-B*15:02* carriers

(continued)

Table 15.1 (continued)

S. No.	Drug	Gene	Phenotypic outcome	Recommendations
29.	Peginterferon Alfa (PEG-IFN-α)	*IFNL3*	Favorable response genotype (CC genotype): Approximately 70% chance for sustained virologic response (SVR) after 48 weeks of treatment	Consider implications before initiating PEG-IFN-α- and ribavirin (RBV)-containing regimens
			Unfavorable response genotype (CT or TT genotypes): Approximately 30% chance for SVR after 48 weeks of treatment	Consider implications before initiating PEG-IFN-α- and RBV-containing regimens
30.	Phenytoin	*HLA-B*	*HLA-B*15:02* positive: Increased risk of phenytoin-induced SJS/TEN	Do not initiate phenytoin or fosphenytoin
		CYP2C9	Intermediate metabolizer: Reduced phenytoin metabolism and, hence, may result in higher plasma concentrations with subsequent increased occurrence of toxicities	Consider 25% reduction of recommended starting maintenance dose. Subsequent maintenance doses should be adjusted according to therapeutic drug monitoring and response
			Poor metabolizer: Reduced phenytoin metabolism and, hence, may result in higher plasma concentrations with subsequent increased occurrence of toxicities	Consider 50% reduction of recommended starting maintenance dose. Subsequent maintenance doses should be adjusted according to therapeutic drug monitoring and response
31.	Rasburicase	*G6PD*	Deficient (<10–60% of normal enzyme activity): At risk of acute hemolytic anemia	Rasburicase is contraindicated, and alternatives like allopurinol can be used
			Deficient with chronic nonspherocytic hemolytic anemia (<10% activity): At risk of acute hemolytic anemia	Rasburicase is contraindicated, and alternatives like allopurinol can be used
32.	Ribavirin	*IFNL3*	Favorable response genotype (CC genotype): Approximately 70% chance for SVR after 48 weeks of treatment	Consider implications before initiating PEG-IFN-α- and RBV-containing regimens
			Unfavorable response genotype (CT or TT genotypes): Approximately 30% chance for SVR after 48 weeks of treatment	Consider implications before initiating PEG-IFN-α- and RBV-containing regimens

33.	Sevoflurane	*CACNA1S* *RYR1*	Malignant hyperthermia	Sevoflurane is relatively contraindicated, and it should not be used, except in extraordinary circumstances in which the benefits outweigh the risks
34.	Simvastatin	*SLCO1B1*	Heterozygous CT genotype (intermediate function): Intermediate myopathy risk	Prescribe a lower dose or consider an alternative statin (e.g., pravastatin or rosuvastatin). Consider routine creatine kinase monitoring
			Homozygous mutant CC genotype (low function): High myopathy risk	Prescribe a lower dose or consider an alternative statin (e.g., pravastatin or rosuvastatin). Consider routine creatine kinase monitoring
35.	Succinylcholine	*CACNA1S* *RYR1*	Malignant hyperthermia	Succinylcholine is relatively contraindicated, and it should not be used, except in extraordinary circumstances in which the benefits outweigh the risks
36	Tacrolimus	*CYP3A5*	Extensive metabolizer: Lower dose-adjusted trough concentrations of tacrolimus and decreased chance of achieving target tacrolimus concentrations	Initiate the starting dose by 1.5–2 times more than the recommended dose. However, the total starting dose should not exceed 0.3 mg/kg/day
			Intermediate metabolizer: Lower dose-adjusted trough concentrations of tacrolimus and decreased chance of achieving target tacrolimus concentrations	Initiate the starting dose by 1.5–2 times more than the recommended dose. However, the total starting dose should not exceed 0.3 mg/kg/day

(continued)

Table 15.1 (continued)

S. No.	Drug	Gene	Phenotypic outcome	Recommendations
37.	Tamoxifen	*CYP2D6*	Intermediate metabolizer: Lower endoxifen concentrations and, hence, may result in higher risk of breast cancer recurrence and event-free and recurrence-free survival	Consider hormonal therapy such as an aromatase inhibitor for postmenopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of CYP2D6 genotype. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher but FDA approved tamoxifen dose (40 mg/day). Avoid CYP2D6 strong to weak inhibitors
			Poor metabolizer: Lower endoxifen concentrations and, hence, may result in higher risk of breast cancer recurrence and event-free and recurrence-free survival	Recommend alternative hormonal therapy such as an aromatase inhibitor for postmenopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women given that these approaches are superior to tamoxifen regardless of CYP2D6 genotype and based on knowledge that CYP2D6 poor metabolizers switched from tamoxifen to anastrozole do not have an increased risk of recurrence
38.	Thioguanine	*TPMT*	Poor metabolizer: Extremely high concentrations of thioguanine metabolites; fatal toxicity possible without dose decrease. Greatly increased risk of thiopurine-related leukopenia, neutropenia, and myelosuppression	For malignancy, reduce both dose (10 times) and frequency (thrice weekly instead of daily), and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Consider alternative nonthiopurine immunosuppressant therapy for nonmalignant conditions
39.	Tramadol	*CYP2D6*	Ultrarapid metabolizer: Higher risk of toxicity including respiratory depression and eventually death	Reduce dose by 30% and be alert to adverse drug reactions (e.g., nausea, vomiting, constipation, respiratory depression, confusion, urinary retention) or select alternative drug (e.g., paracetamol, NSAID, morphine—not oxycodone or codeine)
			Poor metabolizers: Higher (20%) tramadol concentrations and lower (40%) M1 concentrations	Select alternative drug—not oxycodone or codeine—or be alert to symptoms of insufficient pain relief

40.	Trimipramine	*CYP2D6*	Ultrarapid metabolizer: Increased probability of pharmacotherapy failure	Avoid use of amitriptyline due to potential lack of efficacy, and consider alternative drug not metabolized by CYP2D6
			Poor metabolizer: Increased probability of side effects	Avoid use of amitriptyline due to potential for side effects, and consider alternative drug not metabolized by CYP2D6
41.	Warfarin	*CYP2C9*	*CYP2C9*2* (homozygous or heterozygous): Decreased enzyme activity and, hence, may result in greater risk of bleeding	The dose of warfarin needs to be lowered or consider an alternative agent. If warfarin is used, a more prolonged time (>2–4 weeks) is required to achieve a maximum international normalized ratio (INR) effect for a given dosage regimen. [For *2/*2, use 65% of the standard initial dose, and for *2/*3, use 45% of the standard initial dose]
			*CYP2C9*3* (homozygous or heterozygous): Decreased enzyme activity and, hence, may result in greater risk of bleeding	The dose of warfarin needs to be lowered or consider an alternative agent. If warfarin is used, a more prolonged time (>2–4 weeks) is required to achieve a maximum INR effect for a given dosage regimen. [For *3/*3, use 20% of the standard initial dose, and for *1/*3, use 65% of the standard initial dose]
		VKORC1	*VKORC1* -1693G>A (homozygous, AA, or heterozygous, AG): Increased sensitivity to warfarin and, hence, may result in greater risk of bleeding	The dose of warfarin needs to be lowered or consider an alternative agent. If warfarin is used, a more prolonged time (>2–4 weeks) is required to achieve a maximum INR effect for a given dosage regimen. [For AA, use 60% of the standard initial dose, i.e., approximately 3 mg/day for those with the AA genotype, 5 mg/day with the AG genotype and 6–7 mg/day with the GG genotype]
		CYP4F2	*CYP4F2*3*: Increased metabolism of vitamin K and, hence, may result in pharmacotherapeutic failure	Increase warfarin dose by 5–10%

Collated based on the Clinical Pharmacogenetics Implementation Consortium (CPIC) and by the PharmGKB Level of Evidence (LOE). Only those drug-gene combinations with CPIC level A or B and PharmGKB LOE 1A or 1B are included. Refer to the text for details regarding the level or strength of evidence. *HLA-B* Human Leukocyte Antigen-B, *CYP2D6* Cytochrome P450 2D6, *CYP2C19* Cytochrome P450 2C19, *UGT1A1* Uridine diphosphate glucuronosyltransferase 1A1, *TPMT* Thiopurine methyltransferase, *DPYD* Dihydropyrimidine dehydrogenase, *G6PD* Glucose-6-phosphate dehydrogenase, *RYR1* Ryanodine receptor isoform 1 protein, *CACNA1S* Calcium voltage-gated channel subunit alpha1 S, *UGT1A1* Uridine diphosphate-glucuronosyltransferase 1A1, *CFTR* Cystic fibrosis transmembrane conductance regulator protein, *IFNL3 (or IL28B)* Interferon-λ 3, CYP2C9 Cytochrome P450 2C9, *SLCO1B1* Solute carrier organic anion transporter family member 1B1, *CYP3A5* Cytochrome P450 3A5, *VKORC1* Vitamin K epoxide reductase, *CYP4F2* Cytochrome P450 4F2

- Intermediate Metabolizers (IM)
  Homozygous for two reduced activity enzyme gene alleles or heterozygous for an inactive allele (codominance) and, hence, individuals may suffer a lesser degree of adverse effects at usual doses.
- Extensive Metabolizers (EM)
  The normal complement of two fully active enzyme allele genes and, hence, individuals may exert a standard response to the usual dose.
- Ultrarapid Metabolizers (UM)
  More than two copies of active enzyme allele genes and, hence, the optimal therapeutic concentration may not be reached due to increased metabolism in the concerned individuals.

15.3.2 Pharmacogenetics and Drug Transport

- Likewise, polymorphisms in drug transporter genes can also happen at various organ levels; transport proteins encoded by these genes play a significant role in regulating the absorption (across the gastrointestinal tract and, also, uptake into target cells), distribution (across the blood-brain barrier), and excretion (into the bile and urine) of many drugs.
- Genetic polymorphisms tend to occur in both the major class of drug transporters, namely, ATP-binding cassette (ABC) and solute carrier (SLC) superfamilies of transporters. However, genetic variations in the ABC superfamily of transporters, particularly the subtype multidrug resistance protein 1 (MDR1) – also known as the P-glycoprotein (P-gp), are more commonly studied. P-gp usually functions as an efflux transporter at both tumor and normal cell types like intestinal enterocytes, hepatocytes, renal proximal tubule cells, and endothelial cells.
- *MDR1 (ABCB1)* is the gene coding for the P-glycoprotein. There are around 50 SNPs and 3 insertion/deletion polymorphisms found to be existent in the *MDR1* gene. Out of 50, 19 are found to be cSNPs, 11 of which are non-synonymous.
- A particular synonymous SNP 3435C>T (rs1045642) in exon 26 of the *MDR1* gene is studied more exhaustively and is found to be associated with many diseases including cancers and their consequent pharmacotherapy. The other common ones are the SNPs 1236C>T (exon 12) and 2677G>T (exon 21) both occurring at the coding regions.
- Some of the substrates of P-gp are docetaxel, paclitaxel, doxorubicin, daunorubicin, vinblastine, and vincristine (anticancer drugs); digoxin, quinidine, and verapamil (antiarrhythmics); diltiazem and losartan (antihypertensive drugs); erythromycin, levofloxacin, rifampicin, indinavir, and itraconazole (antimicrobials); cyclosporine, sirolimus, and tacrolimus (immunosuppressants); amitriptyline (antidepressant); phenobarbitone and phenytoin (antiepileptics); ranitidine (H_2 blocker); and morphine (opioid). Hence, genetic polymorphisms in the *MDR1* gene can result in the alteration of drug concentration – depending

on whether the polymorphism is a "gain-of-function" or "loss-of-function" mutation.

- Among the SLC superfamily of transporters, the pharmacogenetic variation with respect to the *SLC01B1 (solute carrier organic anion transporter family member 1B1)* gene coding for the OAT polypeptide B1 is clinically significant; this transporter is involved in the uptake of statins into the liver, and patients with the reduced function *SLC01B1* 521T>C SNP are associated with increased risk for myopathy.

15.3.3 Pharmacogenetics and Drug Target

- Variation in drug response could be due to genetic alteration in the molecular drug targets including receptors, enzymes, ion channels, and intracellular signaling proteins. Genes code for the function of the molecules with which drugs interact to produce their desirable or adverse effects.
- The classical example of drug target-related pharmacogenetics is the variation occurring at the *VKORC1* gene coding for the Vitamin K epoxide reductase enzyme that is inhibited (targeted) by warfarin. The genetic variation at the *VKORC1* gene along with variation in the *CYP2C9* gene (which codes for the CYP2C9 enzyme involved in the formation of the more active *S*-enantiomer of warfarin) accounts for more than 50% of the variability in warfarin doses needed to achieve the optimal coagulation level.
- Sometimes the pharmacogenetic variability in drug response can be due to an indirect effect on the target, i.e., modification of the biologic milieu rather than the direct alteration of the target. For example, polymorphisms in the genes coding for the ion channels, namely, *KCNQ1*, *KCNE1*, *KCNE2*, are indirectly associated with prolonged QT intervals and consequent ventricular tachyarrhythmias in patients on certain antiarrhythmics, macrolide antibiotics, and antihistamines.

15.4 Types of Pharmacogenetic Studies

- The genetic constitution of an organism or cell is referred to as *genotype*, and the observable physical or biochemical characteristic of the expression of a gene is referred to as *phenotype*.
- Likewise, analysis of functionally important mutations in the gene coding for specific enzymes and transport proteins is known as *genotyping*, and measurement of specific enzyme activity or transport protein by use of a probe drug is known as *phenotyping*.
- The data about a given genotype is not complete without the information about the associated phenotype; hence, both the genotype and phenotype complement each other, and *genotype-phenotype association studies* are the seminal pharmacogenetic approach.

15.4.1 Candidate Gene Approach

- In candidate gene approach, the genes involved in the various pharmacokinetic and pharmacodynamic pathways of a drug of interest are identified and studied elaborately. *Candidate gene* refers to the gene that is primarily involved in the drug response.
- The occurrence of variants of these candidate genes is then screened in the particular population and is correlated with the differential pharmacological response.
- Finally, the association between these functional variants and drug response is elucidated by this "genotype to phenotype" approach. The converse of this method is "phenotype to genotype" approach wherein variation in the drug response is measured initially and followed by the upstream search in single genes in drug pathways, or across the entire genome for ascertaining the reason for this variability.

15.4.2 Haplotype Analysis

- Group of SNPs located in a chromosome are studied in haplotype analysis. *Haplotype* refers to a set of closely associated (linked) genetic markers located within a particular region (locus) of a single chromosome and which are usually inherited together; they are not easily separable by genetic recombination (crossing-over). These haplotypes are, sometimes, referred to as "molecular fossils" – as they provide snapshots of human evolutionary history.
- Blocks of haplotypes are formed by clustering selected SNPs, and then, the blocks are tested for possible association with the clinical outcomes.
- Haplotype analysis is superior to candidate gene approach. In disease association studies, the number of genetic tests to be carried can be drastically reduced by haplotype analysis rather than testing multiple individual SNPs.
- Haplotype analyses can be utilized in clinical trials for identifying adverse effects-prone high-risk subset of populations. They can also be used for obtaining potential explanation for the outliers in a study.

15.4.3 Genome-Wide Association Studies (GWAS)

- GWAS is a systematic and elaborate search for tagging SNPs (tag SNPs) across the genome to detect new association with common diseases and is not restricted by any prefixed hypothesis (like the candidate gene and haplotype methods). Tag SNPs refer to those SNPs at two different loci that are expressed together due to lack of genetic recombination; the genotypes (alleles) at these two loci are said to be in *linkage disequilibrium*.

- As the expected effect sizes are small and the chances of false-positive findings are high, very large study populations (usually over 1000) and replication samples are mandatory in GWAS.
- GWAS are based upon the "common disease/common variant hypothesis" which states that genetic risk for a common and complex disease is attributable to a relatively common (minor allele frequency >0.05) polymorphism.
- SNPs in the range of 2,00,000–3,00,000 per human genome (sometimes, at more than 5,00,000 SNP sites) are analyzed, and polygenic determinants of drug response are found.
- With the help of GWAS, new insights on associations between possible genetic risk factors and complex diseases have been revealed. However, the cost of GWAS is higher than that of the other two approaches.
- There is another counterpart for GWAS that is in its nascent stage of development known as the phenome-wide association study (PheWAS). PheWAS refers to what human phenotype is a particular genetic variant associated; this has particular utility for "repurposing" marketed drugs.

15.5 Pharmacogenetics in Research

The objectives of pharmacogenetics are to optimize drug efficacy and limit drug toxicity based on an individual's genetic makeup. Hence, research activities in the field of pharmacogenetics are flourishing all along these years.

15.5.1 Pharmacogenomics Research Network

- The NIH-funded activities endorsing pharmacogenetic research was well initiated through the Pharmacogenomics Research Network (PGRN) in the year 2000.
- The mission of PGRN is "to catalyze and lead research in precision medicine for the discovery and translation of genomic variation influencing therapeutic and adverse drug effects."
- After passing through PGRN I–III from 2000 to 2015, the new network is currently in its IV phase of development. Along with PharmGKB, the recently established PGRN-Hub is also being supported by the PGRN IV. The PGRN website (www.pgrn.org) is maintained by the PGRN-Hub, and the aim of PGRN-Hub is to collect, organize, and display PGRN resources and activities.
- The PGRN comprises of a community-driven network of scientific and clinical investigators to boost basic and translational research in pharmacogenomics. Currently, there are over 300 members spanning across academia and industry focusing upon 14 main diseases including cardiovascular, endocrine, and carcinomatous conditions.

15.6 Pharmacogenetics in Drug Discovery and Development

- Pharmacogenetic research can aid in the development of optimal drugs, i.e., drugs with higher efficacy and lesser side effect potential. Furthermore, the application of pharmacogenetics has been found to expedite the process of drug discovery and development.
- The spectrum of pharmacogenetic application in drug discovery and development stretches from drug target identification, through selecting favorable subpopulation for clinical trials, choosing appropriate doses and their modifications, assessing the clinical trial results based on the pharmacogenetic data, and, finally, to imparting necessary drug label changes after regulatory approval.
- Utilization of the huge genomic knowledge, available currently, had broadened the scope of developing drugs exclusively for a particular subset of population with distinct genetic profiles.
- As well known, the branch of oncology has its strong foothold in the application of genomic medicine. Currently, however, there are other therapeutic areas including hematology, endocrinology, and cardiovascular systems which are garnering rapid pace in pharmacogenetic application.
- Nowadays, world over, the gamut of pharmacogenetics is very well integrated in the drug regulatory review process and decision making in patient selection and clinical trial design. For instance, the US-FDA has earmarked a special team of scientists to actively implement pharmacogenetics in drug development; the team named as the Genomics and Targeted Therapy Group is operating under the ambit of the Office of Clinical Pharmacology. The main functions of this group are regulatory review, scientific review, guidance and policy development.
- A major ethical issue due to the application of pharmacogenetics in drug research could be the development of "orphan genotypes," i.e., drugs being developed only for populations with certain genotypes whereas the other nonresponding genotypes are abandoned without access to potential pharmacotherapeutics, thus violating one of the basic principles of medical ethics, namely, the principle of equality.

15.7 Pharmacogenetics in Clinical Practice

- The ultimate objective of pharmacogenetics is the shift from a "trial-and-error" pharmacotherapeutic approach to a more pre-optimized individualized therapy – thereby overcoming patient discontent, inferior clinical outcomes, and higher costs.
- Therefore, patients get the *right* drug or the *right* dose and, if necessary, appropriate therapeutic monitoring sooner than later – at the *right* time.
- Besides, the genetic factors remain stable and fixed throughout the lifetime of an individual unlike the non-genetic determinants of drug response.
- There are three categories of clinical pharmacogenetic testing based on the testing purpose:

  - **Indication-based testing**
    testing "to identify patients likely or unlikely to respond to an intended drug (i.e., indication)"
  - **Contraindication-based testing**
    testing "to identify patients likely to develop harm from a drug (i.e., contraindication/harm)"
  - **Dosing-based testing**
    testing "to guide drug dosing"
- Currently, more than 100 drugs possess pharmacogenetic data in the FDA drug labels. The pharmacogenetic information contained in the US-FDA drug label can elucidate any of the following:
  - Drug exposure and clinical response variability
  - Risk for adverse events
  - Genotype-specific dosing
  - Mechanisms of drug action
  - Polymorphic drug target and disposition genes
  - Trial design features
- With the guidance of PharmGKB, both the FDA and EMA incorporate the following *PGx Level* tagging to their drug labels:
  - **Genetic testing required**
    "Some sort of gene, protein, or chromosomal testing, including genetic testing, functional protein assays, and cytogenetic studies, *should be conducted* before using this drug."
  - **Genetic testing recommended**
    "Some sort of gene, protein, or chromosomal testing, including genetic testing, functional protein assays, cytogenetic studies, etc., is *recommended* before using this drug."
  - **Actionable PGx**
    "The label does not discuss genetic or other testing for gene/protein/chromosomal variants but does contain information about changes in efficacy, dosage, or toxicity due to such variants."
  - **Informative PGx**
    "The label mentions a gene or protein that is involved in the metabolism or pharmacodynamics of the drug, but there is no information to suggest that variation in these genes/proteins leads to different response."

15.7.1 Pharmacogenomics Knowledgebase

- The Pharmacogenomics Knowledgebase (PharmGKB) is a comprehensive open-source database that accumulates, curates, and disseminates information about the impact of human genetic variation on drug responses. It was initiated by the National Institutes of Health (NIH) in 2000 and exists as an interactive tool for clinicians and researchers (www.pharmgkb.org).

- Clinically relevant information, including dosing guidelines, annotated drug labels, and potentially actionable gene – drug associations and genotype – phenotype relationships are provided by PharmGKB. Hence, it is a useful source of high-quality information supporting personalized medicine-implementation projects.
- The genotype-based drug dosing guidelines are presented as a part of the PharmGKB *PGx Prescribing Info* – hierarchically categorized based on the Anatomical Therapeutic Chemical (ATC) classification system given by WHO.
- The PharmGKB Knowledge Pyramid extends from the base with primary pharmacogenomic literature through knowledge extraction, annotation, aggregation, and integration and, ultimately, to clinical interpretation and implementation.
- Clinical Annotation Levels of Evidence given by the PharmGKB are as follows:
  - **Level 1A**
    "Annotation for a variant-drug combination in a CPIC or medical society-endorsed PGx guideline, or implemented at a PGRN site or in another major health system (high evidence)."
  - **Level 1B**
    "Annotation for a variant-drug combination where the preponderance of evidence shows an association. The association must be replicated in more than one cohort with significant *p*-values, and preferably will have a strong effect size (high evidence)."
  - **Level 2A**
    "Annotation for a variant-drug combination that qualifies for level 2B where the variant is within a VIP (Very Important Pharmacogene) as defined by PharmGKB. The variants in level 2A are in known pharmacogenes, so functional significance is more likely (moderate evidence)."
  - **Level 2B**
    "Annotation for a variant-drug combination with moderate evidence of an association. The association must be replicated but there may be some studies that do not show statistical significance, and/or the effect size may be small (moderate evidence)."
  - **Level 3**
    "Annotation for a variant-drug combination based on a single significant (not yet replicated) study or annotation for a variant-drug combination evaluated in multiple studies but lacking clear evidence of an association (low evidence)."
  - **Level 4**
    "Annotation based on a case report, non-significant study or *in vitro*, molecular or functional assay evidence only (preliminary evidence)."

15.7.2 Clinical Pharmacogenetics Implementation Consortium

- The Clinical Pharmacogenetics Implementation Consortium (CPIC) is an international confederation of a small group of dedicated volunteers who are interested in facilitating the use of pharmacogenetic tests for patient care. It was propounded as a shared partnership project between the PGRN and the PharmGKB in 2009 (www.cpicpgx.org).
- The goal of CPIC is to "to address this barrier to clinical implementation of pharmacogenetic tests by creating, curating, and posting freely available, peer-reviewed, evidence-based, updatable, and detailed gene/drug clinical practice guidelines."
- CPIC elucidates genotype-based drug-dosing guidelines thereby extending support to the clinicians to extrapolate the importance of available pharmacogenetic tests. The prime motive of CPIC guidelines is to provide access to clinically high-throughput and preemptive (pre-prescription) genotyping for physicians to have firsthand information – even before they narrow down to any particular drug.
- The CPIC publishes guidelines regarding incorporation of pharmacogenetic knowledge in routine clinical practice. As of now, the CPIC guidelines have been published for 21 drugs. The prioritization of framing CPIC guideline for a particular drug is based on the following eight criteria:
  - Is there prescribing actionability?
  - What is the severity of the clinical consequences (adverse effects, lack of response) if genetics are not used to inform prescribing?
  - Is the gene already subject to other CPIC guidelines?
  - Is there an available genetic test for that gene?
  - How commonly used are the affected drugs?
  - How common are the high-risk genetic variants?
  - Is there mention of genetic testing in drug labeling?
  - Are there pharmacogenetically based prescribing recommendations from professional organizations or others?

15.7.3 Clinical Genome Resource

- The Clinical Genome Resource (ClinGen) is another NIH-funded resource dedicated "to build an authoritative central resource that defines the clinical relevance of genes and variants for use in precision medicine and research" (www.clinicalgenome.org).
- The ClinGen's critical questions are as follows:
  - Is this gene associated with a disease? – *Clinical Validity*
  - Is this variant causative? – *Pathogenicity*
  - Is this information actionable? – *Clinical Utility*

- The ultimate objective of ClinGen is to build a genomic knowledge base, thereby improving patient care through genomic medicine. Hence, its motto is "Sharing Data, Building Knowledge and Improving Care."

15.8 Pharmacogenomics Research in India: Current Status

- There is a substantial surge in the number of pharmacogenetic studies conducted in India in the last few years. However, there are disparities in the populations, diseases, or drugs studied.
- More number of pharmacogenomic investigations were done in the North and South Indian ethnicities compared to the North-Eastern and Tibeto-Burman populations.
- Similarly, studies on drugs used in diabetes, tuberculosis, AIDS, and breast cancer were comparatively few in number than studies on drugs for breast cancer (and other anticancer drugs), anticoagulants (warfarin and acenocoumarol), and antiplatelets (clopidogrel).

15.9 Conclusion

The "multi-omics" approach integrating pharmacogenetics with other large-scale measures of gene function like *transcriptomics*, *proteomics*, *metabolomics, epigenomics*, and *microbiomics* may herald new vistas in the better understanding of intractable diseases and their consequent pharmacotherapeutic management.

The researchers working in the field of pharmacogenetics should explore those functionally important genetic variants. "Point-of-care" genotype testings are the need of the hour. Furthermore, it is in the hands of the practicing physicians to choose appropriate pharmacogenetic testing by judiciously sieving through from the vast pharmacogenomic knowledge.

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Chronopharmacology

16

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Abstract

Rhythmic variations in various physiological, biochemical, and behavioral parameters occur in all living organisms including humans. These variations optimize energy usage by prioritizing certain body functions at certain times of the day and conserving energy at other times. Circadian rhythm, in which the cycle length is about 24 h, regulates many functions in humans. Central and peripheral circadian clocks are involved in the regulation of circadian rhythm in response to the environmental cues like sunlight and feeding called the *zeitgebers*. Melatonin and hypothalamo-pituitary-adrenal (HPA) axis play key roles in the regulation of circadian homeostasis. Chronopharmacology refers to the study of biological rhythm dependencies of drugs to optimize drug therapy by selecting the appropriate time of drug administration, which is associated with maximum efficacy and minimal adverse effects. Circadian variations have also been observed in various diseases like hypertension, myocardial infarction, bronchial asthma, and cancer. Chronopharmacotherpeutic strategies in optimizing the timing of drug administration have been implemented in various diseases exhibiting circadian variations. Innovative chronopharmacological drug delivery systems have also been developed to circumvent the need for administering the drugs at odd timings. However, interindividual variations, interspecies variations, high cost of drug trials incorporating chronopharmacological approaches, and absence of a reliable chronobiological biomarker to guide chronopharmacotherapy are major limitations in this field and warrant further research.

Keywords

Circadian · Chronobiology · Chronokinetics · Chronesthesy · Chronotherapy

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16.1 Introduction

Humans, like other mammals, demonstrate significant rhythmic variations in various biological functions. The field of biology that examines cyclical phenomena in living organisms and their adaptation to various biological rhythms is called as chronobiology. There are different types of biorhythms, namely, circadian, ultradian, infradian, circaseptan, circalunar, and circannual.

Circadian – Cycle length is around 24 h.
Ultradian – Cycle length is less than 24 h.
Infradian – Cycle length is more than 24 h.
Circaseptan – Cycle length is around 7 days.
Circalunar – Cycle length corresponds with the lunar cycle (approximately 29.5 days).
Circannual – Cycle length is around 1 year.

16.2 Circadian Rhythm

- The circadian rhythm regulates many of the physiological processes in humans.
- The term "circadian" was derived from the Latin words "circa" meaning "about" and "diem" meaning "day."
- Circadian rhythm is maintained by the central and peripheral clocks. Melatonin and hypothalamo-pituitary-adrenal (HPA) axis are also involved in its regulation.

16.2.1 Role of Circadian Clocks in Circadian Regulation

- Circadian clocks are present in almost all the cells of our body.
- The main aim of the circadian clock is to optimize metabolism and energy utilization for sustaining life processes in an organism.
- They regulate the homeostatic processes such as sleep-wake cycle, appetite, levels of hormones, and other bodily functions with the 24-h cycle.
- There are two types of circadian clocks, namely, central/master clock (located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus) and peripheral/slave clocks present in all other cells of the body (Fig. 16.1).
- They are organized in hierarchical manner with the central clock controlling the peripheral clocks.
- Both clocks contain molecular oscillators which are regulated by the environmental cues called as *zeitgebers*. Some examples of zeitgebers are light, food, activity, and others.
- The central and peripheral clocks are synchronized by different environmental cues or zeitgebers.

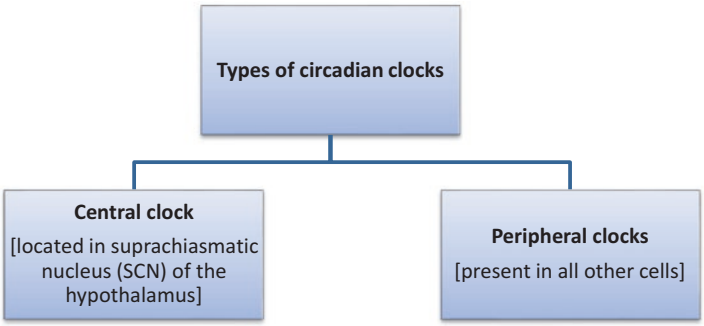

Fig. 16.1 Circadian clocks

Table 16.1 Direct and indirect signaling from the suprachiasmatic nucleus (SCN) (central clock) to the peripheral clocks

Direct signals from SCN to peripheral clocks	Indirect signals from SCN to peripheral clocks
1. Neuronal signals including activation of autonomic nervous system and hypothalamo-pituitary-adrenal (HPA) axis	1. Changes in body temperature
2. Hormonal signals	2. Feeding behavior

- The sunlight is the major regulator of central clock. Sunlight falls on the retina, and the signal is transmitted to the SCN via the retinohypothalamic tract.
- The peripheral clocks are regulated by direct and indirect signals from the SCN. The direct signals are relayed by the neuronal networks (activation of autonomic nervous system and hypothalamo-pituitary-adrenal axis) and through hormonal signaling (e.g., cortisol). The indirect signals are relayed by the SCN on the peripheral clocks by producing changes in body temperature and feeding behavior (Table 16.1).
- Various transcription factors are involved in circadian regulation. The important transcription factors are CLOCK, BMAL1, and NPAS2.

16.2.2 Role of Melatonin in Circadian Regulation

- The SCN contains MT1 and MT2 melatonin receptors.
- Melatonin is released from the pineal gland during the dark phase of the light/dark cycle and acts on its receptors in the SCN to align it in phase with the external light/dark cycle.
- Exogenous administration of melatonin in the morning shifts the endogenous clock out of phase, whereas evening administration synchronizes the endogenous clock according to the light/dark cycle.

16.2.3 Role of Hypothalamo-Pituitary-Adrenal (HPA) Axis in Circadian Regulation

- HPA axis plays a very important role in synchronizing the peripheral clocks with the central clock.
- SCN communicates to the anterior pituitary gland, resulting in rhythmic release of adrenocorticotropic hormone (ACTH) and thus release of cortisol from the adrenal glands.
- The peripheral clocks have glucocorticoid receptors (GCRs), and its activation can alter the transcription of circadian genes present in them, synchronizing them with the central clock.
- However, central clock lacks GCRs, and thus it is devoid of alteration due to cortisol levels.

16.3 Chronopharmacology

Chronopharmacology is the study of biological rhythm dependencies of drugs with respect to its pharmacological effects and/or pharmacokinetics/dynamics of drugs in both animals and in humans. Hence, it has been divided into study of the following three different aspects:

1. Chronokinetics
2. Chronesthesy
3. Chronotherapeutics

16.3.1 Chronokinetics

- Chronopharmacokinetics is the study of predictable rhythmic variations in pharmacokinetic parameters of drugs.
- Thus, the pharmacokinetic parameters like drug absorption, distribution, metabolism, and excretion demonstrate circadian variations.
- Chronopharmacokinetic information can be used in choosing the proper timing of drug administration to optimize drug therapy.
- Circadian rhythms in absorption:
  - Parameters that regulate drug absorption and bioavailability like gastric acid secretion, gastric motility, gastric emptying time, and gastrointestinal blood flow exhibit circadian variations.
  - Most lipophilic drugs are better absorbed in the morning compared to evening due to better blood supply to the gastrointestinal tract and faster gastric emptying time in the morning.

- Circadian rhythms in distribution:
  - Distribution of a drug depends on body size, body composition, blood flow, protein binding, and membrane permeability, of which mainly blood flow and protein binding have shown to possess circadian variability.
  - Blood flow is regulated by the activities of autonomic nervous system with a more prevalent diurnal effect of the sympathetic system. Thus, blood flow increases during daytime due to predominant sympathetic activity and decreases at night time due to low sympathetic activity.
  - The levels of plasma proteins like albumin and globulin produced from the liver exhibit variations during the day and night because of the circadian rhythmic variations in the activities of the liver. Their blood levels are very low during the night, increase by day, and reach to a very high value during the afternoon.
- Circadian rhythms in metabolism:
  - Hepatic drug metabolism depends on the activity of metabolizing enzymes and/or hepatic blood flow, both of which exhibit circadian variations.
  - All phases of drug metabolism are under circadian control.
  - Both the microsomal and non-microsomal enzymes exhibit circadian variations.
  - Circadian variation in the hepatic blood flow affects the metabolism of drugs like propranolol.
  - Oxidative microsomal reactions reach their maximum activity during the day and lowest during the night. On the contrary, sulfate conjugation reactions are much faster during night than during the day.
- Circadian rhythms in excretion
  - Renal excretion depends on renal blood flow, glomerular filtration rate (GFR), tubular secretion, and urinary pH.
  - Glomerular filtration rate (GFR) is maximum during midday and minimum in the night.
  - Urinary pH is acidic in the evening and alkaline in the morning.

16.3.2 Chronesthesy

- It deals with the study of the rhythmic changes in the susceptibility and sensitivity of the target system to a drug.

16.3.3 Chronotherapeutics

Chronotherapeutics refers to the science that deals with optimization of efficacy and minimization of adverse effects of drugs by timing the administration of drugs in accordance with the biological rhythm, i.e., rhythmicity of physiological, biochemical, and behavioral processes.

16.4 Circadian Rhythms in Physiological Functions and Diseases

Some of the physiological functions (Table 16.2) and pathological states (Table 16.3) are partially determined by the circadian rhythm patterns.

16.5 Chronobiological Implications of Drug Treatment

The chronobiological disease-based characteristics with the clinical implication of time-based frequency of dosing are elaborated in Table 16.4.

Table 16.2 Circadian rhythms in various physiological functions

S. no.	Circadian rhythms	Physiological functions
1.	Peaks during morning	Secretion of adrenocorticotropic hormone (ACTH), cortisol, aldosterone, and testosterone
		Platelet adhesiveness, blood viscosity, NK-cell activity
2.	Peaks during afternoon	Hematocrit
		Airway caliber (FEV_1)
3.	Peaks during evening	Secretion of insulin, cholesterol, triglycerides, and uric acid
		Platelet numbers
4.	Peaks during night	Basal gastric acid secretion
		Secretion of prolactin, melatonin, growth hormone (GH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH)
		White blood cells (WBC) including lymphocytes and eosinophils

Table 16.3 Circadian rhythms in various diseases

S. no.	Organ system	Disease	Circadian rhythm
1.	Cardiovascular system	Hypertension	Early morning increase in BP
		Myocardial infarction and sudden death	Early morning increase in incidence
		Thrombotic and hemorrhagic stroke	Early morning increase in incidence
		Symptoms of congestive heart failure	Worst nocturnally
2.	Respiratory system	Bronchial asthma attack	Worst in the early morning
3.	Nervous system	Migraine headaches	Worst in the early morning
		Epilepsy	Common around sleep onset at night and offset in the morning
4.	Musculoskeletal system	Rheumatoid arthritis	Worst in the early morning
		Osteoarthritis	Worst later in the day
5.	Gastrointestinal system	Pain of peptic ulcer disease	Worst in late evening and early morning
6.	Immune system	Allergic rhinitis	Worst in the late night or early morning

Table 16.4 Chronobiological implications of pharmacotherapy

S. no.	Disease	Chronobiological characteristic	Chronotherapy
1.	Bronchial asthma	Precipitation of attacks during late night or at early morning hour (from 2 to 6 a.m.) due to increased bronchial hyperreactivity during this period	Inhaled salbutamol given early morning
			Evening dose of a sustained release preparation of theophylline produces peak drug concentrations in late night and early morning
2.	Hypertension	Blood pressure increases briskly in the morning after awakening, decreases in the evening, and is lowest during sleep (lower values of systolic and diastolic pressure occur between midnight and 4 a.m.)	If given only as once daily early in the morning, many antihypertensive drugs do not reduce the early morning blood pressure
		Rapid rise in blood pressure in the early morning is due to increased vascular tone and total peripheral resistance (corresponding to increased secretion of catecholamines and increased plasma rennin activity in early morning)	However, they achieved greater reduction in blood pressure and risk of adverse cardiovascular events when administered at bedtime
			Extended release formulation of L-type calcium channel blocker, verapamil is administered orally at bedtime, and it produces therapeutically effective plasma levels in the early morning
			Modified release formulation of valsartan administered at bedtime is used to achieve late night/early morning exposure to the drug

(continued)

Table 16.4 (continued)

S. no.	Disease	Chronobiological characteristic	Chronotherapy
3.	Cardiovascular diseases including stroke and myocardial infarction	Commonly occur during the initial hours of morning between 6 a.m. and 12 noon; because of following reasons:	Low-dose administration of aspirin in the evening or bedtime reduces morning platelet reactivity compared to taking aspirin in the morning
		As the platelet aggregation is increased and fibrinolytic activity is decreased in the morning, a state of relative hypercoagulability of the blood prevails	Evening or bedtime administration of angiotensin-converting enzyme inhibitors (enalapril, ramipril, lisinopril) or angiotensin receptor blockers (valsartan, telmisartan) or calcium channel blocker (amlodipine) produces better reduction in BP, and the incidence of adverse cardiovascular events is reduced
		Rapid rise in blood pressure and heart rate, thus augmenting the oxygen demand of the heart in early morning	Administration of beta-blockers prevents the morning increase in the incidence of angina, myocardial infarction, and sudden death
		Coronary blood flow decreases in the morning	
4.	Allergic rhinitis	Symptoms are worst in the late night or early morning	Anti-histamines are usually given once daily at bedtime
5.	Rheumatoid arthritis	Pain is maximum in the early morning	Long-acting NSAIDs like flurbiprofen, ketoprofen, and indomethacin are administered at bedtime
6.	Osteoarthritis	Pain is maximum in the evening	Analgesics like ibuprofen are administered in afternoon
7.	Hypercholesterolemia	HMG CoA reductase enzyme activity is maximum in the night, and thus cholesterol synthesis is usually greater during night time than during day	Statins like simvastatin given in the evening or night are more effective
8.	Diseases where systemic steroids are given	Endogenous secretion of adrenocorticotropic hormone (ACTH) and cortisol peaks in the early morning	Prednisolone and other corticosteroids are given early morning to mimic the release from HPA axis
9.	Acid peptic disease	Basal gastric acid secretion peaks during the midnight	H_2 blockers are given before bedtime to inhibit basal acid secretion at midnight
			Chronopharmaceutical preparation of famotidine is available

(continued)

Table 16.4 (continued)

S. no.	Disease	Chronobiological characteristic	Chronotherapy
10.	Epilepsy	Seizure attacks are common around the onset of sleep at night and the offset of sleep in the morning	Patients in whom the evening dose was double that of the morning dose, without altering the total dose of medication, showed better seizure control
11.	Cancer	Normal cells and cancer cells vary in their chronobiological rhythm. The DNA synthesis peaks in the normal human bone marrow at noon and in lymphoma cells at midnight	S-phase active cytotoxic drugs are administered at late night, providing selective suppression of the lymphoma cells over normal bone marrow cells. Thus, it produces a decrease in tumor cell count with reduced propensity to cause bone marrow suppression

16.6 Chronotherapeutic Drug Delivery Systems

Innovative chronopharmacological drug delivery systems have been developed to circumvent the need for administering the drugs at odd timings. The various drug delivery systems are:

- Spheroidal oral drug absorption system (SODAS) – It is based on production of controlled release beads which release the drugs according to the circadian rhythm.
- Chronotherapeutics oral drug absorption system (CODAS) – This system has enteric release-controlling polymer applied to drug-loaded beads, which facilitates drug release after a prolonged period of time after ingestion. For example, Verelan® PM capsules taken at bed time release verapamil approximately 4–5 h after ingestion, in the next day's morning hours, when the blood pressure is generally high.
- Container Technology – This technology has semipermeable matrix technology with uniform porosity, releasing the drug in a controlled release manner.
- TIMERx Technology (hydrophilic system) – This technology has a combination of Xanthan and Locust bean gums mixed with dextrose, which forms a gel when in contact with water and aids in controlled drug release.

16.7 Advantages of Chronopharmacology

- Optimization of drug therapy by controlling the timing of drug administration, thus maximizing the efficacy and minimizing the adverse effects of a drug.

16.8 Limitations of Chronopharmacology

- Interindividual variations make it difficult to design a common dosing regimen, and thus individual dosing regimen is required.
- Interspecies variations in diurnal cycle make it inappropriate to extrapolate the results of animal studies to humans.
- Increased cost of trials in which chronopharmacological studies are included because it will increase the duration, sample size, and cost of the trial.
- Absence of a reliable marker of biorhythm to guide chronotherapy.

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Pharmacoepidemiology

17

Nishanthi Anandabaskar

Abstract

Pharmacoepidemiology is defined as "the study of the use and effects/side-effects of drugs in large numbers of people with the purpose of supporting rational and cost-effective drug use in the population, thereby improving health outcomes." Pharmacoepidemiology studies can be classified into drug-oriented research or utilization-oriented research (drug utilization research). Drug utilization research aims to promote rational use of drugs by providing description of drug use patterns, helping to identify early signals of irrational drug use, aiding in implementation and assessment of the efficacy of interventions to promote rational drug use, and also assisting in continuous improvement in the quality of drug use. There are various types of drug use information like drug-based information, problem/encounter-based information, patient information, and prescriber information. The major units of measurement in drug utilization studies are defined daily dose (DDD), prescribed daily dose (PDD), and consumed daily dose. The various sources of data on drug utilization are from large databases, data from drug regulatory authorities, supplier/distribution data, community setting data, and practice setting data. In order to maintain uniformity and compare drug utilization data among various countries, the anatomical therapeutic chemical (ATC) classification developed by Norwegian researchers is commonly employed. The World Health Organization/International Network for Rational Use of Drugs (WHO/INRUD) drug use indicators (core and complementary indicators) are used in describing and measuring the patterns of drug use.

Keywords

Anatomical therapeutic chemical (ATC) classification · Defined daily dose (DDD) · Drug utilization · Drug use pattern

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17.1 Definition of Pharmacoepidemiology

According to the World Health Organization (WHO), pharmacoepidemiology is defined as "the study of the use and effects/side-effects of drugs in large numbers of people with the purpose of supporting rational and cost-effective drug use in the population, thereby improving health outcomes" (Fig. 17.1).

17.2 Drug Utilization Studies

- Drug utilization research was defined by WHO in 1977 as "the marketing, distribution, prescription, and use of drugs in a society, with special emphasis on the resulting medical, social and economic consequences."
- The main aim of drug utilization research is promotion of rational drug use.

17.3 How Drug Utilization Research Contributes to Rational Drug Use?

- Provides a description of drug use patterns as follows:
  - Estimate the numbers of patients exposed to specified drugs within a given time period.

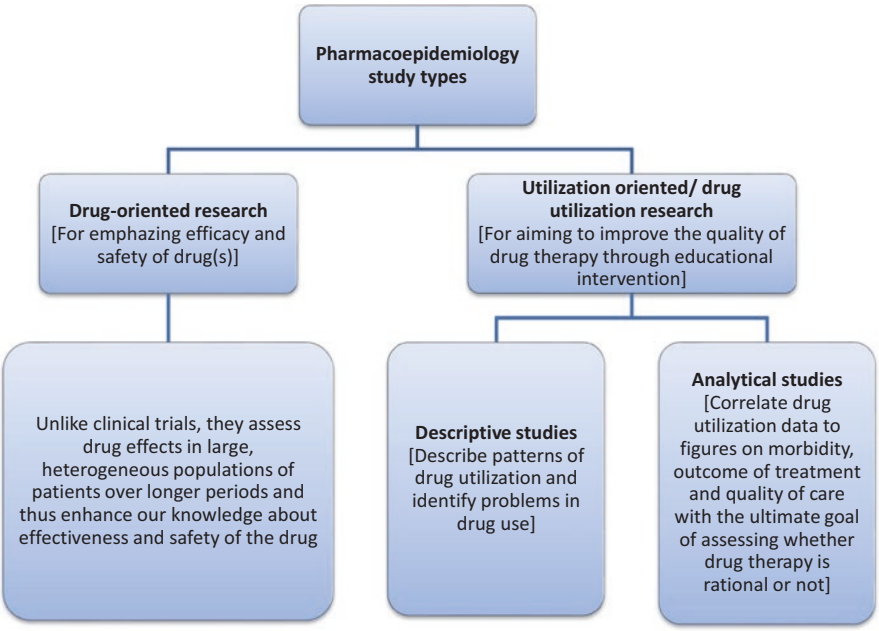

Fig. 17.1 Study types in pharmacoepidemiology

  - Describe the extent of drug use at a certain time and/or in a certain area (e.g., in a country, region, community, or hospital).
  - Help to identify patterns or trends in drug use.
  - Estimate over-usage and under-usage of drugs.
  - Compare the observed patterns of drug use for the treatment of a certain disease with current recommendations or guidelines.
  - Determine the pattern or profile of drug use and the extent to which alternative drugs are being used to treat particular conditions.
  - Apply quality indicators to patterns of drug utilization.
- Provides early signals of irrational drug use. Signs of drug under-use, overuse, or misuse can be identified by the following:
  - Drug utilization pattern and cost can be compared between different geographical regions.
  - Drug utilization pattern can be compared with standard treatment guidelines.
- Helps to implement and assess the efficacy of interventions to promote rational drug use
  - The interventions could be provision of local formulary, educational intervention, or implementation of regulatory policies.
- Helps in continuous improvement in quality of drug use
  - Quality control cycle provides framework for continuous improvement in quality of drug use.
  - Components of quality control cycle are:

    Plan – Work out a plan for improvement after in-depth analysis of the situation at hand.

    Do – Implement the plan on small scale.

    Check – Assess if expected results are obtained.

    Act – Revise the plan or implement the plan on a large scale depending on the outcome (Fig. 17.2).
  - The cycle continues with setting of new targets and performing new analysis.

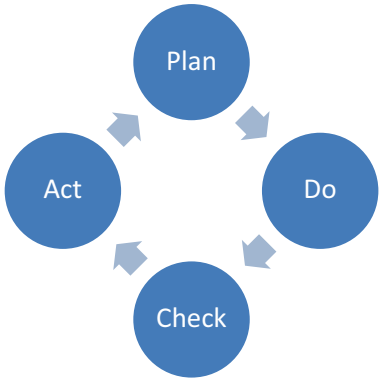

Fig. 17.2 Components of quality control cycle

17.4 Types of Drug Use Information

17.4.1 Drug-Based Information

- Level of drug use aggregation, e.g., comparison of individual drugs or groups of drugs
- Indication of drug use
- Dose of drug
- Drug costs

17.4.2 Problem/Encounter-Based Information

- Cause for the problem/encounter
- Severity of problem/encounter
- Drug therapy vs non-drug therapy
- Duration of consultation

17.4.3 Patient Information

- Age
- Gender
- Comorbidities
- Ethnicity
- Knowledge, beliefs, and perceptions

17.4.4 Prescriber Information

- Age
- Gender
- Educational background – type of medical college, specialist/family care physician
- Practice area (rural/urban) and practice size
- Knowledge about drugs and factors influencing prescribing behavior

17.5 Sources of Data on Drug Utilization

- Large databases, e.g., pharmacy billing database and diagnosis-linked database.
- Data from drug regulatory authorities – It provides information on approved and banned drugs.
- Supplier/distribution data – Drug importers and local manufacturers provide information on drug suppliers.
- Community setting data – Household surveys can be conducted to assess compliance to the treatment regimen.

- Practice setting data – It includes the following types of data:
  - Prescribing data (outpatient and inpatient prescription forms) – It provides information on patient demography, diagnosis, drug name, dosage form, strength, dose, frequency of administration, and duration of treatment. The prescriptions are a good source for evaluation of WHO indicators of drug use, which includes the following:
    Average number of drugs per prescription
    % of drugs prescribed by generic name
    % of prescriptions with an antibiotic
    % of prescriptions with an injection
    % of drugs prescribed from the essential drugs list or formulary
    Average drug cost per prescription
  - Dispensing data – The following data can be obtained from drug dispensers:
    Number of drugs per prescription
    Drug names with dosing schedule
    Quantity of drugs dispensed
    Cost of each drug
    % of drugs adequately labeled
    Dispensing of over the counter medications
  - Aggregate data – The data can be collected from drug procurement records, pharmacy stock and dispensing records, medication error records, adverse drug reaction records, and medical records of patient. These sources provide aggregate data on drug utilization in a hospital. They provide data on:
    Cost of various drugs
    Comparisons of drugs used for the same indication
    Prevalence of medication errors
    Prevalence of adverse drug reactions

17.6 Drug Classification Systems

- An international standard classification system is required for comparing drug utilization data among countries.
- The two main systems of classification which are being used in drug utilization studies are the following.

17.6.1 Anatomical Therapeutic (AT) Classification

- The anatomical therapeutic (AT) classification was developed by the European Pharmaceutical Market Research Association (EphMRA).
- The first classification system recommended for drug utilization research.
- The drugs are classified into groups at three or four different levels.
- Not commonly used as it is superseded by the ATC classification system.

17.6.2 Anatomical Therapeutic Chemical (ATC) Classification

- Developed by Norwegian researchers by modifying the EphMRA classification.
- The drugs are classified into groups at five different levels:
  - First level – main anatomical group
  - Second level – main therapeutic group
  - Third level – therapeutic/pharmacological subgroup
  - Fourth level – chemical/therapeutic/pharmacological subgroup
  - Fifth level – chemical substance
- The drugs are divided into 14 main groups based on the first level of classification (Table 17.1). For example, ATC classification of glibenclamide is depicted in Box 17.1.

Table 17.1 Fourteen main groups according to the first level of ATC classification

S. No.	Code	Main groups of ATC classification
1	A	Alimentary tract and metabolism
2	B	Blood and blood forming agent
3	C	Cardiovascular system
4	D	Dermatologicals
5	G	Genitourinary system and sex hormones
6	H	Systemic hormonal preparation except insulin and sex hormones
7	J	Anti-infectives for systemic use
8	L	Antineoplastics and immunomodulating agents
9	M	Musculoskeletal agents
10	N	Nervous system
11	P	Antiparasitic agents, insecticides, and repellents
12	R	Respiratory system
13	S	Sensory organs
14	V	Various

ATC anatomical therapeutic chemical classification

Box 17.1: ATC Classification of Glibenclamide

A	Alimentary tract and metabolism (first level, main anatomical group)
A10	Drugs used in diabetes (second level, main therapeutic group)
A10B	Oral blood-glucose-lowering drugs (third level, therapeutic/pharmacological subgroup)
A10B B	Sulfonamides, urea derivatives (fourth level, chemical/therapeutic/pharmacological subgroup)
A10B B01	Glibenclamide (fifth level, subgroup for chemical substance)

Table 17.2 Different ATC codes for various formulations of prednisolone

S. No.	ATC code	Formulation of prednisolone
1.	A07E A01	Intestinal anti-inflammatory agents (enemas and rectal foams)
2.	C05A A04	Antihemorrhoidals for topical use (rectal suppositories)
3.	D07A A03	Dermatological preparations (creams, ointments, lotions)
4.	H02A B06	Corticosteroids for systemic use (tablets, injections)
5.	R01A D02	Nasal decongestants (nasal spray, drops)
6.	S01B A04	Ophthalmologicals (eye drops)
7.	S02B A03	Otologicals (ear drops)

- Different formulations with different indications may also be given separate ATC codes. For example, different ATC codes for various formulations of prednisolone are enlisted in Table 17.2.
- ATC classification is being done only for those drugs with an established international nonproprietary name (INN).
- Request for inclusion of new drugs in the ATC system is entertained only after it gets marketing authorization in at least one country.
- Fixed-dose combination containing more than one ingredient will be given separate ATC code different from individual ATC code by use of parallel fifth level classification. For example, code for diazepam is N05BA**01**, and its combination is N05BA**51**.
- Prodrugs and their active drugs will be coded with different ATC codes.
- Immediate- and slow-release preparations will have the same ATC codes.
- Obsolete drugs or drugs withdrawn from the market are retained in the ATC system, since their exclusion may create confusion.
- Alterations of ATC code of the drug will be made in the following circumstances:
  - Clear change in the main indication of drug
  - Splitting of a large group into further generations
  - Correction of incorrect classification
- Disadvantages of ATC classification system are:
  - Requires competence to assign ATC codes to the products.
  - Requires constant updating with the latest version of ATC codes.
  - Does not provide any information regarding the efficacy and superiority of drugs.
  - ATC code is given based on the main indication of the drug, which may vary from country to country.

17.7 Units of Measurement in Drug Utilization Studies

17.7.1 Defined Daily Dose (DDD)

- Defined daily dose (DDD) is defined as "the assumed average maintenance dose per day for a drug used for its main indication in adults." It is a unit of measurement.
- It is not in concordance with prescribed daily dose.

- DDD is assigned only to those drugs that have been coded with ATC code.
- Only one DDD is assigned per ATC code.
- For fixed-dose combinations, the ATC determines which is the main ingredient, and the DDD will be according to the main ingredient.
- All newly assigned DDDs are reviewed after 3 years.
- DDDs are not assigned for pediatric doses, and WHO recommends use of PDD instead of DDD in drug utilization studies in children.
- Drug utilization data are represented as:
  - DDDs per 1000 inhabitants per day or DDDs per inhabitant per year (for drugs used on "outpatient" basis)
  - DDDs per 100 bed-days (for drugs used on "inpatient" basis)
- Main use of DDD – aids in comparing drug utilization patterns since DDDs do not vary with price, currencies, package size, or strength.
- Limitations
  - DDDs do not correspond to prescribed daily dose.
  - DDDs are not established for topical products, sera, vaccines, antineoplastic agents, allergen extracts, general and local anesthetics, and contrast media.
  - DDD may be revised periodically in case of changes in drug dosages over time.

17.7.2 Prescribed Daily Dose (PDD)

- Prescribed daily dose (PDD) is defined as the "average daily dose prescribed, as obtained from a representative sample of prescriptions."
- There can be discrepancies between PDD and DDD, which needs to be considered when interpreting drug utilization data.
- PDD can vary depending on the type of disease, severity of disease, and country of study.

17.7.3 Consumed Daily Dose

- It is a measure of actual drug consumption at the patient level.
- Obtained from patient interviews.

17.7.4 Other Units for Presentation of Volume

- Grams of active ingredient
- Number of tablets
- Numbers of prescriptions

17.8 WHO/INRUD Drug Use Indicators

- Developed by the WHO (World Health Organization) and International Network for Rational Use of Drugs (INRUD).
- Helps in describing and measuring the patterns of drug use.
- The drug use indicators measure performance in three general areas:
  - Prescribing practices by health care providers
  - Key elements of patient care encompassing clinical consultation and pharmaceutical dispensing
  - Availability of facility specific factors favoring rational drug use
- The drug use indicators are classified into core indicators and complementary indicators.

17.8.1 Core Drug Use Indicators

- Include only a small number of basic indicators.
- Act as a simple, quick, and reliable tool to measure drug use. The 12 core drug use indicators are enlisted in Box 17.2.

Box 17.2: Core Drug Use Indicators

Prescribing Indicators

1. Average number of drugs per encounter
2. Percentage of drugs prescribed by generic name
3. Percentage of encounters with an antibiotic prescribed
4. Percentage of encounters with an injection prescribed
5. Percentage of drugs prescribed from essential drugs list or formulary

Patient Care Indicators

6. Average consultation time
7. Average dispensing time
8. Percentage of drugs actually dispensed
9. Percentage of drugs adequately labeled
10. Patient's knowledge of correct dosage

Facility Indicators

11. Availability of copy of essential drugs list or formulary
12. Availability of key drugs

Box 17.3: Complementary Drug Use Indicators

1. Percentage of patients treated without drugs
2. Average drug cost per encounter
3. Percentage of drug costs spent on antibiotics
4. Percentage of drug costs spent on injections
5. Prescription in accordance with treatment guidelines
6. Percentage of patients satisfied with care they received
7. Percentage of health facilities with access to impartial drug information

17.8.2 Complementary Drug Use Indicators

- Complementary drug use indicators include drug use indicators which can be measured in addition to the core indicators. These indicators are not less important compared to core indicators.
- However, they are difficult to measure and/or interpret.
- The seven complementary drug use indicators are provided in Box 17.3.

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Ethnopharmacology

18

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Abstract

The terms ethnopharmacology, ethnobotany and pharmacognosy are interrelated. Ethnopharmacology deals with the traditional use of phytoactive molecules by humans while ethnobotany focuses on cultural use of plants and beliefs involved in their use by various human society. All these branches essentially focus on the chemistry, biological action and uses of active phytochemicals obtained from the plants. Based on their role, phytochemicals are classified into primary and secondary metabolites. The majority of drugs obtained until now are almost the secondary metabolites of the plants. Secondary metabolites like various alkaloids, glycosides, non-protein amino acids, lignins, tannins, polyphenolic compounds and others are isolated, purified, structurally modified and promoted as 'active drug' in modern medicine. Such plant-derived drugs are immense and affect almost all the organ systems in the human body. Bronchodilators like theophylline, purgatives like senna, cardiac glycosides like digoxin, antihypertensives like reserpine, anticholinergics like atropine and number of antibiotics from fungi constitute the tip of the iceberg for the potential use of plant-derived drugs. Discovery of these phytochemical drugs requires comprehensive knowledge about the ethnicity of plant, basic principles in drug extraction process from plants, choice of selection of solvents for extraction and purification steps involved in isolation of active phytochemicals.

Keywords

Ethnopharmacology · Ethnobotany · Pharmacognosy · Phytochemicals · Secondary metabolite · Drug extraction

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18.1 Introduction

Paracelsus penned in his book *Arcanum*, as early as in 1524, that 'It does not matter that rhubarb is a purgative. The question is: What purges?'. This statement had created the impetus search for 'plant-derived drugs' and formed the basis of ethnopharmacology.

As per WHO's survey between 2002 and 2005, almost 80% people in Africa, 75% in France, 70% in Canada, 42% in the USA and 40% in India and China use complementary and alternative medicine or traditional medicine once in their lifetime. Various medicinal agents like aspirin, digitoxin, ephedrine, atropine, vincristine, cocaine, morphine, caffeine, d-tubocurarine, physostigmine and others were used for thousands of years in crude form. They have been scientifically analysed, purified, rediscovered and introduced into allopathic medicinal system as distinct drugs. This indicates the importance of plants in the role of medicine and their contribution to the modern medicine.

18.2 Definitions

18.2.1 Ethnobotany

- The term 'ethnobotany' was first introduced in 1896 by John Harshberger.
- Ethnobotany studies the entire intricate relationship between plants and humans with focus on cultural practices and beliefs involved in the use of plants by the various societies. Ethnopharmacology is a branch of ethnobotany.
- Ethnobotanists connect the scientific community to the indigenous cultures.

18.2.2 Quantitative Ethnobotany

- The domain of quantitative ethnobotany was first introduced in 1987 by Prance.
- Quantitative ethnobotany is defined as 'the application of quantitative techniques to the direct analysis of usage of contemporary plant data'.
- As rapid disappearance of knowledge about use of wild plant resource in folk communities and habitat loss of several wild plants have become a major concern at present, conservation and sustainable use of wild plant resource are mandatory. Quantitative ethnobotany incorporates appropriate quantitative methods in research for data collection, analysis and interpretation in the ethnobotanical studies and improves the indicative value of such studies.

18.2.3 Applied Ethnobotany

- Applied ethnobotany was first coined by 'People and Plants' initiative in 1992.
- Applied ethnobotany aims to:

  - Connect the gap between the scientific knowledge and traditional knowledge
  - Recognize the relationship between native practices and policies and rules set by knowledge system at national and international level

18.2.4 Ethnopharmacology

- Ethnopharmacology was first mentioned in 1967 in the title book on hallucinogens.
- It is defined as 'the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man'.
- The role of ethnopharmacology is not limited by its definition. This is because ethnopharmacology also studies the cognitive foundations of plant use besides analysing the empirical aspects of indigenous plant use.

18.2.5 Pharmacognosy

- The term pharmacognosy was introduced in 1811 by Dr. Schmidt in Austria.
- While ethnopharmacology studies the pharmacological qualities of traditional medicinal drugs, pharmacognosy deals with the physical, chemical and biological properties of drugs obtained from all plants with additional focus on the search for new drugs from the modifications of naturally derived drugs.
- In Germany, the word 'pharmacognosy' is used synonymously for 'drogenkunde' which means science of crude drugs.

18.2.6 Phytochemicals and Secondary Metabolites

- Any chemical moiety that is obtained from the plant source is called as a phytochemical.
- The phytochemicals are broadly classified into two types:
  - Primary metabolite
  - Secondary metabolite
- The primary metabolites constitute the chemicals that are responsible for the basic survival, growth and reproduction of the plants, e.g. minerals, water, fat, carbohydrates, proteins, chlorophylls, nucleic acid and others.
- The secondary metabolites are the chemicals which are not immediate necessity for the survival or growth and do not play direct role in reproduction but are produced by plants in order to accomplish the following:
  - Protect the plant from the predators (toxic alkaloids).
  - Attract the pollinators like bees and other insects for pollination (colourants and fragnants) and spread of seeds to the distant areas.
  - Maintain the symbiotic relationship with other organisms.
  - Compete with the same species for better survival.

- Almost all of the drugs derived from the plants till now are secondary metabolites. They are classified into the following categories based on its chemical nature:
  - Nitrogen-containing compounds
    Alkaloids (e.g. atropine, cocaine, morphine and vincristine)
    Glucosinolates (e.g. glucobrassicin)
    Glycosides (e.g. digitoxin, ouabain and digoxin)
    Nonprotein amino acids (e.g. djenkolic acid)
  - Isoprenoids or terpenes (e.g. artemisinin, tetrahydrocannabinol and plant steroids)
  - Phenolic and polyphenolic compounds
    Natural phenols (e.g. resveratrol)
    Lignin and its derivatives (e.g. lignophenols and lignosulfonic acid)
    Tannins and its derivatives (e.g. gallic acid)
    Flavonoids (e.g. silibinin, quercetin and luteolin)
    Coumarins (e.g. scopoletin, warfarin and brodifacoum)

18.3 Role of Plants in Medicine

- Until 2006, it was estimated that more than 12,000 compounds have been isolated from the plants that have proven beneficiary effects in human health.
- Moreover, it was also predicted that the isolated medicinal compounds from the plants so far constitute only 10–20% of the resources and more than 70% are yet to be discovered.
- Traditional medicines like Ayurveda, Siddha, Unani and Chinese medicine use only plant products as the major drug source and are being practised with great success.
- Around 30% of the entire plant species are used as a source of medicine at least once till date.
- In developed nations, around 25% of drugs sold are plant-derived, and the percentage escalates to whooping 80% in the developing nations. This indicates that the herbal medicine plays a vital role in the management of ailments in the developing nations. The list of plant-derived drugs and their putative role in medicine is tabulated in Tables 18.1, 18.2, 18.3, 18.4 and 18.5 based on the organ system.

18.4 Discovery of Drugs from the Plants

- The discovery of drugs from the plants is a multilevel chained process which starts with the identification of potential use of plant products based on ethnopharmacological knowledge.
- The following are the steps involved in the discovery of drugs form the plants:
  - Basic principles in extraction
  - Collection of plant parts rich in phytochemicals

Table 18.1 List of plant-derived drugs with established/proposed medicinal use in ANS

Autonomic nervous system				
S. no	Class	Drug name	Plant source	Use
1	Cholinergic (muscarinic)	Muscarine	*Amanita muscaria* *Amanita phalloides* *Inocybe cinnamomea* *Inocybe lacera* *Clitocybe dealbata*	1. No direct therapeutic use nowadays 2. Analogues are used as miotics, in xerostomia, post-operative urinary retention, etc.
		Pilocarpine	*Pilocarpus pennatifolius* *Pilocarpus microphyllus*	1. Xerostomia 2. Glaucoma
		Arecoline	*Areca catechu*	1. Euphoriant 2. Antihelminthic
2	Cholinergic (nicotinic)	Nicotine	*Nicotiana tabacum* *Nicotiana rustica* *Duboisia hopwoodii* *Asclepias syriaca*	1. Euphoriant 2. Produces psychological and physical dependence
		Anabasine	*Nicotiana glauca*	1. Insecticide
3	Antimuscarinic	Atropine (d,l-hyoscyamine)	*Atropa belladonna* *Datura innoxica* *Datura metel* *Datura stramonium*	1. Antidote in organophosphorous compound poisoning 2. AV block 3. Midriatics in children 4. Preanaesthetic medication
		l-Hyoscine (scopolamine)	*Hyoscyamus niger* *Datura stramonium*	1. Motion sickness 2. Renal, abdominal colic 3. PONV 4. bowel syndrome
		Hyoscyamine (l-atropine)	*Mandragora officinarum* *Atropa belladonna* *Datura stramonium*	1. Renal, abdominal colic 2. Parkinsonism 3. Irritable bowel syndrome
		Anisodine (daturamine)	*Anisodus tanguticus*	1. Circulatory shock
4	Antinicotinic	d-Tubocurare	*Chondrodendron tomentosum*	1. Peripheral skeletal muscle relaxant
		Toxiferine	*Strychnos toxifera*	1. No therapeutic use
5	Ach-esterase inhibitors	Physostigmine	*Physostigma venenosum* *Hippomane mancinella*	1. Glaucoma 2. Alzheimer's disease 3. Anticholinergic poisoning
		Galantamine	*Galanthus caucasicus* *Galanthus nivalis*	1. Alzheimer's disease

(continued)

Table 18.1 (continued)

Autonomic nervous system				
S. no	Class	Drug name	Plant source	Use
6	Adrenergic agonists	Ephedrine	*Ephedra sinica* *Catha edulis*	1. Hypotension in spinal anaesthesia 2. Stokes Adams attack 3. Asthma and bronchitis
		Pseudoephedrine	*Ephedra sinica*	1. Nasal decongestant
7	Adrenergic antagonist	Reserpine	*Rauwolfia serpentina* *Rauwolfia vomitoria*	1. Hypertension 2. Dyskinesia in Huntington's disease
		Deserpidine	*Rauwolfia serpentina*	1. Hypertension
		Ergotamine, Ergonovine	*Claviceps purpurea*	1. Hypertension 2. Postpartum haemorrhage
		Yohimbine	*Pausinystalia johimbe*	1. Aphrodisiac

Table 18.2 List of plant-derived drugs with established/proposed medicinal use in CNS and PNS

Central and peripheral nervous system				
S. no	Class	Drug name	Plant source	Use
1	CNS stimulants	Caffeine	*Coffea arabica* *Coffea canephora* *Camellia sinensis* *Cola acuminata*	1. Improve attention 2. CNS stimulants
		Strychnine	*Strychnos nux-vomica*	1. Emetic 2. Convulsants
			Strychnos ignatii	
		Cocaine	*Erythroxylum coca*	1. Local anaesthetic 2. CNS stimulant
2	Antidepressant	Glasiovine	*Octea glaziovii*	1. Depression
3	Sedative/ tranquilizer	Kavain	*Piper methysticum*	1. Anxiety 2. Insomnia
		Rescinnamine	*Rauwolfia serpentina*	
		Rhomitoxin	*Rhododendron molle*	
		Tetrahydropalmatine (rotundine)	*Stephania rotunda*	
4	Analgesic	Morphine	*Papaver somniferum*	1. Post-operative analgesia 2. Recreational drug
		Codeine		
		Salicin	*Salix alba*	1. Anti-inflammatory and analgesic
		Tetrahydrocannabinol and cannabidiol	*Cannabis sativa*	1. Appetite suppressant 2. Alzheimer's 3. Epilepsy (LGS)

(continued)

Table 18.2 (continued)

Central and peripheral nervous system				
S. no	Class	Drug name	Plant source	Use
5	Analeptic	Picrotoxin (Cocculin)	*Anamirta cocculus*	1. Respiratory stimulant
6	Local anaesthetic	Cocaine	*Erythroxylum coca*	1. Local anaesthetic 2. Recreational drug
7	Antitussive	Codeine	*Papaver somniferum*	1. Tiresome dry cough 2. Psychological dry cough
		Noscapine		
8	Cognitive enhancers	Ginkolides	*Ginkgo biloba*	1. Alzheimer's disease
		Bilobides		
9	Hallucinogenic	Lysergic acid amide	*Ipomoea violace*	1. Recreational use
		Psilocybin	*Psilocybe mexicana*	
		Harmine	*Banisteriopsis caapi*	
		Mescaline	*Lophophora williamsi*	
		Tetrahydrocannabinol and cannabidiol	*Cannabis indica*	1. Appetite suppressant 2. Alzheimer's disease 3. Epilepsy (LGS)

Table 18.3 List of plant-derived drugs with established/proposed medicinal use in CVS

Cardiovascular system				
S. no	Class	Drug name	Plant source	Use
1	Inotropic agents	Digoxin	*Digitalis lanata*	1. Congestive heart failure 2. Antiarrhythmic agent (SVT)
		Digitoxin	*Digitalis purpurea*	
		Ouabain	*Strophanthus gratus*	
		Oleandrin	*Nerium oleander*	1. Toxic compound 2. Tried as anticancer agent
		Antiarin	*Antiaris toxicaria*	1. Tried as anticancer agent
2	Antihypertensive	Reserpine	*Rauwolfia serpentina* *Rauwolfia vomitoria*	1. Resistant hypertension (obsolete)
3	Antiarrhythmic	Quinidine	*Cinchona officinalis*	1. SVT and VT (obsolete)
		Atropine	*Atropa belladonna*	1. Atrioventricular block
		Digoxin and digitoxin	*Digitalis lanata* *Digitalis purpurea*	1. SVT
4	Anticoagulant	Bishydroxy-Coumarin	*Dipteryx odorata* *Galium odoratum* *Melilotus officinalis*	1. Anticoagulant 2. Produce uricosuric effect 3. Lymphedema management 4. Component of rodenticide

Table 18.4 List of plant-derived drugs with established/proposed medicinal use in chemotherapy

Chemotherapy				
S. No.	Class	Drug name	Plant/fungal source	Major therapeutic use
1	Antibacterial	Penicillin	*Penicillium chrysogenum*	Syphilis, leptospirosis
		Cephalosporin	*Cephalosporium acremonium*	Various Gram +ve and −ve infections
		Neomycin	*Streptomyces fradiae*	Topical wounds, hepatic coma
		Streptomycin	*Streptomyces griseus*	Tuberculosis, Gram –ve infections
		Gentamicin	*Micromonospora purpurea*	Gram –ve infections
		Netilmicin	*Micromonospora inyoensis*	Various Gram +ve and −ve infections
		Tobramycin	*Streptomyces tenebrarius*	
		Oxytetracycline	*Streptomyces rimosus*	Broad spectrum antibiotic
		Demeclocycline	*Streptomyces aureofaciens*	Broad spectrum antibiotic
		Chloramphenicol	*Streptomyces venezuelae*	Broad spectrum antibiotic
		Erythromycin	*Streptomyces erythraeus*	Gram +ve infections
		Vancomycin	*Streptomyces orientalis*	MRSA
		Teicoplanin	*Actinoplanes teichomyetius*	Reserve drug used against resistant *Staph. aureus* infection
		Daptomycin	*Streptomyces roseosporus*	Reserve drug
		Rifamycin	*Streptomyces mediterranei*	GIT infections
		Thienamycin	*Streptomyces cattleya*	Precursor for carbapenems
		Aztreonam	*Chromobacterium violaceum*	Reserve drug
		Clavulanic acid	*Streptomyces clavuligerus*	Beta-lactamase inhibitor
		Pristinamycins	*Streptomyces pristinaespiralis*	Vancomycin-resistant *E. faecium* infection
		Spectinomycin	*Streptomyces spectabilis*	Alternative to cephalosporins for gonorrhoea in pregnancy

2	Antifungal	Griseofulvin	*Penicillium griseofulvum*	Dermatophytosis
		Amphotericin B	*Streptomyces nodosus*	Systemic fungal infections
		Caspofungin	*Glarea lozoyensis*	Candidiasis, aspergillosis
		Micafungin	*Coleophoma empedri*	Deeply invasive candidiasis
		Anidulafungin	*Aspergillus nidulans*	Candidiasis
		Nystatin	*Streptomyces noursei*	Dermatophytosis
3	Anti-protozoal	Artemisinin	*Artemisia annua*	Malaria
		Quinine	*Cinchona officinalis*	Complicated malaria
		Emetine	*Carapichea ipecacuanha*	Anti-amoebic agent
		Fumagillin	*Aspergillus fumigatus*	Microsporidial infections, *Encephalitozoon hellem* keratoconjunctivitis
4	Antihelminthic	Ivermectin	*Streptomyces avermitilis*	Onchocerciasis, lymphatic filariasis, strongyloidiasis, scabies
5	Antiviral	Shikimic acid (precursor of oseltamivir)	*Illicium verum* (*star anise*)	Influenza
6	Anticancer	Pentostatin	*Streptomyces antibioticus*	Hairy cell leukaemia
		Vinca alkaloids	*Catharanthus roseus*	Hodgkin's lymphoma, neuroblastoma, Kaposi sarcoma, choriocarcinoma
		Taxanes	*Taxus brevifolia*	Breast, ovarian, head, neck and lung cancers
		Camptothecin	*Camptotheca acuminata*	Ovarian and small cell lung cancer
		Dactinomycin	*Streptomyces parvulus*	Rhabdomyosarcoma, soft tissue sarcoma, Wilms' tumour
		Anthracyclines	*Streptomyces peucetius*	AML
		Epipodophyllotoxin	*Podophyllum peltatum*	Small cell lung cancer, testicular carcinoma, non-Hodgkin's lymphoma
		Bleomycin	*Streptomyces verticillus*	Germ cell tumours of testis and ovary

Table 18.5 List of plant-derived drugs with established/proposed medicinal use in other systems

Miscellaneous system				
S. No	Class	Drug name	Plant source	Use
1	Hypolipidemic drug	Mevastatin	*Penicillium citrinum*	Prototype for all currently available statins
		Lovastatin	*Aspergillus terreus*	
2	Immunosuppressant	Cyclosporine	*Beauveria nivea*	Transplant rejection
		Rapamycin	*Streptomyces hygroscopicus*	Transplant rejection
3	Bronchodilators	Theobromine	*Theobroma cacao*	Asthma
			Ilex paraguariensis	COPD
			Camellia sinensis	
		Caffeine	*Coffea arabica*	
			Coffea canephora	
			Camellia sinensis	
			Cola acuminata	
		Theophylline	*Camellia sinensis*	
			Theobroma cacao	
4	Mucolytics	Vasicine	*Adhatoda vasica*	Precursor for bromhexine
5	Anti-gout	Colchicine	*Colchicum autumnale*	Pain reliever in acute gout
6	Antidiabetic	Biguanides	*Galega officinalis*	Precursor of metformin
		Acarbose	*Actinomyces* sp.	Alpha glucosidase inhibitor
7	Laxatives and purgatives	Ispaghula	*Plantago ovata*	Constipation
		Sennoside A and B	*Cassia acutifolia*	
		Barbaloin	*Cascara sagrada*	
		Chrysaloin	*Cascara sagrada*	
		Rhubarb	*Rheum rhabarbarum*	
		Ricin	*Ricinus communis*	
8	Antidiarrhoeal	Berberine	*Berberis vulgaris*	Diarrhoea
			Berberis aristata	Antidiabetic potential under research

- Identification and authentication
- Choice of solvent
- Pre-extraction preparation of plant samples
- Extraction of phytochemical from the plant parts
- Subjection of pure phytochemical for structure analysis

18.4.1 Basic Principles

The extraction of the drugs from the plants depends upon:

- Partition of components between solid residuals and the solvent phase used
- Diffusion nature of the component
- Quantity and nature of the drug
- Mixing ratio of the solvent
- Rate of equilibrium establishment between the drug and solvent
- Process governing the separation
- Degree of size reduction of plant materials
- Method of preparation of solution from lysed and intact cells
- Interaction between the dissolved constituents and the insoluble support materials of the plant

18.4.2 Collection of Plant Parts Rich in Phytochemicals

- After a deep literature review and the floristic diversity survey, the interested plant should be identified.
- The selection of plant material can be performed by totally random selection or by using ethnopharmacological data or by using natural product alert (NAPRALERT) or literature information selection technique (LIST).
- The first and major requirement in specimen collection is that the plant parts collected should be healthy and free from diseases, infestations and malnutrition. This is because the quality and quantity of the phytochemicals change in these situations.
- The factors influencing the quality and quantity of the phytochemicals in the plants are:
  - Age of the plant
  - Habitat
  - Climate and type of soil
  - Timing of collection of the plant parts
  - Local factors like infection, pesticides and other environmental contaminants

18.4.3 Identification and Authentication

- After collection, the plant and its parts should be correctly identified and vetted with reputed botanists (preferably in standard institute of local area).
- Three specimens should be prepared to the minimum. One specimen should be kept in the National Herbarium in the locality. Remaining two specimens should be kept either in specialist museum or in the suitable place with appropriate protection for further references in future.

Table 18.6 Solvents commonly employed in extractions of phytochemicals

S. No.	Solvent	Nature	Used to dissolve
1	Water	Polar	Polysaccharides, phenols, aldehydes, ketones, amine, other oxygen-containing compounds
2	Petroleum ether, chloroform, diethyl ether, *N*-Hexane, benzene, toluene, cyclohexane, carbon tetrachloride	Non-polar	Lignin, wax, lipid, aglycone, sterols and terpenoids
3	Methanol, ethanol, acetone, ethyl acetate, acetone, *N*-propanol	Semi-polar	Sterol, flavonoids, terpenoids, phenolic compound and alkaloids

- The place of collection, description about the environment, details of the altitude, season timing, parts taken from the plants and the characteristics should be mentioned while keeping in the herbarium.

18.4.4 Choice of Solvent

- The simple rule 'like dissolves like' must be remembered always. Phytochemicals with polar nature are soluble in polar solvents, and the same is true for non-polar phytochemicals too.
- The pH of the solvents also plays an important role in extraction of phytochemicals. Polar aqueous acids are used to extract non-polar alkaloids (as they are basic and form salts in acidic medium).
- Phytochemicals with acidic nature (like fatty acids and phenols) are extracted using solvents at acidic pH.
- For phytochemicals with ester nature, pH should be tightly regulated as esters can hydrolysed in alkaline pH. The same applies for phytochemical with glycoside nature, as the sugar moiety in the glycoside can be lost in acidic pH.
- The solvent should be easy to remove, non-toxic, inert, not inflammable and with minimal or nil chemical interactions (Table 18.6).

18.4.5 Pre-extraction Preparation of Plant Samples (Drying, Grinding and Powdering)

- The main aim of this section is to preserve the biomolecules in the plant parts before the actual extraction process.
- Both fresh and dried samples can be used for the extraction of the phytochemicals.
- A fresh sample is meant by sample subjected to extraction within 3 h of collection from the plant to the extraction. Fresh samples are usually delicate and deteriorate at a faster rate than the dried samples.

- Dried samples are commonly used than the fresh samples as it is convenient and relaxing and allows time needed for experimental designs. Shadow (air) drying rather than direct drying under sunlight is usually employed. Other methods of drying are:
  - Microwave drying (shortens the drying time)
  - Oven drying (yields same quality of phytochemicals like air drying at short duration)
  - Freeze drying (yields high levels of phenolic phytochemicals)
- The phytochemical constituent can vary between fresh and dried samples. For example, dried *Moringa oleifera* leaves have higher flavonoids than the fresh samples.
- The surface contact between samples and the solvents can be increased by lowering the size of particles by grinding or powdering.
- Coarse particles will be seen in grinding, while smaller homogenized particles will be achieved in powdering.
- Grinding or powdering is done by traditional mortar-pestle or electric blenders.
- Particle size less than 0.5 mm is ideal for the successful extraction of the phytochemicals.

18.4.6 Extraction of Phytochemicals: Step 1 – Solvent Penetration into Tissues with Solubilization of Secondary Metabolite

- The process of separation of medicinally active moieties from the plant parts using the appropriate solvents through the scientifically standardized processes is called *extraction*.
- The initial step is to achieve the good penetration of solvents into the plant tissue so that the phytochemicals will be solubilized properly and hence easily extracted.
- Depending upon the component, one solvent with varying solubilizing capacity will be used, or mixtures of solvents are used.
- In this step, large part of unwanted materials like chlorophyll, fats, etc. will be removed. This can be achieved by the following methods:
  - *Maceration*: It involves soaking plant powders with solvent in airtight container for a minimum of 3 days with repeated agitations. It softens the plant cell wall, and phytochemicals are released.
  - *Infusion*: It is the same like maceration except the sample is soaked in cold solvent.
  - *Decoction*: It is the same like maceration except the sample is soaked in boiled water for specified time and reduces the processing time. It is suitable for isolation of heat stable components.
  - *Percolation*: It utilizes specialized equipment called percolator. The same principle like decoction except that the solution percolates at the rate of six to eight drops/min until the extraction. Then the extract is evaporated into concentrated form.

- *Soxhlet extraction (hot continuous extraction)*: The finely ground sample is kept in a porous bag in the thimble chamber of the Soxhlet apparatus. The extraction solvent is heated with Bunsen burner in the bottom flask, and the solvent vaporizes into the thimble and gets condensed in the condenser and finally recycled. It requires only small quantity of solvent. Flammable solvent should be used with high precautions.
- *Microwave-assisted extraction (MAE)*: In this method, heating is achieved in the sample by microwave due to interactions of microwave with dipoles of polar and polarizable materials. Microwave electromagnetic disruption causes breaking of hydrogen bonding and increases the migration of dissolved ions resulting in good solvent penetration into the plant cell. Solvent with high dielectric constant (most polar) can be used for extraction by this method.
- *Ultrasound-assisted extraction (sonication extraction)*: Ultrasound from 20 to 2000 kHz is used in this method to produce acoustic cavitation's mechanic effect which in turn increases surface contact between the sample and the solvent. UAE is a simple method to use; however, this method induces many free radicle formation.
- *Other extraction methods*: Other methods like accelerated solvent extraction, supercritical fluid extraction, supercritical chromatography, hydro-distillation, enfleurage and ecuelle are also employed occasionally.

18.4.7 Extraction of Phytochemicals: Step 2 – Fractionation of Extract with Subsequent Analysis

- The extract obtained in the step 1 will be usually a mixture of various phytochemicals. This is because various phytochemicals have the same degree of polarity and get dissolved in solvents. Many times mixture of solvent is used, and hence extract will also contain various phytochemicals.
- Hence the next step is to fractionalize the crude extract into separate groups of chemical moieties.
- This is achieved by the following methods:
  - Open silica column method
  - Counter current extraction and distribution
  - Fractional distillation (hydro-distillation, hydro-steam distillation and steam distillation)
  - Fractional crystallization
  - Sublimation
- The resultant solutions obtained during the fractional extraction at different time interval are called as *fractions*. Each fraction has one or two closely related phytochemicals and exhibits unique properties like in colour and odour.
- All the fractions should be labelled with date of extraction, time of extraction, process utilized and details of solvents used for future reference. The fractions should be stored in appropriate conditions or can be subjected immediately to the next step 'purification'.

18.4.8 Extraction of Phytochemicals: Step 3 – Separation of Desired Component with Adequate Purity

- This is the final step in the extraction process aimed to obtain the desired phytochemical with adequate purity.
- This can be achieved by the following methods:
  - High-performance liquid chromatography (HPLC)
  - Gas chromatography
  - Nuclear magnetic resonance (NMR)
  - Fluorimetry
  - Liquid chromatography and mass spectrometry (LCMS)
  - LC-NMR
  - Thin-layer chromatography
- The chromatography techniques work by separation of mixture of compounds by their continuous distribution between stationary and mobile phases. The quality of yield between the different chromatographic techniques varies with lowest in thin-layer chromatography and highest with HPLC and LCMS.

18.4.9 Structure Activity Analysis of Obtained Phytochemicals

- After isolation of interested phytochemicals, it is vital to know their detailed structure. Presence of hydroxyl, aryl or alkyl group in the structure may influence the nature of the phytochemicals with regard to biological effects.
- The following techniques are used to determine the structural details of the isolated phytochemical:
  - Ultraviolet spectroscopy
  - Infrared spectroscopy and Fourier-transform infrared spectroscopy (FTIR)
  - NMR spectroscopy
  - Mass spectroscopy
- All the spectroscopic techniques work by passing electromagnetic radiation through an organic molecule which absorbs a part of radiation. A spectrum can be produced by measuring the amount of radiation absorbed by the molecule.
- Since each spectrum is unique to the particular molecules (aryl, hydroxyl or ester), the basic structure can be mapped.
- Generally, spectra from three or more regions (ultraviolet, infrared, radio frequency and electron beam) are compared for the structural clarification.
- UV spectroscopy can detect anthocyanins, tannins, polymer dyes and phenols
- Infrared spectroscopy can detect bonds like C=O, O-H, C-C and C=C.
- Detailed structural analysis can be done by using mass spectrometry.

18.4.10 Development of Lead Molecule After SAR Analysis

- This is the final step before proceeding to the preclinical toxicity studies after a successful isolation of the phytochemical and mapping its structure.
- Various chemical modifications will be done to the isolated phytochemical, and all the molecules will be subjected to initial receptor, protein and enzyme interactions via computer-assisted modelling.
- Few lead molecules amongst the series of closely related phytochemicals will be identified and then selected for the preclinical toxicity studies.

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Pharmacoeconomics

19

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Abstract

Pharmacoeconomics is a field of economics that compares the costs and outcomes of various pharmaceutical products and treatment strategies. The different types of health costs include direct (medical and non-medical costs), indirect and intangible costs. The pharmacoeconomic analyses are usually done based on a single or multiple perspectives; namely patient, health care provider, payer or societal perspective(s). The various methods of pharmacoeconomic evaluation are cost-of-illness analysis, cost-minimization analysis (CMA), cost-effectiveness analysis (CEA), cost-benefit analysis (CBA) and cost-utility analysis (CUA). The results of the pharmacoeconomic analyses can be used in clinical decision making, formulation and management of formulary, framing policies and guidelines for drug use and allocation of scarce health resources.

Keywords

Cost-minimization · Cost-effectiveness · Cost-benefit · Cost-utility

19.1 Introduction

- Pharmacoeconomics is defined as 'the branch of economics that uses cost-benefit, cost-effectiveness, cost-minimization, cost-of-illness and cost-utility analyses to compare pharmaceutical products and treatment strategies'.
- Pharmacoeconomic information is very useful for policy-makers and to health-care decision-makers to identify which treatment alternative provides best outcome for the resources being invested.

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- The two essential components of pharmacoeconomic studies are costs and outcomes.
  - Cost – usually measured in terms of money invested
  - Outcomes – could be positive (efficacy of drug) and/or negative (adverse effects, treatment failure or relapse, development of drug resistance)

19.2 Types of Costs

- The different types of health costs or economic outcomes are described in Fig. 19.1.
- Other types of costs involved in pharmacoeconomic analysis are opportunity costs and incremental costs.
- Opportunity cost – It refers to the economic benefit which is relinquished while choosing one therapeutic alternative over the other.
- Incremental cost – It refers to the extra cost required to buy an additional unit of benefit for one therapeutic alternative compared to the other.

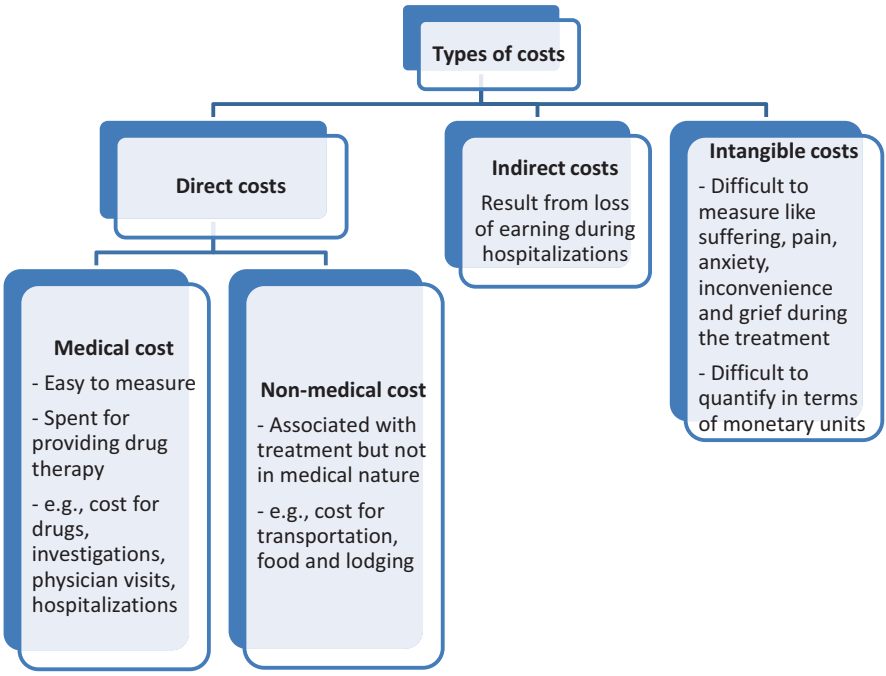

Fig. 19.1 Different types of health costs or economic outcomes involved in pharmacoeconomic analysis

19.3 Perspectives in Pharmacoeconomic Analysis

Pharmacoeconomic analysis is usually done based on single or multiple perspectives. The various perspectives are:

- Patient perspective – used when assessing the impact of the therapy on quality of life of patient
- Healthcare provider (hospitals or private-practice physicians) perspective – used while making decisions regarding formulary management or drug-use policy-making.
- Payer (insurance companies, employers or the government) perspective – used when insurance companies/employers are selecting healthcare benefits for their policy holders/employees
- Society perspective – broadest of all perspectives and is used when a holistic view of impact on society is required

19.4 Methods of Pharmacoeconomic Evaluation

19.4.1 Cost-of-Illness or Burden of Illness Evaluation

- The cost-of-illness analysis measures both the direct and indirect costs attributed with a specific disease, e.g. type 2 diabetes mellitus. Thus, it helps to estimate the financial burden posed by a disease.
- It also helps to estimate the relative value of preventive and therapeutic strategies for that particular disease.

19.4.2 Cost-Minimization Analysis (CMA)

- Cost-minimization analysis helps to identify the least costly therapy among various treatment options.
- Applicable only when outcomes of both the therapies are identical – therapeutically equivalent with similar efficacy and adverse effect profile.
- Involves measuring only cost of the treatments in terms of monetary units – both direct and indirect costs need to be measured.
- It shows the amount of cost saved when choosing one treatment over the other.
- Useful in comparing generic drugs or me-too drugs.

19.4.3 Cost-Effectiveness Analysis (CEA)

- Cost-effectiveness analysis is used for comparing treatment options with differing efficacy and safety (i.e., options that are not therapeutically equivalent).
- One of the most commonly used pharmacoeconomic analysis.

- It is used in the preparation and management of drug formulary and in making individual patient treatment decisions.
- Cost is measured in monetary terms.
- Outcome (effectiveness) is measured in nonmonetary terms (e.g. clinical outcomes such as number of lives saved, complications prevented, diseases cured or reduction in blood pressure).
- The results of this analysis are expressed as a ratio called as 'incremental cost-effectiveness ratio (ICER)'.

$$\text{ICER} = \frac{\text{Cost of drug A} - \text{Cost of drug B}}{\text{Effectiveness of drug A} - \text{Effectiveness of drug B}}$$

- ICER helps to calculate the additional cost required to produce the additional benefit when choosing one alternative over the other, e.g. cost per extra patient cured in choosing one treatment over the other.

19.4.4 Cost-Benefit Analysis (CBA)

- Cost-benefit analysis method incorporates both cost and outcomes (benefit) in terms of monetary units.
- Outcomes like 'gain in life years' and 'quality of life' can be converted into monetary units in terms of 'average wage' and 'willingness to pay' (cost that individuals will be volunteering to pay for the gain in quality of life), respectively.
- The costs and benefits are expressed as 'benefit-to-cost (B:C) ratio' for each treatment option.
- Interpretation of benefit-to-cost (B:C) ratio:
  - If B:C ratio < 1, then the cost of the therapy outweighs its benefits.
  - If B:C ratio = 1, then the benefits of the therapy are equivalent to its cost.
  - If B:C ratio > 1, then the benefits of the therapy outweigh its cost.
  - The treatment option with highest benefit-to-cost ratio will be selected.
- The main advantage of this analysis is that different treatment modalities can be compared, e.g. drug therapy vs. surgery. Also it helps in decision-making regarding resource allocation.
- The main disadvantage of this analysis lies in the difficulty and controversy in assigning monetary values for various health benefits.
- This type of pharmacoeconomic analysis is not commonly used.

19.4.5 Cost-Utility Analysis (CUA)

- In this analysis, outcome is measured in terms of utility like quality-adjusted life years (QALYs) gained.
- QALY helps in assessment of both quantity and quality of life. An increased quality of life is expressed on a scale of 0 (dead) to 1 (best quality of life). For

example, a year of life spent without any disease will be given a QALY of 1, whereas a year of life spent with some disease will be assigned a lower QALY value (e.g. 0.7) depending on the severity of the disease.

- In this method, cost-utility (C:U) ratio is calculated which gives the cost per QALY gained for each treatment option.
- The treatment option with the least cost per QALY gained is usually selected.
- This analysis is complex and not commonly used unless quality of life is the most important outcome being evaluated.
- This analysis is best suited for comparison of therapeutic options which
  - Prolong life expectancy but produce serious adverse effects, e.g. chemotherapeutic drugs
  - Produce reduction in morbidity (improve quality of life) but does not alter the morbidity, e.g. drug treatment for arthritis

19.5 Applications of Pharmacoeconomics

Pharmacoeconomic analysis has the following applications:

- Clinical decision-making
- Formulation and management of formulary
- Framing policies and guidelines for drug use
- Allocation of scarce health resources

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Orphan Diseases and Drugs

20

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Abstract

An orphan disease refers to a disease with a low prevalence of less than 6.5–10 cases in 10,000 people. The National Organization for Rare Disorders (NORD) and Orphanet are the organizations working for the welfare of people with orphan diseases. Orphan drugs are defined as the drugs used for the diagnosis, prevention, or treatment of orphan diseases. The US-FDA provides a list of orphan drug designations and approvals. Partial orphan drugs are those drugs having both orphan and non-orphan indications. Orphan drug development faces many challenges since pharmaceutical companies are generally reluctant in undertaking drug development for orphan diseases due to their low market value. Thus, in order to promote orphan drug development, various strategies have been tried like the enactment of specific legislations and providing incentives for the development of orphan drugs.

Keywords

Neglected diseases · Orphan drugs · Rare diseases

20.1 Definition of Orphan Disease

- The definition of an orphan disease still remains controversial and varies from country to country.
- According to WHO, orphan disease refers to a disease with low prevalence of less than 6.5–10 cases in 10,000 people.
- In the USA, orphan disease is defined as one that affects less than 200,000 individuals.

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- In Europe, it is defined as a disease with prevalence of less than 5 in 10,000 people.

20.2 Organizations for Orphan Diseases

- National Organization for Rare Disorders (NORD) – An American registered nonprofit organization established in 1983 by a group of patient advocates of rare diseases. It is involved in voicing the needs of rare disease community and aided the development of treatments for rare diseases. It played a key role in passing of the Orphan Drugs Act in 1983.
- Orphanet is a global network of 37 countries, coordinated by Orphanet Coordinating team at the French National Institute of Health and Medical Research (INSERM) in Paris. Its website portal provides a rich source of information on rare diseases which are freely accessible like:
  - Inventory of rare diseases and orphan drugs
  - Encyclopedia of rare diseases
  - OrphaNews (a fortnightly newsletter)
  - Orphanet report series (thematic reports)
  - Orphadata (datasets related to orphan diseases and drugs)
  - Orphanet rare disease ontology (ORDO)

20.3 Orphan Drugs

- Orphan drugs are defined as drugs used for the diagnosis, prevention, or treatment of orphan diseases. "Orphan drug" status can also be granted to vaccines and diagnostic agents apart from drugs.
- These drugs generally do not provide a lucrative market, and thus sponsors are reluctant in developing these drugs.
- However, these drugs respond to the healthcare needs of the people with orphan diseases, and these patients have the same right to be cared for as any other patient.
- In other words, these are drugs with one or more indications mentioned in the Orphan Drugs Act of 1983.
- The US FDA (Food and Drug Administration) provides a list of orphan drug designations and approvals. Examples of US FDA-approved orphan drugs are given in Table 20.1.

20.4 Partial Orphan Drugs

- These are drugs having both orphan and non-orphan indications.
- Adalimumab is an example of a partial orphan drug.

Table 20.1 List of US FDA-approved orphan drugs and their indications

S. no.	Orphan drug[a]	Indication
1.	Sebelipase alfa (Kanuma)	Lysosomal acid lipase (LAL) deficiency
2.	Sirolimus (Rapamune)	Lymphangioleiomyomatosis (LAM)
3.	Ivacaftor (Kalydeco)	Symptomatic treatment of cystic fibrosis
4.	Alglucerase (Ceredase)	Gaucher disease type 1
5.	Mogamulizumab (Poteligeo)	Cutaneous T-cell lymphoma
6.	Rucaparib (Rubraca)	Ovarian cancer with BRCA gene mutation
7.	Eltrombopag (Promacta)	Aplastic anemia
8.	Lenvatinib (Lenvima)	Hepatocellular carcinoma
9	Nivolumab (Opdivo)	Small cell lung cancer
10.	Pembrolizumab (Keytruda)	Primary mediastinal B cell lymphoma
11.	Nilotinib (Tasigna)	Chronic myelogenous leukemia
12.	Brentuximab vedotin (Adcetris)	Hodgkin's lymphoma
13.	Elotuzumab (Empliciti)	Multiple myeloma
14.	Lanadelumab-flyo (Takhzyro)	Angioedema
15.	Migalastat hydrochloride (Galafold)	Fabry disease

[a]Brand names are denoted within parentheses

  - Orphan indications – pediatric Crohn's disease, juvenile rheumatoid arthritis, hidradenitis suppurativa, and uveitis
  - Non-orphan indications – rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, plaque psoriasis, and ulcerative colitis

20.5 Challenges in Orphan Drug Development

- It is estimated that there are 7000 rare diseases in the USA, and treatments are available for only 5% of them.
- Pharmaceutical companies generally are hesitant/reluctant in undertaking drug development for orphan diseases due to the following reasons:
  - Insufficient knowledge regarding the disease pathophysiology
  - Lack of validated biomarkers and surrogate end points
  - Small patient population
  - Limited clinical expertise for rare diseases
  - Demand for the drug is very low

20.6 Strategies to Enhance Orphan Drug Development

- Specific legislations have been enacted to encourage orphan drug development, e.g., the Orphan Drug Act of 1983 (which was amended further in the subsequent years) and Rare Diseases Act of 2002 in the USA.
- The OPD (Office of Orphan Products Development) within the US FDA is involved in the promotion and development of safe and efficacious orphan drugs

and devices. It is involved in scrutinizing the applications submitted and grants the "orphan drug" status.

- Various incentives also have been provided for orphan drug development like:
  - Financial subsidies/grants
  - Market exclusivity (7 years of marketing exclusivity upon FDA approval in the USA)
  - Tax credits (50% tax credit on the expenses of undertaking clinical trials in the USA)
  - Regulatory fee waivers
  - Fast track approval
  - Protocol assistance from FDA

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Fixed-Dose Combinations

21

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Abstract

Fixed-dose combinations or FDCs are combinations of two or more active pharmaceutical ingredients in a single dosage form in a fixed ratio usually for the same indication. FDCs aim to improve the therapeutic efficacy of each component of the medication while reducing the adverse drug reactions. When produced appropriately, they have the advantage of decreasing the total cost of the medication while improving the ease of administration and adherence to the medication. FDCs make it difficult to identify the causal relationship of toxicity when it occurs and to make dose changes to the individual components. Further, many irrational combinations increase patient's drug exposure aiming to improve the profit margins of the pharmaceutical companies. Strong and rigid guidelines need to be implemented to make FDCs rational and safe.

Keywords

Fixed-dose combinations · Medication · Combinations

21.1 Introduction

- Fixed-dose combinations or FDCs are combinations of two or more active pharmaceutical ingredients in a single dosage form in a fixed ratio usually for the same indication.
- FDCs are different from *co-blistered combinations* (*CBCs*). When two or more medications are packaged in the same blister pack, they are called co-blistered combinations or CBCs.

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21.2 Rationale for the Use of FDCs

FDCs are considered to be rational when there is adequate scientific evidence showing one or more of the benefits as given below:

- Increases therapeutic efficacy
- Reduces adverse drug reactions
- Lowers individual drug doses and exposure
- Lowers the total cost
- Decreases the development of resistance or tolerance
- Improves ease and therefore improves patient adherence

21.3 Criteria for FDCs

There are few conditions to be met before drugs can be combined in a fixed-dose combination:

- The drugs must act through different mechanisms of action.
- The pharmacokinetic profile of the drugs must be similar.
- The drugs should not cause additive toxicity.
- Benefits of using FDCs over individual drugs should be present.

21.4 Advantages of FDCs

Apart from the benefits for which FDCs are combined as shown in the rationale, there can be other advantages of using FDCs over the individual drugs:

- Reduce medication errors
- Facilitate patient compliance
- Simplify and improve the security of supply chains
- Help upscale national drug programs, for example, the antiretroviral therapy or the antitubercular therapy

21.5 Disadvantages of FDCs

- Difficulty in managing toxicity of one of the drugs in the combination.
- Changing individual drug doses is not possible.
- When one drug in the combination is contraindicated, the whole FDC cannot be used in the patient.
- Lead-in dosing or dose escalation (titration) of one of the components is not possible.

- Irrational combinations increase the cost.
- Irrational antibiotic combinations increase antibiotic resistance.

21.6 Examples of Rational FDCs Listed in the WHO Essential Medicines List

21.6.1 Sulfamethoxazole + Trimethoprim

This combination causes a sequential enzymatic blockade of the production of folic acid in bacteria resulting in an antibiotic effect. The combination has been found to have a synergistic action that the combination is often bactericidal while the individual components are bacteriostatic. Sulfamethoxazole is chosen because of matching the pharmacokinetic profile.

21.6.2 Amoxicillin + Clavulanic Acid

Amoxicillin is an extended-spectrum penicillin which is susceptible to the action of beta-lactamases. Clavulanic acid inhibits the action of beta-lactamase. Hence the combination improves the spectrum of amoxicillin.

21.6.3 Levodopa + Carbidopa

This combination is used in the treatment of Parkinson's disease. Levodopa is converted to dopamine in the body by the action of DOPA decarboxylase. This reaction happens both in the central nervous system and in the periphery. The peripheral dopamine results in cardiovascular and GI side effects while reducing the bioavailability to the brain. Carbidopa prevents the peripheral decarboxylation, and hence the combination is effective for patients with Parkinson's disease.

21.6.4 Imipenem + Cilastatin

The half-life of imipenem is short when administered alone as it is degraded by renal dehydropeptidase I. This degradation is inhibited by cilastatin, and hence the antibiotic effect of imipenem is enhanced.

21.6.5 Ferrous Salt + Folic Acid

Iron and folic acid are two dietary supplements required during pregnancy. The combination improves compliance in pregnant mothers.

21.7 Examples of Irrational FDCs in the Indian Market

21.7.1 Fluoroquinolone + Nitroimidazole Combination

This combination is commonly prescribed for diarrhea and dysentery. Many cases of diarrhea might not require an antibiotic. Bacterial dysentery is treated with fluoroquinolones, while amoebic dysentery is treated with nitroimidazoles. These two rarely coexist. This FDC increases the patient's exposure to antibiotics causing increased resistance and cost.

21.7.2 Amoxicillin + Cloxacillin

Amoxicillin is an extended-spectrum penicillin commonly prescribed for Gram-negative infections. They are susceptible to the action of beta-lactamases. Cloxacillin is active against Gram-positives only and is resistant to the action of beta-lactamases. Amoxicillin + clavulanic acid is a better combination in this situation.

21.7.3 NSAID Combinations

Combinations like aceclofenac and paracetamol are commonly prescribed as the first-line pain-killers for common conditions. While the pharmaceutical industry claims some benefits, the combination is not required for many and often increases the cost and toxicity. There is no rationale in adding other NSAID combinations as well.

21.7.4 H_2 Receptor Blockers/PPI + Antiemetics

One example of such a combination is rabeprazole and domperidone. The common indication of proton pump inhibitors and H_2 receptor blockers is acid peptic disease. Vomiting is not a common feature, and the use of domperidone is not required in majority of patients.

21.7.5 Ramipril + Telmisartan

The use of two drugs with the same mechanism of action is generally irrational. Multiple ACE inhibitors are generally prescribed for patients with hypertension in view of decreasing the number of drugs that particular patient has to consume. However, most patients do not require simultaneous treatment with two drugs with the same mechanism of action.

21.8 Challenges for FDCs

21.8.1 Pharmaceutical Challenges

- Two or more drug to be made into an FDC must be physically and chemically compatible.
- The particle size of one can affect the uniform distribution of the other in the formulation.
- The dissolution profiles of the drugs combined should match.

21.8.1.1 Market Pressure

- Increased market pressure results in numerous irrational fixed-dose combinations being released to the Indian market

21.9 Examples of Some Banned FDCs in the Indian Market

21.9.1 Irrational Combinations of Aceclofenac (Examples)

- Aceclofenac (SR) + paracetamol
- Aceclofenac + paracetamol + famotidine
- Aceclofenac + paracetamol + rabeprazole

21.9.2 Combinations with Ambroxol and Other Cold Preparations (Examples)

- Ambroxol + levocetirizine + phenylephrine + guaiphenesin + menthol
- Ambroxol + guaifenesin + phenylephrine + chlorpheniramine
- Ambroxol + salbutamol + choline theophylline + menthol
- Caffeine + paracetamol + chlorpheniramine
- Caffeine + paracetamol + phenylephrine + cetirizine
- Dextromethorphan + levocetirizine + phenylephrine + zinc
- Levocetirizine + phenylephrine + ambroxol + guaiphenesin + paracetamol
- Azithromycin + ambroxol

21.9.3 Antibiotic Combinations (Examples)

- Azithromycin + cefixime
- Azithromycin + cefpodoxime
- Azithromycin + levofloxacin
- Azithromycin + ofloxacin

- Clobetasol + neomycin + miconazole + clotrimazole
- Ciprofloxacin + fluticasone + clotrimazole + neomycin
- Doxycycline + serratiopeptidase

21.9.4 Diabetic Combinations (Examples)

- Metformin 500/500 mg + gliclazide SR 30/60 mg + pioglitazone 7.5/7.5 mg
- Metformin 850 mg + pioglitazone 7.5 mg + glimepiride 1 mg
- Pioglitazone 30 mg + metformin 500 mg
- Voglibose and metformin + chromium picolinate

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Translational and Reverse Pharmacology

22

Abialbon Paul

Abstract

Translational pharmacology is the branch of pharmacology that deals with the application of results from molecular and preclinical research into clinical practice. Translational pharmacology aims to reduce the high failure rate of drug development process, thereby reducing the cost and time required for bringing a new drug to the market. It does this by elucidating the target properly, improving the predictive value of early preclinical trials and optimizing dose selection. Reverse pharmacology is the field of drug discovery where the drug targets which might act as critical intervention points in the pathogenic process of diseases are first identified and characterized. Reverse pharmacology aims to characterize the target first and fish for an appropriate ligand.

Keywords

Translational pharmacology · Reverse pharmacology · Drug discovery

22.1 What Is Translational Pharmacology?

Translational pharmacology is the branch of pharmacology that deals with the application of results from molecular and preclinical research into clinical practice. In essence, translational pharmacology helps translate the results obtained in the laboratory into clinically meaningful practices. This helps in increasing the number of drug candidates that will succeed as marketable drugs and decrease the number of drug candidates that will fail later in the drug development process, hence, speeding up the drug development process.

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22.2 Why Translational Pharmacology?

- Increased cost of the drug development process.
- High failure rate of the traditional drug development process.
- Poor reproducibility of results from molecular research and other preclinical animal studies.
- Failure to pick up important toxicities observed during the post-marketing surveillance in the preclinical phases.
- The major reason for a drug to fail during the later stages of drug development process is lack of efficacy.

22.2.1 Reasons for Poorly Reproducible Results from Preclinical Testing

- Poorly written methods.
- Not quantifying the context where the tools work.
- Statistically underpowered studies.
- Less rigorous experimental designs.
- Non-suitability of the models being tested.
- No consideration for the presence of comorbidities in the disease process.
- Inadequate modelling from animal to human pathophysiology.
- *P* hacking refers to the process of analyzing data to show statistically significant results, while no such effect exists in reality.

22.3 Main Objectives of Translational Pharmacology

- Improve the predictive value of the tools that assess the success or suitability of drug candidates during preclinical research.
- Bring down the costs of the drug development process.
- Shorten the time frame for bringing a new drug into the market.
- Decrease attrition of the drug candidates late in the drug development process.
- Predict the response of the drug in the diseased population including adverse drug reactions, mode of resistance and long term toxicity.

22.4 Processes of Translational Pharmacology

22.4.1 Better Target Elucidation

- The molecular target is better elucidated with the help of genomic databases and high-throughput assays. The role of the receptor in the pathogenesis of the disease has to be fully understood to create drugs with higher efficacy and lesser side effects.

- Availability of genetic libraries helps in the computational discovery of receptors and receptor structures to be tested as molecular targets in specific pathogenic processes.

22.4.2 Improving Predictive Value of Early Preclinical Trials

The predictive value of early preclinical trials can be improved by:

- Chip-based genetic assays that can improve the accuracy and speed of detection
- Human cell lines instead of small animals
- Better mathematical modelling of pharmacokinetic data from smaller animals to humans
- Improving the disease model to emulate all characteristics observed in the clinics
- In silico studies to better characterize the drug receptor binding and potential off-target side effects

22.4.3 Dose Selection

- Use of in silico assays and mathematical modelling helps in the selection of appropriate dose ranges to be tested in human volunteers thereby helping to increase the efficacy of the drug tested while reducing the number of adverse drug reactions.

22.5 Hurdles to Translational Pharmacology

Some of the hurdles in the deployment of translational pharmacology processes are as follows:

- Lack of cooperation among researchers
- Inadequate financial support
- Difficulties with the use of some genetic databases

22.6 Reverse Pharmacology

Reverse pharmacology is the field of drug discovery where the drug targets which might act as critical intervention points in the pathogenic process of diseases are first identified and characterized. It is followed by high-throughput screening of large chemical libraries to identify suitable chemical ligands with high affinity and specificity for the chosen drug target. Reverse pharmacology is also called as *target-based drug discovery (TDD)*.

22.6.1 Basic Steps in Reverse Pharmacology

- **Target Mining**
  - A suitable receptor target that could play a role in altering the course of the disease in question is identified.
  - Bioinformatics and data from the human genome project are used to computationally compare DNA sequences and three-dimensional protein structures to find novel targets.
  - Targets can also be identified by clinical observation of the effects of various drugs used in alternative medicine like in Ayurveda.
- **Ligand fishing**
  - Ligand-binding studies are carried out with large chemical libraries to find suitable candidates that will bind to the target with higher affinity and specificity.
  - Promising candidates are then subjected to chemical modification, and their biological action is assessed through cellular assays.
  - The final drug candidate is then tested in animals, and extensive clinical trials are done.

22.6.2 Differences from Classical or Forward Pharmacology

- The initial step in classical pharmacology is the identification of functional activity of a compound.
- The mechanism of action of the drugs is elucidated, and proof of concept studies are done to confirm its beneficial activity.
- The molecular target is identified, and further drug candidates may be screened for better affinity and specificity to the target.
- The compound is then modified for better selectivity and pharmacokinetics.
- Preclinical and clinical testing is done before the drug is released in the market.

22.6.3 Advantages of Reverse Pharmacology over the Conventional Approach

- Increased speed of drug delivery
- Better chances of success in clinical trials as the affinity and specificity of the drug to the target are better established
- Decreases drug discovery costs
- An efficient process of drug discovery compared to the traditional method of drug discovery

22.6.4 Reverse Pharmacology and Ayurveda

- Drug discovery from natural products greatly utilizes the process of reverse pharmacology to discover new drugs for clinical use and large-scale manufacturing and marketing.
- Since these preparations already have a known clinical application, the extracts are tested for potential molecular targets they modulate to produce beneficial clinical action.
- Once the receptor is identified, the active principle responsible for eliciting a response from the receptor is identified and modified to make a better drug candidate that can be tested for marketing.
- Examples of application of reverse pharmacology in Ayurveda:
  - *Mucuna pruriens* for Parkinson's disease
  - *Zingiber officinale* for nausea and vomiting
  - *Picrorhiza kurroa* for hepatitis
  - *Curcuma longa* for oral cancer
  - Panchvalkala for burns and wounds
  - *Azadirachta indica* for malaria

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Gene Therapy

23

Arun Chander Yadav and Gopisankar MG

Abstract

Gene therapy technique alters cellular gene expression for therapeutic purposes through diverse methods. Different vectors are used for the transfer of gene into either somatic or germline human cells. Viral vectors are used commonly for this transfer. Adeno-, Herpes-, and Lente-viruses are the most common experimented vectors. Genes are transferred into the cells by in vivo or ex vivo mode of administrations. Non-viral vectors (particle- or chemical-based DNA transfer) are also employed for the same purpose. Gene therapy has various applications in the treatment of malignancy, cardiovascular diseases, SCID, Alzheimer's disease, and many others. Though there are a number of limitations for this mode of treatment, several gene therapy products are being approved recently by regulatory authorities. Of late, the approved drugs are talimogene laherparepvec, voretigene neparvovec, gendicine, sipuleucel-T, tisagenlecleucel, and axicabtagene ciloleucel. Several other drugs are in clinical trials and awaiting regulatory approval.

Keywords

Gene therapy · Viral vector · Non-viral vector · Herpes viral vector · Adeno viral vector

23.1 Introduction

Human gene therapy is an experimental technique that manipulates or modifies the expression of a gene for therapeutic use. It also includes the process of altering biological properties (reprogramming) of living cells or viral vectors for treating a disease.

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23.2 Mechanism of Gene Therapy

- Replacing a mutated gene that causes disease with a healthy copy of the gene
- Inactivating or "knocking out" a mutated gene that is functioning improperly
- Introducing a new gene into living cells to combat a disease
- Introducing modified virus or living cells into the human body that targets and destroys neoplastic cells

23.3 Types of Gene Therapy Products

- Viral vectors: Virus is modified by recombinant DNA technology and used to deliver therapeutic genes into human cells.
- Plasmid DNA: Genetically engineered circular DNA molecules are used for delivery.
- Human gene editing technology: Repairing mutated genes and disrupting harmful genes.
- Patient-derived cellular gene therapy products: Target cells are removed from the patient and modified with the help of vectors and then replaced.
- Bacterial vectors: Bacteria are modified and used to deliver therapeutic genes into humans (Fig. 23.1).

23.4 Viral Vectors

23.4.1 Herpes Simplex Virus

- A highly infectious neurotropic double-stranded DNA virus used in two forms: Replication-defective and replication-competent virus that can carry more than 30 kB genetic material to target cells, e.g., HSV-1 amplicon vectors and replication-defective HSV-1 vectors.

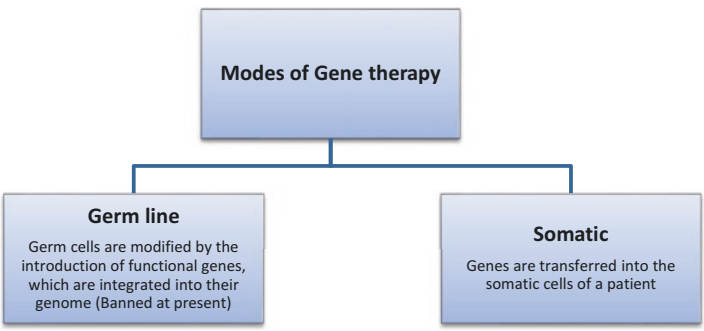

Fig. 23.1 Modes of gene therapy

- Replication-competent herpes viral product, Imlygic®/T-VEC (talimogene laherparepvec), is the first gene therapy drug approved for treatment of malignant melanoma by the US-FDA.
- Advantages:
  - More than 50% of the viral genes are accessory in function, providing more than 30 kB of space for inserting therapeutic genes.
  - The introduced DNA with transgene remains episomal as a closed circular molecule without integration into host DNA.
  - Inside the neurons, HSV can remain life long as the lytic genes become latent. However, in non-neuronal cells, it undergoes lytic replication and results in cell death.
- Disadvantages:
  - Inadequate penetration into the target interstitial space.
  - There is a chance of immunogenicity and reactivation of the virus.

23.4.2 Adeno-associated Virus

- Adeno-associated virus (AAV) is a DNA virus which is nonpathogenic in humans and is naturally replication-defective.
- Luxturna (voretigene neparvovec), is a recently approved gene therapy product for the treatment of patients with confirmed biallelic retinal pigment epithelial 65 (RPE65) mutation-associated retinal dystrophy. It uses AAV as the vector.
- Alipogene tiparvovec (Glybera®) is an adeno-associated virus serotype 1 (AAV1)-based-gene therapy that has been developed for the management of patients with lipoprotein lipase (LPL) deficiency.
- Advantages:
  - They are nonpathogenic in humans with low risk of random insertions causing mutagenesis.
  - AAV depends on the helper virus for their active replication. In the absence of helper virus, its genome remains in a latent state, because its integrative capacity is eliminated by removal of *rep* and *cap* at the inverted terminal repeats (ITRs) region.
  - After the helper virus coinfection/co-transfection, AAV starts replicating and will release the desired therapeutic gene products.
- Disadvantages:
  - It can carry only a small amount of genome (maximum capacity of 30 kB).

23.4.3 Retro Virus

- Retroviral vectors are RNA viruses used as vectors after removal of retroviral *gag*, *pol*, and *env* genes and replaced by the therapeutic gene.
- It has been tried as a vector in many disease conditions like adenosine deaminase deficiency, AIDS, malignancies, familial hypercholesterolemia, Fanconi's anemia, Gaucher's disease, Hemophilia B, and rheumatoid arthritis.

- Advantages:
  - It has the capacity to transform its single-stranded RNA to double-stranded DNA.
  - The host cell nuclear genome can be permanently modified (long transgene expression).
  - Lentivirus is a type of retrovirus with an additional ability to transduce nondividing cells. HIV virus is a type of lentivirus used in gene therapy experiments.
- Disadvantages:
  - Even though they are used in most of the experiments as vectors, most of the studies have revealed the issue of insertion mutagenesis due to the expression of the enzyme integrase.
  - Transgene capacity is only 9 kB.
  - There is a chance of germline transmission.

23.4.4 Adeno Virus

- Most commonly used virus for experiments in gene therapy.
- Gendicine (recombinant human p53 adenovirus, AdRSV-p53) was approved by the China Food and Drug Administration (CFDA) as a first-in-class gene therapy product to treat head and neck cancer.
- Advantages:
  - Adenovirus (DNA virus) can carry about 30 kb DNA and transduce dividing and nondividing cells within 1 or 2 days of administration.
  - Replication-defective virus is used to carry healthy genes, whereas replication-competent virus is used for cancer gene therapy (oncolytic virus).
  - Viral integration into the genome is minimal with no germline transmission.
- Disadvantages:
  - A short duration of expression.
  - Adenovirus is strongly immunogenic.

23.5 Non-viral Vectors

- Non-viral vector technique directly administers naked DNA or through particle-based or chemical-based carriers. It includes lipid-based DNA vectors, polymeric DNA vectors, in vivo mRNA delivery, in vivo siRNA delivery, lipid-based siRNA nanoparticle, and polymeric siRNA-based nanoparticle.
- Initially, direct injection of naked plasmid DNA near or into the site of injury has been the method of choice in gene therapy using non-viral techniques.
- Recent techniques include the following:
  - Lipoplex-mediated gene delivery in which the plasmid is made to interact with lipids and liposomes to form lipoplexes – action by sonoporation

  - Polyplex-mediated gene delivery in which the –ve charged DNA undergo ionic interactions with complexes of cationic polymers – action by proton sponge effect
  - Dendrimer-mediated gene delivery (carriers in siRNA delivery)
  - Graphene-mediated gene delivery (Polyethylenimine, PEI + Graphene oxide + DNA complexes ➔ effectively deliver plasmid DNA into cells)
- Nanoparticle technology using antisense oligonucleotides (ASOs) for RNA-targeting therapies:
  - One of the most promising approaches to modulate mi-RNA expression is the development of single-stranded ASOs that directly bind to the target mi-RNA to inhibit their function and thus causing translational repression of the target gene.
  - ASOs can also modulate pre-mRNA splicing occurring because of mutations. Fomivirsen for CMV retinitis and mipomersen for familial hypercholesterolemia are approved ASOs till date.
- Advantages:
  - Easily manufactured and produced in large numbers using genetic engineering techniques
  - No risk of neutralizing antibodies interference in therapy
  - Can carry a large amount of DNA
  - Currently used in a number of gene therapy trials
- Disadvantages:
  - Short-term gene expression
  - Relatively low transfection efficiency compared to viral vectors
  - Rarely immunogenic reactions

23.6 Methods of Administration of Gene Therapy

23.6.1 Ex Vivo

- Genetic alterations of the target cells happen outside the human body in culture.
- Target cells from patients are infected with a recombinant vector containing the desired therapeutic gene.
- Modified cells are introduced into the patient's body to generate the necessary proteins.

23.6.2 In Vivo

- Therapeutic DNA is directly placed in the patient's body.
- DNA is introduced by cell-specific direct injection using viral or plasmid vectors into the tissue.
- DNA is incorporated into cells of specific tissue that will produce the needed proteins by the encoded genes.

23.7 Gene Therapy and Diseases

23.7.1 Gene Therapy in Malignant Diseases

- Immunotherapy uses boosting of the immune system to destroy cancer cells or suppress its spread and uses gene therapy to create recombinant cancer vaccines to achieve this.
- Harvested cancer cells (either from patients; autologous or from cell lines; allogenic) are genetically engineered by including cytokine genes/highly antigenic proteins to make them more recognizable by the immune system. These altered cells are grown in vitro and killed and subsequently, the contents are used as vaccine.
- Trials are in place for pancreatic cancer, prostate cancer, lymphoma, melanoma, and renal cell cancer incorporating genes like IL2, carcinoembryonic antigen (CEA), and granulocyte-monocyte colony-stimulating factor (GM-CSF).
- Sipuleucel-T (Provenge®) is an FDA-approved cancer vaccine that is a type of autologous cellular immunotherapy indicated for the treatment of asymptomatic or minimally symptomatic metastatic castration-resistant (hormone refractory) prostate cancer.
- Another method in immunotherapy is in vivo administration of inflammatory genes to tumor cells so that the tumor cells become more antigenic and get killed by immune cells.
- The third approach is to alter the patients' immune system to target cancer cells by adding a tumor antigen or a stimulatory gene. Immune cells from the patients are collected and modified, or the same procedure can be done in vivo by viral vectors that target immune cells.
- Kymriah® (tisagenlecleucel) is an FDA-approved treatment which is a CD19-directed genetically modified autologous T-cell immunotherapy indicated for the treatment of B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse and for relapsed or refractory (r/r) large B-cell lymphoma after two or more lines of systemic therapy including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma.
- Yescarta® (axicabtagene ciloleucel) is an FDA-approved CD19-directed genetically modified autologous T-cell immunotherapy indicated for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including DLBCL not otherwise specified, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma.
- Oncolytic virotherapy utilizes the replication-competent virus to target and destroy the cancer cells with minimal effect on the normal cells.
- Gene transfer method
  - Cytotoxic/suicidal gene therapy with herpes simplex thymidine kinase (HS-tk) sensitizes the tumor cells to ganciclovir which is a natural substrate for HS-tk.

23.7.2 Hemophilia

- Hemophilia is an X-linked monogenetic coagulation disorder which presents as abnormal bleeding in various forms.
- Hepatic in vivo transfer of FVIII genes (Hemophilia A) and FXI (Hemophilia B) using AAV are executed in many preclinical studies as well as clinical trials. The patients achieve stable and long-term protein expression, well tolerated, and with less immune activation.
- Gene editing is another evolving area in the treatment of hemophilia using clustered regularly interspaced short palindromic repeats-Cas system (CRISPR-Cas) that creates DNA breaks to insert the required gene and repair using homology-directed repair (HDR).

23.7.3 Severe Combined Immunodeficiency (SCID)

- SCID-X1 is characterized by the absolute lack of T cells and natural killer cells although B cells are present.
- In SCID-X1, a mutation occurs in the *IL2R* gene which is a critical component for the expression of IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21.
- Studies done with ex vivo retrovirus-mediated transfer of gamma chain to autologous CD34 cells were found to be effective. The success of gene therapy in management of SCID-X1, reflecting as a correction of immunodeficiency, was elucidated after nearly 10 years of follow up.

23.7.4 Cystic Fibrosis

- Cystic fibrosis is caused by a mutation in a gene that code for a sodium chloride transporter called CFTR which is found on the surface of the epithelial cells that line the lungs and other organs.
- Cationic lipid-mediated CFTR gene transfer to the patients through nebulization resulted in significant correction of chloride abnormality in the patients receiving treatment compared to placebo. Bacterial adherence was also reduced.

23.7.5 Duchenne Muscular Dystrophy

- Duchenne muscular dystrophy (DMD) occurs due to loss of function mutations in the X-linked *dystrophin* (*DMD)* gene resulting in almost absolute deficiency of dystrophin in cardiac, skeletal, and smooth muscles.
- Premature termination codon (PTC) mutations are found in *dystrophin* gene, and this knowledge led to the discovery of ataluren (Translarna) that can correct this PTC mutation and was found effective in restoring functional *dystrophin* expression in mice.

- ASOs are also useful in restoring the *dystrophin* expression by the method of exon splicing. Two ASOs, namely, drisapersen and eteplirsen, are currently in clinical trials.

23.7.6 Cardiovascular Diseases

- Around 25% of cardiomyocytes are wiped out in a few hours of myocardial infarction.
- Vascular endothelial growth factor-A, by using AAV1 and AAV6, has shown improvement in tissue viability and cardiac function along with reduced apoptosis in animal studies.
- After 12 weeks of infarction, usage of fibroblast growth factor 4 using adenovirus 5 in stress-induced myocardial ischemia in animal studies has shown to increase regional myocardial function and perfusion.
- In patients with end-stage heart failure, incorporation of sarcoplasmic reticulum Ca^{2+}-ATPase using adenovirus 5 in human ventricular myocytes has resulted in enhanced pump activity and contraction velocity; it also boosted calcium concentrations in systole and diastole.
- S100A1, primarily expressed in myocardial tissue, causes modulation of cardiomyocyte calcium homeostasis and myocardial filament function resulting in profound inotropic actions which are independent of β-adrenergic stimulation. Cardiomyocytes that overexpress S100A1 seem to have a higher content of ATP in them. Introduction of S100A1 using AAV9 has shown restoration of S100A1 levels with improved calcium handling, energy homeostasis, and improvement in cardiac contractility in animal studies.

23.7.7 AIDS

- RNA interference is a newer concept in the treatment of HIV-1 infection which involves the pairing of short mi RNAs to an endogenous mRNA target; this process leads to silencing of the gene, thus impeding the further transcription of mRNA thereby preventing the active viral replication. These techniques can be executed both in ex vivo and in in vivo using a lentivirus vector and AAV vector, respectively. Since these studies are still in preclinical stages, detailed explorations of these concepts are not possible currently.

23.7.8 Alzheimer's Disease

- Alzheimer's disease is an age-related neurodegenerative disorder characterized by loss of cholinergic neurons affecting memory and cognition. Pathological hallmarks include accumulation of amyloid beta and tau proteins.

- Nerve growth factor (NGF) protects cholinergic neurons, and its delivery via AAV is tried in preclinical studies.
- FK506-binding protein (FKBP1b) is a small protein that regulates calcium and declines with aging in the hippocampus. Microinjections of AAV bearing a transgene encoding FKBP1b when given into the hippocampus of aged rats confirmed hippocampal FKB1b overexpression at 4–6 weeks after the injections.

23.8 Regulatory Bodies of Gene Therapy

The major guidelines and regulatory bodies are:

1. National Institutes of Health (NIH) guidelines for research involving recombinant or synthetic nucleic acid molecules
2. Center for Biologics Evaluation and Research (FDA – CBER)
3. European Union – Biotechnology
4. European Medicines Agency (EMA)
5. Gene Therapy Discussion Group of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)

23.9 Limitations of Gene Therapy

- Viral carriers are usually costly and difficult to produce in large quantities compared to the non-viral vectors.
- Use of viral vectors can lead to severe or fatal immunogenic reactions during administration.
- Many of the transgenes achieve only short-term expression by the vectors.
- Development of malignancies can be a major adverse outcome as shown by the development of T-cell leukemia in patients undergone gene therapy.
- Lack of tissue selectivity by the vectors can be a challenge that can hamper specificity of transduction.
- Intracellular degradation of gene carriers can reduce the efficacy of the vectors.
- Pre-existing neutralizing antibodies of viral carriers in the circulation can cause immediate destructions of the vectors before its action.
- Some pathophysiological mechanisms are proven effective only in animal studies, and hence, it is difficult to use them in clinical trials.
- Most of the human clinical trials under progress are in the early phase of development, and a very few trials have reached successfully to phase 3; further, these gene therapeutics have a great task ahead to get the FDA approval.

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Stem Cell Therapy

24

Panneer Devaraju, Damayandhi Kaliyaperumal, and Thirumurthy Madhavan

Abstract

Stem cells are a reserve of unique cells with self-renewal property. It differentiates into organ-specific cells to replace the dead tissues to restore the physiological functions. Based on their ability to differentiate into the whole organism, cells of all the three germ layers, cells of particular germline, or only the target cells, they are termed as totipotent, pluripotent, multipotent, or progenitor stem cells, respectively. Based on their source, they are classified as embryonic, non-embryonic, mesenchymal, perinatal, cancer, and induced pluripotent stem cells. The self-renewal and differentiation properties of stem cells made them an ideal therapeutic tool to treat diseases associated with tissue loss. Hence, the major neurological, musculoskeletal, skin, hematological, cardiac diseases, and diabetes mellitus once thought to be nontreatable has now gained the hope of remedy on the use of organ-specific stem cells. In addition, stem cells have been engineered for targeted enzyme prodrug delivery for the treatment of deep-seated tumors like glioblastoma. The major limitation in the use of stem cells for therapy is its propensity to form teratoma and distribution in nonpreferred sites. Hence, tailoring the stem cells to reach the desired niche and to restore the physiological functions is underway to develop a promising therapeutic modality for treating the various challenging diseases in the medical field.

Keywords

Stem cells · Stem cell therapy · Teratoma · Targeted enzyme prodrug delivery

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24.1 Introduction

Stem cells are precursor cells with self-renewal property and can develop into a whole organism or differentiate into mature specialized cells in an organ system. They can renew without undergoing significant changes in their stem cell characteristics such as self-renewal and differentiation into mature lineage of cells. The term *plasticity* refers to the ability of stem cell to divide into cells of all the three germ layers. Based on the property of differentiation the stem cells can be classified as follows (Fig. 24.1 and Table 24.1):

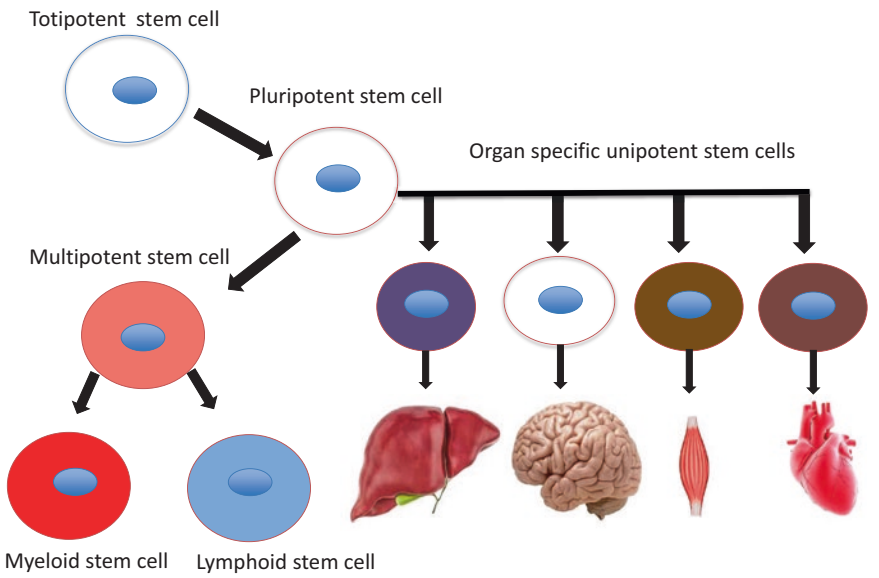

Fig. 24.1 Types of stem cells

Table 24.1 Classification of stem cells based on their potential of differentiation

Stem cell type	Description	Example
Totipotent	Stem cells can develop into an entire organism	Cells from 1 to 3 day old embryo
		Each cell can develop into a complete organism
Pluripotent	Stem cells that can differentiate to cells originating from all the three germ layers	Inner cell mass of the blastocyst
Multipotent	Stem cells that can differentiate into closely related family of cells	Hematopoietic stem cells
Oligopotent	Stem cells that can differentiate into few cell types	Lymphoid and myeloid stem cells
Unipotent	Stem cells that can differentiate into organ of origin	Cardiac stem cells

- **Totipotent Stem Cells:** The stem cells that can develop into a complete organism, e.g., cells of the zygote (1–3 days of an embryo).
- **Pluripotent Stem Cells:** The stem cells with an ability to differentiate into all types of cells in the body. They can differentiate into cells of all germinal layers such as endoderm, mesoderm, and ectoderm, e.g., inner cell mass of a blastocyst (5–14 days blastocyst).
- **Multipotent Stem Cells:** Multipotent stem cells are descendants of pluripotent stem cells but with limited ability to differentiate into cells of particular germline origin. These cells are usually found in the gastrulation phase of the embryo. These cells can be restricted to become progenitor or precursor cells, e.g., pluripotent hematopoietic stem cells (HSCs), mesenchymal stem cells, and neural stem cells.
- **Progenitor, Precursor, or Oligopotent Stem Cells:** These stem cells can differentiate into a few different cell types, e.g., lymphoid and myeloid stem cells.
- **Unipotent Stem Cells:** They are usually found in the organs and develop into cells of organs of their origin, e.g., cardiac stem cells and muscle stem cells.

24.2 Classification of Stem Cells Based on Their Origin (Table 24.2)

- **Embryonic Stem Cells:** Around 10–15% of the inner cell mass in an embryo blastocyst prior to implantation was observed to have totipotent stem cell property, and they are termed as embryonic stem cells.
- **Non-embryonic Stem Cells:** These are the multipotent stem cells commonly present within the organs. They have limited replication capacity and differentiate into committed progenitor cells, e.g., bone marrow stem cells (BMSCs), cord blood stem cells, and stem cells in adipose tissues, heart, and muscles.
- **Mesenchymal Stem Cells (MSCs):** These are a type of non-embryonic stem cells found in the bone marrow which has the ability to differentiate into cells of mesodermal germline origin such as liver epithelium, lung, gastrointestinal tract, and skin cells.
- **Induced Pluripotent Stem Cells (iPSCs):** Induced expression of embryonic transcription factors transforms an adult cell into an embryonic stem cell. Usually, retroviruses are used to induce the expression of embryonic transcription factors such as Sox2, Oct4, Klf4, cMyc, and Lin28, which then induces the transformation of an adult cell into a pluripotent stem cell.
- **Perinatal Stem Cells:** Stem cells present in the amniotic fluid and umbilical cord blood also have the ability to change into specialized cells.
- **Cancer Stem Cells (CSCs):** Cancer stem cells are a subpopulation of tumor cells, which can self-renew and differentiate and ultimately, develop into a tumor if implanted on an animal.
- **Stem Cell Lines:** Stem cell lines are developed from single parent stem cells. They can be cultured under in vitro conditions and used for conducting research on stem cells.

Table 24.2 Stem cell-specific transcription factors

Type of stem cells	Transcription factor
Embryonic stem cells	HNF-3α, c-Jun, c-maf, c-myc, Oct3/4, P53, Smad
	Sox
Induced pluripotent stem cells	Oct3/4, KLF4, Sox2, and c-myc (pluripotency induction in somatic cells)
	Sox-17, Pox-16 (induces pluripotency)
	KLF2, KLF4, c-maf, c-myc, Nanog, Oct3/4, P53, SOX1,2,3
	TBX-18
Mesenchymal stem cells	Dux4, EBF1–3, ETV-5, c-Jun, myocardin, P53, Pax3, Twist1–2
	Smad-chondrogenic differentiation
	Fox-osteogenesis
	Sox-2-self renewal and multipotency
Neural stem cells	Ascl1/Mash1, CREB, CSL, FOXO3, c-Jun, KLF4, Neurogenin-1-2
	NKX2.2, P53, Pax3–6, Smad 3,4,7
	Olig2-induces differentiation to neural lineage
	Pax6 and HES-1-regulate expression of genes necessary for neural
	Stem cell proliferation and multipotency
Hematopoietic stem cell	SCL/Tal-1
	LMO2-hematopoiesis during embryogenesis
	GATA-2-hematopoiesis in liver
	PU.1/Spi-1-HSC differentiation and lineage commitment
	Aiolos/KZF3, CDX4, CREB, Helios, HMGB3, Ikaros, c-JUN
	c-maf, c-myc, P53, smad2–4,7, and TSC 22

24.3 Stem Cell Therapy

The capability of stem cells to renew the dead or damaged tissue in an organ system and to restore the organ function has given high hopes for its therapeutic use in treating organ injuries and chronic diseases. The field of regenerative medicine is on the growing phase to treat many diseases, which were once considered as incurable. The ray of hope given by stem cells to treat these diseases is a new triumph in the field of medicine. The stem cells not only replaced the lost tissue but also they actively protect the existing tissues from degeneration by producing trophic factors required for the survival. The following are the organ-specific diseases, for which the stem cells are used to treat.

24.3.1 Stem Cell Therapy for Neurological Disorders

Detailed knowledge of the disease pathology will be helpful in imparting stem cell therapy to restore the function of a specific organ. The major neurological diseases, for which the pathophysiology is well documented, are Parkinson's disease,

Alzheimer's disease, Huntington's disease, and multiple sclerosis. Demonstration of the development of neurons and glial cells from the embryonic stem cells and stem cells from fetal or adult CNS envisage a permanent cure for the abovementioned major neurological disorders.

- **Parkinson's Disease**
  - Progressive depletion of nigrostriatal dopamine-producing neurons leads to Parkinson's disease (PD) which is characterized by bradykinesia, tremors, and postural changes. The current therapies offered to PD are aimed to replenish dopamine, which offers a transient symptomatic relief. Also, conventional therapy fails to prevent disease progression.
  - The in vitro culture of dopamine-secreting neurons from embryonic stem cells, BMSCs, and fetal brain predicts a permanent cure which will replenish and restore the functions of nigrostriatal dopamine-producing neurons. Hence, in the future, stem cell therapy may develop as a recommended therapy to cure PD.
  - Transplantation of human fetal dopaminergic neurons yielded a sustained improvement of function in a few patients. However, experimental transplantation of human dopamine-producing neurons in animal models resulted in poor survival of the grafts.
  - The progressive loss of dopaminergic neurons in PD may be controlled by transplanting engineered stem cells programmed to secrete glial cell line-derived neurotrophic factor (GDNF), which is a neuroprotective molecule. However, a major hurdle in stem cell therapy for PD is the site of implantation of the stem cell graft.
- **Alzheimer's Disease**
  - Alzheimer's disease (AD) remains a major cause of memory loss in geriatric patients. AD is characterized by the progressive cognitive loss which is the outcome of the accumulation of AB-amyloid neuritic plaques in the neuronal cytoplasm and deposition of Aβ amyloid in the vessel walls of cerebral arteries. The major site of neuritic plaque accumulation is noticed in the hippocampus, temporal cortex, and nucleus basalis.
  - The neuritic plaques are the derivatives of the amyloid precursor protein. APP exerts neurotrophic and neuroprotective properties, and it is associated with the protection of neurons.
  - The key feature in AD is the progressive loss of acetyl choline, acetylcholinesterase, and nicotinic receptors. The potential treatment proposition under stem cell therapy for AD is limited to replenishment of cholinergic neurons. However, the loss of other cells in AD requires genetically programmed stem cells to produce neuroprotective growth factors.
  - In animal models, transplantation of forebrain fibroblasts nerves was observed to reduce the loss of cholinergic neurons by secreting nerve growth factor, and thus the animals were observed to have improved cognitive functions. The results of this study appear to be promising in the putative management of AD.

- **Huntington's Disease**
  - Huntington's disease (HD) is an autosomal dominant disorder of CNS. Patients with HD manifest motor, behavioral, and cognitive dysfunctions due to the death of projection neurons in the striatum.
  - A sign of recovery in HD animal models was observed upon treatment with grafts of fetal striatal neurons. However, complete restoration striatal neural circuitry did not develop in these animals. Hence in HD, stem cell therapy should address the replacement of projection neurons in the striatum, development of neural circuitry, and production of neuroprotective and neurotrophic factors to reduce the death of neurons.
- **Amyotrophic Lateral Sclerosis**
  - Amyotrophic lateral sclerosis (ALS) is the most devastating neurodegenerative disorder which arises as a result of the loss of neurons in the spinal cord, cerebral cortex, and brainstem (upper and lower motor neurons).
  - The major issue in the treatment of ALS is to restore the loss of motor neurons and to integrate them in the neural circuitries. Demonstration of development of motor neurons from various stem cell sources, formation of neuronal synapse with muscle fibers under in vitro conditions, and evidence of extension of axons to ventral roots in animal models demonstrates a route of treatment for ALS.
- **Stroke**
  - Acute occlusion of CNS vasculatures leading to reduced or no blood flow to neural tissues may result in ischemic death of neurons and glial cells. Stroke will end up in motor, sensory, and cognitive impairment.
  - Animal models of stroke on treatment with embryonic and adult stem cells revealed a detectable magnitude of motor and sensory improvements.
  - A clinical trial using stem cells to treat human patients with stroke yielded promising results of restoration of functions. It was observed that the trophic factors released by the stem cells extended cell survival and functions.
  - The salient neuro-reconstructive features observed upon using stem cells for treating stroke are as follows:

    In the stroke model of rats, treatment using stem cells enhanced the migration of new neurons to the ischemic foci.

    Re-establishment of neural circuitries and improvement of motor functions.
  - It has also been observed that the endogenous neural stem cells do contribute to the restoration of dead neurons in patients with stroke. Therefore, complementing the endogenous repair process with exogenous stem cells foresees a treatment modality for stroke in the near future.
- **Multiple Sclerosis**
  - Multiple sclerosis (MS) is characterized by demyelination of neurons, inflammation, and gliosis which ends up in conduction deficits, a plethora of manifestations and disability. Though the etiology of MS is obscure, the current use of immunomodulation and immunosuppression prevents further loss of axons.

  - Though the oligodendrocytes come for rescue by re-myelinating the axons, their activity is outlasted by the exaggerated inflammatory responses during the course of the disease.
  - Adult and embryonic oligodendrocyte progenitor cells of human origin were reported to re-myelinate the affected neurons in animal models of MS. However the prevailing inflammatory processes hinder the protective activity of oligodendrocytes. Hence, stem cell therapy coupled with immunosuppressive or anti-inflammatory therapy might, therefore, be essential for an effective outcome from MS.
- **Spinal Cord Injuries**
  - Injuries in the spinal cord may lead to loss of motor, sensory, and autonomic functions. The clinical manifestations of spinal cord injury are the outcomes of the inflammation, demyelination, and loss of neurons or glial cells.
  - Treatment of spinal cord injury with stem cells have ensured functional benefits due to the secretion of trophic factors by the stem cell transplants.
  - Locomotor recovery in animal models due to the development of oligodendrocytes and newer neurons was reported on animal models of spinal cord injury. A positive correlation between the extent of recovery and the number of oligodendrocyte was found as a result of stem cell therapy.
  - However, the uncontrolled proliferation of astrocytes produced hyperesthesia in animal models. Hence, transplantation of stem cell-derived oligodendrocyte might help in the re-myelination process. Extensive work has to be carried out to standardize the therapeutic modality for treatment of spinal cord injuries.

24.3.2 Stem Cell Therapy for Musculoskeletal System

- The limitations in the use of autologous cancellous bone grafts for the treatment of bone defects and post-traumatic skeletal defects have led to the use of stem cells to develop bone tissue.
- Development of bone grafts from stem cells can be achieved by growing them on an osteoconductive carrier.
- A distal phalanx avulsion of thumb in a patient was successfully treated using MSCs. Similarly, autologous BMSCs in porous hydroxyapatite (HA) were used to treat segmental defects and demonstrated reversal of function. A combination of BMSC and HA was used to treat long bone tumors.
- Likewise, BMSCs and platelet-rich plasma (PRP) was utilized for the reconstruction of distraction osteogenesis. The polymerization of thrombin in the PRP was done using 10% calcium chloride before the bone marrow implantation. PRP produced osteoconductive fibrin clot, which favors the development of bone. The same procedure was applied to repair the cleft palate without resorting to surgical correction procedures. Promising results were obtained for reconstruction of skeletal architecture using stem cells.

- An increase in bone mineral content in an osteogenesis imperfecta patient treated with BMSCs was noted. The outcome of that study was encouraging and lead to a platform for the development of a protocol to treat this genetic disorder.
- Reconstruction of ruptured tendons and ligaments was also achieved by implanting stem cells on the polyglycolic acid scaffolds in animal models. Though the development of a long tendon remains unachievable now, it has been found that the stem cells have enhanced the healing in ruptured tendons and ligaments.
- Stem cell therapy in combination with tissue engineering is a large field of research that promises reconstruction of damaged and underdeveloped musculoskeletal system.

24.3.3 Stem Cell Therapy for Skin Diseases

- Stem cells in the skin belong to the somatic stem cell group and are named based on their location in the skin. Epidermal stem cells reside in the basal layer of the epidermis and can differentiate into epidermal cells. Follicular stem cells present in the follicular bulge region may develop into hair follicle epithelium, inner and outer root sheath, and hair shaft. Stem cells in sebaceous gland and melanocyte cells were also isolated from the skin.
- The major application of skin stem cells in therapy is to restore the skin damages such as extensive burns, postinfection lesions of the skin, inflammatory skin diseases, and trauma.
- In vitro cultured keratinocytes was used to treat patients with severe burn injuries and chronic cutaneous ulcers.
- A phase I clinical trial on using epidermal stem cells to treat recessive dystrophic epidermolysis bullosa resulted in increased deposition of collagen at dermal-epidermal junction and reduced blistering lesions.
- Transplantation of murine bulge region cells showed the repopulation of epidermis, sebaceous glands, and epithelial layer of the hair follicle.
- The immunomodulatory properties of HSC transplantation have offered beneficial results on its use in treating many inflammatory skin diseases such as atopic dermatitis, psoriasis, scleroderma, graft-versus-host disease (GvHD), and lupus.
- BMSC transplantation in rodent and baboon models of GvHD was observed to reduce the graft rejection. Steroid-resistant GvHD upon treatment with BMSCs showed promising results of disease remission. MSC based therapies induced clinical remission in refractory lupus patients with skin manifestations. Another study revealed an amelioration of disease activity of severe nephritis and cutaneous lupus conditions after BMSC therapy. Upregulation of regulatory T cells was attributed to the clinical remission in lupus.
- BMSC treatment in rodent model of scleroderma revealed amelioration of fibrosis in the lungs and skin.
- Autologous BMSC therapy in psoriasis patients was found to enhance the incidence of other autoimmune diseases such as autoimmune thyroiditis, diabetes mellitus, myasthenia gravis, and ulcerative colitis. This was due to the inherent

impaired anti-inflammatory functions observed in these patients. However, allogeneic BMSC transplantation yielded remission.

- In animal models of psoriasis, amelioration of disease symptoms was due to the immune modulatory effects of the stem cells by upregulation of regulatory T cells and reduction of reactive oxygen species and infiltration of immune cells in the skin.
- Hence to treat psoriasis, engineered stem cells with overexpression of superoxide dismutase might inhibit differentiation of Th17 cells, thereby reducing the induction and amplification of autoimmune responses.
- Clinical trials on the use of umbilical cord-mesenchymal stem cells (UC-MSCs) in psoriasis vulgaris patients in the USA and Philippines revealed disease remission, and the observed therapeutic outcome was equivalent to methotrexate treatment and without any serious adverse effects. The potential use of stem cells for the treatment of atopic dermatitis and allergic contact dermatitis has also been explored.

24.3.4 Stem Cell Therapy or Diabetes Mellitus

- More than 400 million people worldwide have developed diabetes mellitus (DM). The current therapeutic management of DM is achieved by the use of oral hypoglycemic drugs and subcutaneous use of insulin.
- Transplantation of whole pancreas and islets yielded encouraging results for a shorter duration, but these procedures are laborious to perform. Attempts have been made to treat DM by using mesenchymal stem cells.
- Clinical trials on infusion of umbilical cord blood cells in young diabetic patients indicated that the therapy is safe, but no significant changes in the levels of C-reactive peptide and HbA_{1c} were observed between baseline and post-therapy. However, UC-MSC therapy yielded promising results of insulin independence with a 50% reduction in insulin dependence. Poor prognosis of this trial was observed in patients with diabetic ketoacidosis, as this condition disfavored the stem cell survival due to an excessive inflammatory environment. Treatment efficacy was reported to be higher when diabetes is diagnosed relatively earlier.
- Blinded randomized trial using bone marrow-mesenchymal stem cells (BM-MSCs) to treat DM disclosed a moderate response.
- Trials using the combination of two stem cell sources revealed that the use of HSCs was beneficial, as it exerted immunomodulatory activity to protect the existing beta cells from the immune-mediated destruction and regeneration of beta cells from autologous marrow stem cells.
- Infusion of stem cells via dorsal pancreatic artery showed better results than the intravenous perfusion of the stem cells.
- Recently, the generation of functional islet beta cells was achieved from cultured pluripotent stem cells.
- Single hormone-positive beta cells capable of glucose-stimulated insulin secretion were developed in vitro; however, implantation of these cells in the pancreas

did not yield the desired results. The lack of intracellular communications and immune-mediated destruction of the grafts were the major cause of failure. Hence, immune engineering of the in vitro developed islet beta cells might be helpful in the maintenance of the graft.

24.3.5 Stem Cell Therapy for Cardiac Diseases

- Every year, cardiovascular diseases (CVDs) serve as the major cause for the 3.9 million deaths. Currently available therapies for CVDs mainly alleviate the symptoms but fail to reduce the irreversible loss of cardiac tissues.
- In 2001, the first preclinical study using stem cells was carried out to repair the infarcted tissue and to improve the ventricular function. In the last two decades, the results of several preclinical trials have demonstrated that the stem cells were safe and feasible to treat CVDs.
- Skeletal myoblasts, which resides in the basal lamina of muscle fibers, were first used to treat CVDs. The major drawback of this modality was the development of ventricular arrhythmia, which was due to the lack of electromechanical capacity between myoblasts and cardiomyocytes. In a large double-blinded MAGIC trial, no improvement of LV function, 6 years post-therapy, was observed on use of myoblasts to treat CVDs. Amiodarone therapy was warranted in these patients to treat arrhythmia.
- Bone marrow-derived mononuclear cells, HSCs and MSCs, mobilized stem and progenitor cells, when used to treat CVDs, resulted in reduction of infarct size. However, controversial reports on the improvement of cardiac functions were reported. In the clinical trial CARDIO 133, after bone marrow-derived HSCs were infused intramyocardially, there was an improvement in infarct size and reperfusion but no changes in global function and clinical symptoms. Another study using the mesenchymal stem cells, PERFECT (double-blinded phase III trial), also demonstrated no significant difference in left ventricular ejection function (LVEF).
- In acute myocardial infarction, infusion of exogenous granulocyte-colony stimulation factor (G-CSF) to mobilize the stem cells and progenitor cells from bone marrow did not yield any beneficial results. Use of adipose tissue-derived stem cells to restore cardiac functions in CVDs also yielded poor results; nevertheless, there was an improvement in maximal oxygen consumption by the use of such cells.
- The demonstration of self-renewing cardiac stem cells from the adult heart and its ability to develop into cardiomyocytes, endothelial cells, and smooth muscles changed the scenario of stem cell therapy for CVDs.
- In animal models, the use of cardiac stem cells to treat CVDs enhanced the LVEF. In a first-of-a-kind clinical trial by the name SCIPIO, usage of cardiac stem cells was found to be safe and produced MRI-detectable improvement of global and regional heart functions.

- The beneficiary effect of cardiac stem cells was once again reiterated in the CADUCEUS trial which supported improvement in the viable myocardium, regional contractility, and systolic wall thickening.
- A reduction in cardiac fibrosis and heart failure status was reported in a phase III clinical trial PERSEUS. The major clinical benefits obtained on the use of cardiac stem cells are due to:
  - The endocrine or paracrine effects exerted by the grafts
  - Differentiation into cardiac and endothelial cells
  - Integration into the myocardium
  - The immunomodulatory properties of the stem cells
- Teratoma formation in experimental studies in animals using embryonic stem cells to treat CVDs precluded its further use. The major beneficial effect observed with the use of embryonic stem cell was the electromagnetic integration of graft with the host cells and cardiac remodeling process.
- A phase I clinical trial using the ESC-derived progenitor cells, which was earlier primed with bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) and fixed using fibrin sheets, produced a 10% improvement in LVEF and improvement of symptoms from NYHA III to I in a 1-year follow-up period. Also, no teratoma and arrhythmia development was reported. On the whole, the ethical consideration on use of stem cells, teratoma formation, genetic instability, development of immune response against the grafts, and tumorigenic property of embryonic stem cells impedes the use of embryonic stem cells for therapy.

24.3.6 Stem Cell Therapy for Blood Diseases

- Since the 1950s, the intravenous infusion of whole bone marrow has been used to treat acute leukemia, aplasia of bone marrow, and congenital immune deficiencies. The stem cells present in the bone marrow was later identified as the cause for producing the benefits. Less than one hundred purified HSCs can reconstitute the blood system. In addition, the methodology to mobilize the stem cells from the marrow into the circulation greatly reduced the adverse effects of allogeneic stem cell transplantation.
- The use of HSCs to treat leukemia, leading to the immunological recognition and elimination of leukemic cells, is called as *graft* versus *leukemia effect*. The major limitation in HSC therapy is the availability of appropriate or matched donors as a mismatched transplantation may result in the development of GvHD with trivial to life-threatening complications.
- Thalassemia and sickle cell anemia (SCA) are the most common hereditary hemoglobinopathies distributed widely around the world. RBC infusion remains as the major therapy for most of the thalassemic patients. Allogeneic HSC therapies were carried out, which resulted in poor outcome as a result of GvHD. However, HSC therapy in HLA-matched individuals produced higher survival rates.

- In SCA, HSC transplantation with immunosuppression therapy resulted in disease-free survival in 85% of the recipients. The success of transplantation was higher if the HSC therapy is initiated before the onset of the irreversible sickle cell vasculopathy. Monitoring the mixed chimerism in patients' post-HSC therapy might be helpful to predict the acceptance of the graft and development of GVHD.
- Genetic engineering of hematopoietic stem cells to replace the aberrant gene with the normal copy of the gene leads to the successful treatment of X-linked severe combined immunodeficiency (SCID). Despite the clinical success of SCID gene therapy, gene therapy for other genetic disorders was not successful due to the development of insertional mutagenesis, tumor formation, and others.

24.3.7 Stem Cell Therapy for Leukemia

- Allogeneic hematopoietic stem cell transplantation (HSCT) has been observed to be a very effective treatment for leukemia which is characterized by the lack of relapse posttreatment. The remission is brought about by the *graft* versus *leukemia effect*. However, this modality will not be applicable in a HLA mismatch transfer, as the graft cells may induce GVHD. A new modality named as *dual transplantation* in which co-infusion of cord blood and haploidentical HSCs was reported to enhance the reconstitution of hematopoietic system of the recipient and reduced incidence of GVHD.

24.3.8 Other Uses of Stem Cells

- Neural and mesenchymal stem cells can be engineered to express enzymes that act on nontoxic prodrugs and produce cytotoxic products, which form the basis for the targeted enzyme-prodrug delivery.
- Cytidine deaminase is a major enzyme used for prodrug therapy. It converts nontoxic 5-fluorocytosine to cytotoxic 5-fluorouracil.
- In mouse models, use of engineered neural stem cells inhibited the development of glioblastoma.
- In humans, a clinical trial was carried out to test the efficacy of HB1.F3 cells in the expression of the enzyme cytidine deaminase following the surgical resection of the glioblastoma. Herpes simplex virus-thymidine kinase (HSV-TK) was used to phosphorylate the nontoxic monophosphorylated ganciclovir to toxic triphosphate ganciclovir. Intra-tumoral injection of the engineered neural stem cells with HSV-TK into the gliomas, followed by intraperitoneal injection of monophosphate ganciclovir induced protective effect against the tumor.
- TNF-α-related apoptosis-inducing ligand (TRAIL) is a secretor molecule that induces apoptosis in tumor cells. The use of stem cells engineered to secrete

TRAIL in animal models with surgical resection of glioblastoma delayed metastasis.

- IFN-β is a growth inhibitory cytokine; stem cells engineered to overexpress this cytokine upon injected into breast tumor in animal models reduced the pulmonary and hepatic metastasis.
- Targeting the cancer stem cells might be helpful in reducing the activation of tumor and metastasis. The use of neural and hematopoietic stem cells to control cancer stem cells in glioblastoma revealed that HSCs were superior to control the tumor growth by inhibiting the cancer stem cells.
- iPSCs can be used to screen the newly developed anticancer drugs. The iPSCs derived from cancer tissues are biologically similar to patient tumor and can be used to assess the tumor-specific efficacy of the newly developed drug. The iPSCs developed from the liver will be highly useful for carrying out in vitro hepatotoxicity assays.

24.4 Safety Concerns and Regulations of Stem Cell Therapy

- The ability of stem cells to regenerate and restore the function of damaged tissues has raised immense expectations of its use as a reliable therapeutic modality. The utility of stem cells for clinical use is still under the process of discovery, but the safety and limitations of their use are still uncovered.
- The major limitation of stem CT is to monitor the bio-distribution and to limit their replication in non-preferred organ sites. Hence, technologies need to be developed and validated for tracking the bio-distribution and removal of stem cells in nontherapeutic areas.
- The tumorigenic property of stem cells, which is evident with the formation of teratomas in animal models treated with stem cells, is another major setback in its implication for therapy.
- The destruction of allogeneic stem cell grafts by the host immune system is also a major blockade for stem cell therapy.
- Based on the lessons learned from the past experiments, the following are the parameters to be tested:
  - Demonstration of consistency
  - Assessing the genetic stability of the stem cells
  - Dose and route of administration
  - Assessment tools for monitoring the bio-distribution and elimination of stem cells by programmed self-destruction if lodged in unnatural site
  - Assessment of tumorigenic capability of stem cells
- The currently used assays for testing the safety of stem cells are as follows:
  - Assessment of purity of stem cells to avoid the stem cells which may develop into a teratoma. Currently, flow cytometry-based assays, immunohistochemistry (IHC), and quantitative PCR (qPCR) are used to identify the stem cells with tumorigenic property.

- As stem cells are passaged multiple times in vitro, there are more chances for the development of chromosomal aberrations. Hence, karyotypic analysis of stem cells is carried out before initiation of the therapy for monitoring the occurrence of these chromosomal aberrations.
- Engineered stem cells must be evaluated for insertional mutagenesis by array comparative genomic hybridization and SNP arrays.
- Currently, bioluminescence and IHC are being used for tracking cell migration.

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Principles of Prescription Writing

25

Heta Shah

Abstract

In 2016, the Government of India passed a legislation requiring practitioners to prescribe by generic name, written in capital letters. It is still not clear whether this was a well-thought-out decision and is not followed by all. Nevertheless, around the world, a prescription is an order by a licensed practitioner to the pharmacist to dispense medicines or other medicinal products by generic or brand name. It can be handwritten or electronic. The basic components include patient demographics, the main body, and doctor's details including name, registration number, and contact details. Many countries do not hand over the prescription back to the patient once the drug is dispensed. Non-prescription drugs are those sold over the counter and should ideally not belong to Schedule H, X, or G of the Drugs and Cosmetics Act. Misuse of over-the-counter (OTC) drugs has given rise to menace such as drug interactions, antibiotic resistance, and abuse of addictive drugs.

Keywords

Prescription · Non-prescription drugs · Over-the-counter (OTC) drugs

25.1 Introduction

Prescription is an order written by a licensed practitioner (qualified health care professional) who is authorized to prescribe medications, non-medicinal products, and other health-related services. The prescription stands as a medium of communication between the prescriber and the pharmacist regarding an individual patient's needs. Prescriptions can be presented as written, verbal (by telephone), or electronic (i.e., via fax or computer network) format to the pharmacist. Depending on the national regulations, the requirements of the prescription may vary.

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25.2 The Structure of a Prescription

The prescription should include the following information as depicted in Fig. 25.1.

- **Patient information** including full name and address
- **Date** on which the prescription was issued
- **Name and dosage form of the product**
- The name can be any of the following:
  - Proprietary (brand)
  - Nonproprietary (generic)
  - Chemical
- **Product strength**
  - The strength of the product is not necessary if one and only strength is commercially available or if the product contains a combination of more than one active ingredient.
  - It is advisable to include the strength to reduce the chance of misinterpretation.
- **Quantity to be dispensed**
  - This should include the amount and the units of measure (e.g., milligrams, grams, milliliters, tablets).
  - If the amount is not stated, the directions should at least specify the dose to be taken and the duration of therapy so that the pharmacist can calculate the quantity required for the patient.
- **Directions for the pharmacist**
- Directions may be required for:
  - Preparation (e.g., compounding)
  - Labelling (i.e., information to be put on the blister pack)
- **Directions for the patient**
  - These should comprise explicit instructions on the amount, frequency, and duration for proper use.
  - The phrase "as directed" should be avoided. If at all the directions vary, the lowest and highest dose should be suggested.
- **Refill information**
  - If refill information is not supplied, it is generally assumed that no refills are authorized.
  - In the Western countries, the "as needed" [pro re nata (*prn*)] refills are usually construed as allowing for refills for at least 1 year unless laws or regulations restrict the amount or period in which a prescription is valid.
- **Prescriber information**
  - This should include the name, office address, signature of the prescriber, and the license number (the national or state medical council registration number).

Dr. John Dave
Consulting Physician
MBBS. MD (Medicine)
Rean. No. XXXXX
Address: _____
Phone no. XXXXXXXXXX. Email: _____

Date:

Patient name:

Age/date of birth:

Address/Phone:

Gender:

Diagnosis:

℞

1. Cap. AMOXICILLIN 500 mg, tid X 7 days

   Take after breakfast, lunch and dinner

Signature

*Regn. No.*_____

Order to pharmacist:

Dispense 21 capsules, no refills

Fig. 25.1 A standard prescription format

- The physician and patient details along with the diagnosis are referred to as the *superscription*; the drugs' name, formulation, dose, frequency, and duration comprise the body of the prescription also known as the *inscription*, and finally, the instructions given to the pharmacist or advice given to the patients with the prescriber's signature come under *subscription*.

25.3 Over-the-Counter Drugs

Over-the-counter (OTC) drugs or non-prescription drugs are those that can be purchased without a prescription because they have been considered safe for the public to use without restrictions.

25.3.1 OTC Drugs Use in India

- OTC drugs have not got any legal recognition in India. Usually, prescription drugs belong to Schedules H (prescription only), X (narcotics), and G (hormonal and anti-cancer preparations). OTC drugs, hence, must not belong to any of these schedules of the Indian Drugs and Cosmetics Act.
- Some commonly used non-prescription medicines (irrespective of legal status) are:
  - Antacids and acid suppressants
  - Laxatives and stool softeners (Senna is relatively harmless than others)
  - Antidiarrheals
  - Cold and allergy remedies: antihistamines like chlorpheniramine and diphenhydramine
  - Analgesics such as paracetamol and diclofenac
  - Antitussives
  - Antibiotics
  - Oral contraceptives
  - Multivitamin preparations
- Over 50% of OTC drugs belong to Schedule H. In order to curb indiscriminate use, new regulations have been formed which incorporates a new schedule, i.e., the Schedule H1. This schedule contains drugs which should not be dispensed by chemists without a prescription, and chemists have to maintain a sales register with a copy of the prescription.
- Schedule H1 includes antibiotics and sedatives and, hence, aims to prevent antibiotic resistance and side effects from narcotics.

25.3.2 Toxic Potential of Non-prescription Drugs

- These drugs can interact with other medicines. For example, antacids can form complexes with drugs. An acidic environment in the stomach is needed for many drugs for their absorption.

- Antitussives like dextromethorphan and codeine and antidiarrheals like loperamide are opioids and pose a risk of abuse if used in large doses.
- Gastrointestinal bleeding and renal toxicity with NSAIDs and hepatotoxicity with paracetamol.
- Antibiotic resistance.
- Risk of toxicity in infants or young children (high dose) or to pregnant women (unaware of the teratogenic risk).
- Vitamins and iron preparations are one of the best examples for commonly misused medicinal products. Multivitamins are used for conditions or symptoms like lethargy, stress, memory enhancer, growth stimulant, and immunity booster rather than for the actual vitamin deficiency.
  - Frequently, community pharmacists sell and recommend these products without considering whether there is a genuine need for the treatment. Two important factors are the patient's desire for treatment and the pharmacist's desire to make a sale.
  - Patient and community education on appropriate medication use is the key solution for rational use of prescription or non-prescription drugs.

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Part IV

Toxicology

Environmental Toxicology

26

Nishanthi Anandabaskar

Abstract

Environmental toxicology is the "science and practice of the adverse effects, mainly of chemicals and other man-made agents in the environment and through the environment." Metals are an important source of environmental contamination. The top three heavy metals of concern due to their toxic profile listed under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) are arsenic, lead, and mercury. Chronic exposure to arsenic produces peripheral vascular disease leading to cyanosis of the extremities, particularly the feet, progressing to gangrene called the *blackfoot* disease. It can also lead to hyperkeratinization of the skin, gastrointestinal toxicity, peripheral neuropathy, anemia, leucopenia, and carcinogenesis. Lead toxicity can lead to neurotoxic effects; adverse cardiovascular, hematological, renal, and gastrointestinal effects; and also predisposes to carcinogenesis. Minamata disease is a classic example of organic mercury poisoning following ingestion of fish and shellfish contaminated by methylmercury. Chelators like ethylenediaminetetraacetic acid (EDTA), British anti-lewisite (BAL), succimer, sodium 2,3-dimercaptopropane sulfonate (DMPS), penicillamine, deferoxamine, and deferasirox are used in the management of heavy metal poisoning.

Keywords

Chelators · Ecotoxicology · Heavy metal poisoning

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26.1 Definitions

26.1.1 Toxicology

- It is defined as the "study of the adverse effects of agents such as chemicals, radiation, dusts and endo- or exotoxins produced by microorganisms on living organisms."

26.1.2 Environmental Toxicology

- Environmental toxicology is the "science and practice of the adverse effects, mainly of chemicals and other man-made agents in the environment and through the environment."

26.1.3 Ecotoxicology

- It is defined as the "field of study which integrates the ecological and toxicological effects of chemical pollutants on populations, communities and ecosystems with the fate including transport, transformation and breakdown of such pollutants in the environment."
- The publication of Rachel Carson's book on *Silent Spring* in 1962 was a landmark event in the field of ecotoxicology.
- The book gave an insight into the harmful effects of man-made synthetic chemicals like pesticides on humans and the ecosystem.

26.1.4 Occupational (Industrial) Toxicology

- Occupational toxicology is the "study of the adverse effects of agents that may be encountered by workers, during the course of their employment."
- For example, workers involved in:
  - Mining and quarrying, stone cutting, construction, and sandblasting are exposed to silica, leading to silicosis.
  - Textile manufacture inhale cotton dust, leading to byssinosis.
  - Manufacture of asbestos products, construction, and shipbuilding are exposed to asbestos, leading to asbestosis, lung cancer, pleural plaques, and mesothelioma.
  - Rubber manufacturing, aluminum and metal production, and textile and dye manufacturing are at increased risk of bladder cancer due to their exposure to aromatic amines, including 2-naphthylamine, benzidine, 4-aminobiphenyl, and o-toluidine

26.2 Heavy Metals

- Metals are an important source of environmental contamination.
- The top three heavy metals of concern due to their toxic profile listed under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) are:
  - Arsenic
  - Lead
  - Mercury

26.2.1 Arsenic

- **Source**
  - Common sources of exposure – drinking water and seafood contaminated with arsenic
  - Human activities leading to the release of arsenic into the environment – utilization of arsenic-containing pesticides, mining, and burning of coal
  - Occupational exposure – manufacture and use of organic arsenicals (herbicides and insecticides) and generation of computer chips and semiconductors
- **Chemistry** – exists in elemental, trivalent (arsenites and arsenious acid), and pentavalent (arsenates and arsenic acid) forms
- **Mode of action** – inhibition of the electron transport chain
- **Toxicokinetics**
  - Absorption – by inhalation and ingestion
  - Deposition – nails, hair, liver, and kidney
  - Excretion – urine (major route), feces, sweat, hair, nails, skin, and exhaled air
- **Adverse health effects**
  - Cardiovascular system – cardiac arrhythmias and ischemic heart disease with acute and chronic exposure; peripheral vascular disease, leading to cyanosis of the extremities, particularly the feet, progressing to gangrene (called as the *blackfoot disease*) occurs with chronic exposure.
  - Skin
    Hyperkeratinization of the skin leading to the formation of multiple corns or warts, especially in palms and soles (occurs many years after exposure).
    Hyperpigmentation interspersed with spots of hypopigmentation (occurs after 6 months of exposure).
  - GI tract – mild cramping, diarrhea, vomiting, GI hemorrhaging and death (occurs by ingestion of high-dose arsenic – acute or subacute exposure).
  - Nervous system – peripheral neuropathy related to both motor and sensory neurons (occurs with acute or subacute arsenic exposure).
  - Other non-cancer toxicities – anemia and leukopenia (due to cytotoxic effects on blood cells and bone marrow suppression), fatty liver and liver cirrhosis, renal damage and increased risk of diabetes.

  - Carcinogenesis – increased risk for skin cancer (basal cell and squamous cell carcinomas), bladder cancer and lung cancer. Its mechanism of carcinogenesis is by altering the gene expression, DNA methylation, interruption of DNA repair, generation of oxidative stress and/or altered signal transduction pathways
- **Treatment of acute poisoning**
  - Termination of further exposure
  - Supportive measures – especially monitoring of fluid levels (as it can cause fatal hypovolemic shock)
  - Chelation therapy
  - Exchange transfusion

26.2.2 Lead

- **Source**
  - Common sources of exposure – paint (lead carbonate and lead oxide) and gasoline (tetraethyl lead).
  - However, the use of lead in paints and gasoline has been banned since 1978 and 1996, respectively.
  - Lead does not degrade and stays back in the environment in dust, soil, and the paint of older homes.
  - Screening of children at 6 months of age for increased blood lead levels is recommended by the Centers for Disease Control and Prevention (CDC).
    For children with blood lead levels >10 μg/dL, aggressive lead abatement is required.
  - Occupational exposure – inhalation of lead dust and fumes by lead smelters, workers of storage battery factories, steel welding or cutting, firing ranges, rubber and plastic industries, printing, radiator repair shops.
- **Chemistry** – exists as divalent or tetravalent cations
- **Mode of action** – alters protein structure and can activate or inactivate proteins in the human body
- **Toxicokinetics**
  - Absorption – by ingestion or inhalation.
  - Nearly 99% of lead present in the blood binds to hemoglobin.
  - Distribution – occurs in the kidney and liver.
  - Redistribution and deposition – bone, teeth, and hair.
  - Almost 95% of the adult body load of lead accumulates in the bones (lead lines visible by radiography in growing bones). Its half-life in bones is 20–30 years.
  - Excretion – major route (urine), minor route (biliary excretion, milk, and sweat).
- **Adverse health effects**
  - Neurotoxic effects – cognitive delays and behavior changes in children, lead-induced encephalopathy in children (blood lead levels of >70 μg/dL) and adults (blood lead levels of >100 μg/dL).

  - Cardiovascular effects – increased blood pressure and increased risk of mortality due to cardiovascular and cerebrovascular diseases.
  - Renal effects – depresses glomerular filtration, proteinuria, proximal tubular nephropathy, and glomerulosclerosis.
  - Hematological effects – *hypochromic microcytic anemia* (due to reduced erythrocyte life span and inhibition of heme synthesis).
  - Gastrointestinal effects – muscle discomfort, malaise, headache, persistent metallic taste, mild anorexia and usually constipation (rarely diarrhea), and severe intestinal pain (lead colic).
  - Carcinogenesis – lead exposure is associated with cancers of the lung, kidney, brain, and stomach (non-genotoxic carcinogen).
- **Treatment of lead poisoning**
  - Termination of further exposure
  - Supportive measures
  - Chelation therapy – for children (blood lead levels >45 μg/dl) and adults (blood lead levels >70 μg/dl) with or without acute symptoms of lead poisoning

26.2.3 Mercury

- **Source**
  - Release of mercury vapor – volcanic activity and combustion of fossil fuels
  - Metallic or elemental mercury – vaporization of mercury in dental amalgam and broken thermometers
  - Organic mercury – consumption of fish contaminated with mercury
  - Occupational exposure – workers in the chloralkali industry involved in the manufacture of alkaline batteries, fluorescent bulbs, and thermometers
- **Chemistry** – it exists in three forms
  - Metallic or elemental mercury (Hg^0)
  - Inorganic mercury – either as monovalent (mercurous, Hg^{1+}) or divalent (mercuric, Hg^{2+})
  - Organic mercury (divalent mercury complexed with one or two alkyl groups) – e.g., methylmercury ($MeHg^+$) formed by marine organisms
- **Mode of action** – it disrupts functions of proteins in the human body.
- **Toxicokinetics**
  - Absorption – by inhalation or ingestion
  - Deposition – in hair and nails
  - Excretion
    Major route in chronic exposure – urine
    Major route in acute exposure – feces
    Minor routes – exhaled air, sweat, and breast milk
- **Adverse health effects**
  - Metallic mercury

Acute exposure leads to weakness, metallic taste, nausea, vomiting, diarrhea, and *respiratory symptoms* like cough, tightness in the chest, and dyspnea (can lead to interstitial pneumonitis).

Chronic exposure – *renal damage*, tachycardia, labile pulse, severe salivation, gingivitis, and *neurotoxic effects like* tremors (especially of hands), emotional lability, insomnia, memory loss, muscular atrophy, weakness, paresthesia, and cognitive deficits.

- Inorganic salts of mercury
  Gastrointestinal toxicity like vomiting, diarrhea, and abdominal pain
  Renal toxicity – acute tubular necrosis and renal failure
- Organic mercury – *CNS toxicity* like visual disturbances, ataxia, paresthesia, hearing loss, slurring of speech, cognitive deficits, muscle tremor, movement disorders, paralysis, and ultimately death in case of severe exposure Minamata disease is a classic example of organic mercury poisoning following ingestion of fish and shell fish contaminated by methylmercury.

- **Treatment of mercury poisoning**
  - Termination of further exposure.
  - Supportive measures (respiratory support, maintenance of fluid, and electrolyte balance).
  - Emesis (within 30–60 min of exposure to inorganic mercury).
  - Chelation therapy can be tried in patients with acute mercury (inorganic or metallic) exposure.

26.3 Chelation Therapy for Heavy Metal Poisoning

- A chelator is a "compound that forms stable complexes with metals, typically as five- or six-membered rings."
- Mechanism of action – chelators form complexes with heavy metals and thereby prevent or alter the binding of the heavy metals with the biological ligands.
- Properties of an ideal chelator are enumerated in Box 26.1.
- Examples of chelators are enlisted in Box 26.2.

Box 26.1: Properties of an Ideal Chelator

- High water solubility
- Resistant to biotransformation
- Capacity to reach sites of metal storage
- Capacity to form stable and nontoxic complexes with toxic metals
- Easy excretion of the metal-chelator complex

Box 26.2: Examples of Chelators
- Ethylenediaminetetraacetic acid (EDTA)
- Dimercaprol or British anti-Lewisite (BAL)
- Succimer
- Sodium 2,3-dimercaptopropane-1-sulfonate (DMPS)
- Penicillamine
- Deferoxamine
- Deferasirox

26.3.1 Ethylenediaminetetraacetic Acid (EDTA)

- Effective chelators of divalent and trivalent metals
- Calcium disodium EDTA ($CaNa_2EDTA$) – preferred salt
- Used for treatment of *acute lead poisoning*
- Administered intramuscularly or slowly by intravenous drip
- Adverse effects – damage to proximal tubular cells of kidney, nausea, vomiting, frontal headache, sneezing, nasal congestion, lacrimation, glycosuria, anemia, dermatitis, prolonged prothrombin time, and T-wave inversion on the electrocardiogram

26.3.2 Dimercaprol or British Anti-Lewisite (BAL)

- Used for treatment of acute *arsenic*, gold, and mercury poisoning
- Used in combination with CaNa2EDTA to treat lead poisoning
- Deep intramuscular injection as a solution in peanut oil (contraindicated in patients with peanut allergy)
- Contraindicated in patients with hepatic insufficiency (except in arsenic poisoning induced hepatic dysfunction)
- Adverse effects
  - CVS – increase in systolic and diastolic arterial pressures, tachycardia, and chest pain
  - GIT – nausea and vomiting, salivation, a burning sensation in the mouth and throat, and abdominal pain
  - CNS – headache and tingling of the hands
  - Eye – conjunctivitis, blepharospasm, and lacrimation
  - Others – rhinorrhea, a burning sensation in the penis, and sweating

26.3.3 Succimer

- Orally effective chelator
- Chemically similar to dimercaprol

- Used for treatment of *lead*, arsenic, cadmium, mercury, and other toxic metal poisonings
- Advantage – does not significantly chelate essential metals such as zinc, copper, or iron
- Adverse effects – nausea, vomiting, diarrhea, loss of appetite, and rashes

26.3.4 Sodium 2,3-Dimercaptopropane-1-Sulfonate (DMPS)

- Orally effective chelator
- Clinically effective chelator of lead, arsenic, and mercury
- Less toxic than dimercaprol but more toxic than succimer

26.3.5 Penicillamine

- Orally effective chelator
- Inexpensive
- Uses
  - Wilson's disease (hepatolenticular degeneration due to an excess of copper)
  - Acute heavy metal intoxications with lead, mercury, or arsenic (not a first-line drug)
  - Cystinuria
  - Rheumatoid arthritis (rarely)
- Adverse effects
  - Skin – dryness and scaling, urticaria, macular or papular reactions, pemphigoid lesions, lupus erythematosus, and dermatomyositis
  - Cross-reactivity with penicillin
  - Hematological system – leukopenia, aplastic anemia, and agranulocytosis
  - Renal toxicity – reversible proteinuria and hematuria, which can result in nephrotic syndrome with membranous glomerulopathy
  - Pulmonary system – dyspnea due to bronchoalveolitis (rare)
  - Myasthenia gravis
  - GIT – nausea, vomiting, diarrhea, dyspepsia, anorexia, and a transient loss of taste for sweet and salt (relieved by dietary copper supplementation)
- Contraindications – pregnancy, renal failure, past history of penicillamine-induced agranulocytosis, or aplastic anemia

26.3.6 Deferoxamine

- Iron chelator
- Removes iron from hemosiderin, ferritin, and transferrin
- Cannot remove iron from hemoglobin or cytochromes
- Administered by intramuscular or intravenous route

- Dose: 10–15 mg/kg/h by slow intravenous infusion
- Also used in chelation of aluminum in dialysis patients
- Adverse effects
  - *Allergic reactions* – pruritus, wheals, rash, and anaphylaxis
  - Cataract formation
  - Neurotoxicity (on long-term use)
  - Pulmonary syndrome (with a high dose of 10–25 mg/kg/h) – tachypnea, hypoxemia, fever, and eosinophilia
  - Others – dysuria, abdominal discomfort, diarrhea, fever, leg cramps, and tachycardia
- Contraindications – renal insufficiency, anuria, and pregnancy

26.3.7 Deferasirox

- Orally administered chelator of iron
- FDA approved for the treatment of chronic iron overload in patients receiving therapeutic blood transfusions

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Basic Principles of Management of Drug Poisoning

27

Nishanthi Anandabaskar

Poisons and medicine are oftentimes the same substance given with different intents.

– Peter Latham

All things are poisons, for there is nothing without poisonous qualities. It is only the dose which makes a thing poison.

– Paracelsus

Abstract

Poisoning refers to the damaging physiological effects occurring as a result of exposure to pharmaceuticals, illicit drugs, or chemicals. Poisoning is a medical emergency and requires immediate care. The various steps involved in the management of a patient with poisoning are resuscitation or stabilization of the patient, evaluation of the patient, decontamination of the patient, poison elimination, antidote administration, and nursing and psychiatric care. Analytical toxicological methods can aid in the diagnosis, management, prognosis, and prevention of poisonings. Analytical toxicology is defined as the "detection, identification, and measurement of foreign compounds (xenobiotics) in biological and other specimens." Also, toxicovigilance activities are required to monitor the toxic effects of drugs in humans. Toxicovigilance is defined as "the active process of identifying and evaluating the toxic risks existing in a community and evaluating the measures taken to reduce or eliminate them." Poison information center refers to a specialized division providing information on poisoning to healthcare professionals and the community. They are located at centers having facilities for emergency and intensive care services. Their main functions include the provision of toxicological information and advice, management of patients with poisoning, provision of laboratory analytical services, toxicovigilance activities and research, and education on prevention and treatment of poisoning.

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Keywords
Drug overdosage · Poison information center · Toxicovigilance · Toxicology

27.1 Introduction

- Poison refers to any substance that can cause severe organ or tissue damage or death when it comes into contact with or enters the body. It is also known as a *toxicant*.
- Poisoning refers to the damaging physiological effects occurring as a result of exposure to pharmaceuticals, illicit drugs, or chemicals.
- Poisoning can be caused by industrial or household chemicals, heavy metals, environmental toxins, overdose of drugs, toxic plant, and animal products.
- Poisoning is a major global public health problem. According to WHO data, in 2012, an estimated 1,93,460 people died worldwide from inadvertent poisoning.
- Thus, proper management of these cases of poisonings is of paramount importance since they determine the patients' prognosis.

27.2 Management of Poisoning

The various steps involved in the management of a patient with poisoning are enlisted in Box 27.1.

27.2.1 Resuscitation or Stabilization of Patient

- It involves identification and correction of life-threatening problems.
- ABCD (attention paid to airway, breathing, circulation, and depression of CNS) of resuscitation:

Box 27.1: Steps Involved in the Management of a Patient with Poisoning
- Resuscitation or stabilization of patient (the most important step)
- Evaluation of the patient
- Decontamination of the patient
- Poison elimination
- Antidote administration
- Nursing and psychiatric care

- **Airway** – opening up the airway (chin lift and jaw thrust technique); clearing the airway of secretions, vomit, or foreign body with the patient placed in lateral position; and endotracheal intubation, if required.
- **Breathing** – supplemental oxygen by venture mask or through an endotracheal tube.
- **Circulation** – intravenous fluids may be administered.
- **CNS depression**
  Assessed by Glasgow Coma Scale.
  If etiology of coma is not known, the following three antidotes (called coma cocktail) can be administered:
  - Dextrose – 100 ml of 50% solution
  - Thiamine (vitamin B_1) – 100 mg
  - Naloxone – 2 mg

27.2.2 Evaluation of the Patient

- Done after resuscitation and when the patient is no longer in crisis.
- A thorough clinical examination is performed to identify and correct (if present) the following parameters.
  - Hypoglycemia
  - Hypothermia or hyperthermia
  - Acid-base disorders
  - Seizures
  - Electrolyte disturbances (hypokalemia, hyperkalemia, hyponatremia, hypernatremia, and hypocalcemia)
- Prediction of the possible clinical progress and impending complications for the individual patient by taking into account the following:
  - Causative agent (poison)
  - Dose
  - Time of poisoning
  - Current clinical position
  - Distinct patient factors

27.2.3 Decontamination of the Patient

- **Skin decontamination** (if applicable) – remove contaminated clothes, and wash the contaminated area with soap and water.
- **Eye decontamination** (if applicable) – wash eyes with running tap water or normal saline.
- **Gastrointestinal decontamination or evacuation** (Box 27.2)

Box 27.2: Gastrointestinal Decontamination Procedures

- **Emesis**
- **Gastric lavage**
- **Catharsis**
- **Administration of activated charcoal**
- **Whole bowel irrigation**

These procedures aim to reduce absorption of an ingested agent and they include:

- **Emesis**
  - Rarely employed.
  - Vomiting is induced using syrup of ipecacuanha.
  - Dose: 30 ml (adult) and 15 ml (child).
  - Used only in conscious patients within 4–6 h of ingestion of poison.
  - Absolute contraindications – ingestion of convulsant poison, coma or altered mental status, corrosive ingestion, and foreign body ingestion.
  - Complications of procedure – aspiration pneumonia, esophageal mucosal tears (Mallory-Weiss tears), and cardiotoxicity (bradycardia, atrial fibrillation, myocarditis).
- **Gastric Lavage (Stomach Wash)**
  - Performed only when the patient presents within 1–3 h of ingestion of poison (however this period can be extended up to 12–18 h post-ingestion in case of poisoning with salicylates, tricyclic antidepressants, phenothiazines, and antidepressants)
  - Absolute contraindications – corrosive poisoning (due to risk of perforation)
  - Relative contraindications – convulsant poison, comatose patients, volatile poison, patients with esophageal varices, hypothermia, and bleeding diathesis
  - Procedure:

    The patient is placed in a left lateral position and head tilted down so as to prevent aspiration of stomach contents into the respiratory tract.

    Ewald tube or Boas tube or any soft, non-collapsible rubber tube of 1 cm diameter and 1.5 m length with a funnel attached to one end and a mark 50 cm from the other end with the mid-part of the tube having a suction bulb (to pump out stomach contents) is used for stomach wash.

    The inserting end of the tube is lubricated with Vaseline or glycerine and inserted orally through the pharynx and esophagus into the stomach, till the 50 cm mark is reached.

    In children, Ryle's tube of diameter 22–28 French sizes can be used, and it should be inserted up to a length of 25 cm.

The position of the tube should be checked by air insufflations while listening over the stomach or by aspiration and pH testing of the aspirate (acidic pH confirms its position in the stomach).

Lavage is carried out by using 200–300 ml of warm (38 °C) saline in adults and 10–15 mL/kg body weight of warm saline in children.

The first aliquot of washing should be sent for chemical analysis.

Lavage should be continued until clear and odorless fluid comes out.

At the end of the procedure, a small amount of antidote or activated charcoal suspension (1 g/kg body weight) and/or a cathartic is left in the stomach.

– Complications – aspiration pneumonia, laryngospasm, sinus bradycardia, and perforation of the stomach or esophagus (rare)

- **Catharsis or Purging**
  - It is a procedure used to decrease the absorption of poison by hastening its expulsion from the GIT (especially, the small intestine).
  - Two main groups of cathartics are used – saccharide cathartics (sorbitol) and saline cathartics (magnesium citrate, magnesium sulfate, and sodium sulfate).
  - These drugs cause osmotic retention of fluid in the intestinal lumen, and this activates GI motility and accelerates expulsion.
  - Contraindications – corrosive poisoning, existing electrolyte imbalance, paralytic ileus, recent bowel surgery, and renal failure. Hence, not used routinely.
- **Administration of Activated Charcoal**
  - Activated charcoal is a fine, black, odorless, and tasteless powder.
  - It is prepared by burning organic materials (especially wood) and treating them with activating agents (like steam and carbon dioxide) at high temperatures.
  - The particles are very small with an extremely large surface area.
  - Mode of action – It adsorbs and retains the poison on to its surface and thus delays the absorption of the poison.
  - Dose: 1 g/kg body weight
  - It is mixed with four to eight times the quantity of water to form a slurry and administered orally.
  - Its efficacy is maximum when administered within 1 h of ingestion of poison.
  - The extent of adsorption of various drugs to activated charcoal is given in Box 27.3.
  - **Multiple-Dose Activated Charcoal**

    Use of repeated doses of activated charcoal facilitates the passage of drugs from plasma into the intestinal lumen.

    Initial loading dose – 1–2 g/kg body weight, followed by repeat doses of 0.5–1 g/kg body weight every 4–6 h.

Box 27.3: Extent of Adsorption of Various Drugs to Activated Charcoal

Well adsorbed		Moderately adsorbed	Poorly adsorbed
Drugs acting on the CNS		Phenol	Corrosives
Barbiturates	Benzodiazepines	Salicylates	Heavy metals
Opiates	Amphetamines	Kerosene	Cyanide
Antidepressants	Phenothiazines	Paracetamol	Hydrocarbons
Antiepileptics			Alcohol
Alkaloids			
Atropine	Quinine		
Chloroquine	Strychnine		
Digitalis	Theophylline		
Other drugs			
Antihistamines	Cimetidine		
Tetracycline			

It is useful for the elimination of certain drugs like theophylline, phenobarbital, quinine, digitoxin, salicylates, and carbamazepine.

- Complications – vomiting, pulmonary aspiration, and intestinal obstruction
- Contraindications – absent bowel sounds/proven ileus, small intestinal obstruction, and caustic ingestion

Whole Bowel Irrigation (Whole Gut Lavage)

- Recommended when the patient presents late, especially after 4 h of ingestion of poison.
- Other indications – overdosage of drugs difficult to remove like:
  Sustained release formulations
  Iron
  Lithium
  Cocaine and heroin
- Polyethylene glycol with electrolyte lavage solution is instilled into the stomach by a nasogastric tube.
- Rate of administration – 0.5 l/h to children less than 5 years of age and 2 l/hour to adults.
- The procedure is continued until a clear rectal effluent passes (takes about 2–6 h).
- Complications – vomiting, abdominal distension and cramps, and anal irritation.
- Contraindications – GI pathologies like obstruction, ileus, hemorrhage, or perfusion.

27.2.4 Poison Elimination

The various methods of elimination include:

Renal elimination – It includes the following methods:

- Forced diuresis
- Urinary pH alteration – urinary alkalinization and urinary acidification

Extracorporeal techniques

- Principle – these techniques remove the poison from the blood compartment.
- Not useful for drugs with a large volume of distribution as they are predominantly present in extravascular compartments.
- Disadvantages – more invasive, more costly, and require relocation to a specialized center.
- It includes the following methods:
  - Hemodialysis
  - Hemoperfusion
  - Hemofiltration
  - Peritoneal dialysis
  - Plasmapheresis

- **Forced diuresis**
  - Principle – enhance elimination of poison by administering large volume of crystalloids with loop diuretics.
  - It can be used for elimination of poisons with renal excretion (Box 27.4).
  - Complications – fluid overload, pulmonary edema, cerebral edema, hypernatremia, and hypokalemia.

Box 27.4: List of Drugs or Poisons with Renal Excretion: Suitable for Forced Diuresis

Cyclophosphamide	Thallium
Isoniazid	Meprobamate
Fluoride	Iodide
5-Fluorouracil	Cisplatin
Bromides	Barium
Chromium	Lithium
Salicylates	Ethylene glycol

Urinary pH alteration

- Most drugs are weak electrolytes, and they exist in both ionized and unionized forms.
- The rate of renal tubular reabsorption of a drug depends on the amount of unionized form of the drug in renal tubules as cell membranes are permeable only to the lipid soluble and unionized forms.
- The urinary pH determines the extent of ionization of the drug in renal tubules.
- Acidic drugs are ionized more in alkaline pH and tubular reabsorption decreases, thus enhancing renal elimination.
- Basic drugs are ionized more in acidic pH and tubular reabsorption decreases, thus enhancing renal elimination.
- Thus, alkalinization of urine increases the elimination of acidic drugs, and acidification of urine enhances elimination of basic drugs.
- **Urinary Alkalinization**

  Urinary alkalinization is performed with i.v. sodium bicarbonate administration.

  Characteristics of poisons for which urinary alkalinization will work are:
  - Primarily eliminated by kidneys
  - Predominantly distributed in the extracellular compartment
  - Minimally protein bound
  - Relatively weak acids (pKa: 3.0–7.0)

  For maximal efficacy – urine pH is maintained between 7.5 and 8.5.

  Drug poisonings for which urinary alkalinization is used – salicylates and phenobarbitone.

  Complications – hypokalemia (most common) and alkalotic tetany (rare).
- **Urinary Acidification**

  Urinary acidification performed with ammonium chloride or ascorbic acid

  Not preferred due to lesser efficacy and enhanced toxicity

  Drug poisonings for which urinary acidification was performed – amantadine, amphetamine, quinidine, and phencyclidine poisoning

  Complication – metabolic acidosis

- **Hemodialysis**
  - Principle – it involves diffusion of the toxin through a semipermeable membrane into the dialysate.
  - It is performed with the help of a hemodialysis machine.
  - Drugs with the following properties are easily dialyzed:

    Low molecular weight

    Low protein binding

    Small volume of distribution

    Low lipid solubility
  - Examples of drug poisonings for which hemodialysis is indicated are:

    Salicylates

    Methanol

    Lithium

    Ethylene glycol

    Theophylline

  - Complications of the procedure include:
    Hypotension
    Risk of infections such as AIDS and hepatitis B
    Thrombosis
    Air embolism
    Bleeding due to the use of heparin as a systemic anticoagulant
- **Hemoperfusion**
  - Principle – it involves adsorption of the drug to the adsorbent, which is usually either activated charcoal or ion exchange resins.
  - It is performed using a hemoperfusion device which contains a cartridge containing the adsorbent. The anticoagulated blood of the patient is made to flow through the cartridge.
  - Examples of drug poisonings for which hemoperfusion is indicated are:
    Paracetamol
    Salicylates
    Barbiturates
    Diazepam
    Dapsone
    Organophosphates
    Theophylline
    Tricyclic antidepressants
  - Complications of hemoperfusion are many and include the following:
    Thrombocytopenia
    Leucopenia
    Hypocalcemia
    Hypoglycemia
    Hypothermia
    Risk of infections such as AIDS and hepatitis B
    Bleeding due to the use of heparin as a systemic anticoagulant
- **Hemofiltration**
  - Principle – Patient's blood is pumped into a hemofilter which removes toxins by convection across a membrane.
  - It efficiently removes high molecular weight toxins (up to 40,000 D).
  - Drugs for which hemofiltration is preferred are:
    Vancomycin
    Methanol
    Procainamide
    Lithium
    Methotrexate
- **Peritoneal dialysis**
  - Rarely used since it is not an efficient method of toxin removal.
  - Principle – It involves diffusion of toxins from mesenteric capillaries across the peritoneal membrane, into the dialysate residing in the peritoneal cavity.
  - Complications:
    Pain
    Hemorrhage from vascular lacerations

Perforation of viscus
Bacterial peritonitis
Volume overload or depletion
Electrolyte imbalance

- **Plasmapheresis**
  - Principle – cellular blood components are separated from plasma. The cells are resuspended in colloids, albumin, or fresh frozen plasma and reinfused.
  - This procedure is suitable for toxins that are highly protein bound with low volumes of distribution.
  - Examples of poisonings for which plasmapheresis is used are:
    Theophylline
    Carbamazepine
    Mercury
  - Rarely used since serious complications like the following can occur:
    Bleeding disorders (DIC, thrombocytopenia)
    Hypercoagulation (myocardial infarction, stroke, and pulmonary embolism)
    Anaphylaxis
    Fluid overload
    Convulsions
    Metabolic alkalosis

27.2.5 Antidote Administration

- Antidotes are drugs given to counteract the effect of poisons (Table 27.1).
- They are required only in a minority of cases of acute poisoning. Majority of the patients benefit from supportive care alone.
- The common modes of action of antidotes are:
  - **Inert complex formation** – these antidotes interact with the poison and form inert complexes which are excreted from the body, e.g., chelating agents for heavy metals and specific antibody fragments for digoxin poisoning.
  - **Accelerated detoxification** – these antidotes accelerate the detoxification of poison. For example, thiosulfate hastens the conversion of toxic cyanide to nontoxic thiocyanates.
  - **Reduced toxic conversion** – these antidotes reduce the formation of toxic metabolites. For example, ethanol inhibits the metabolism of methanol and prevents formation of toxic metabolites.
  - **Receptor site competition** – these antidotes compete with the poison for binding to specific receptor(s). For example, naloxone as an opioid antagonist competes with opioid compounds for the same binding site.
  - **Receptor site blockade** – these antidotes block the receptor on which the poison acts to produce toxic effects. For example, atropine blocks the muscarinic effects of anticholinesterases like organophosphorus and carbamate pesticides.

Table 27.1 List of specific antidotes commonly used for different types of poisoning

Antidote	Type of poisoning
Atropine	Organophosphorus and carbamate pesticide
Desferrioxamine	Iron
Digoxin-specific Fab fragments	Digoxin toxicity
Dimercaprol	Arsenic, mercury, lead
Ethanol	Methanol, ethylene glycol
Flumazenil	Benzodiazepine
Folinic acid	Methotrexate
Fomepizole	Methanol, ethylene glycol
Naloxone	Opioid
N-acetylcysteine	Paracetamol
Phytomenadione (vitamin K)	Warfarin
Pralidoxime	Organophosphate pesticide
Protamine	Heparin
Pyridoxine	Isoniazid
Penicillamine	Copper
Sodium nitrite	Cyanide
Sodium thiosulfate	Cyanide
Succimer (DMSA)	Lead, mercury

– **Toxic effect bypass** – these antidotes bypass the toxic effect of the poison, e.g., use of 100% oxygen in cyanide poisoning.

27.2.6 Nursing and Psychiatric Care

- Most important for unconscious patients.
- Attention to pressure points to prevent the formation of decubitus ulcers.
- Eye protection in the absence of spontaneous blinking.
- Passive physiotherapy to prevent joint stiffness and muscle atrophy.
- Prophylactic antibiotics.
- Prevention of aspiration of gastric contents by placing the patient in semi-prone position.
- Management of fecal and urinary incontinence.
- Psychiatric counseling and care must be provided for patients with suicidal ideation once the patient stabilizes.

27.3 Analytical Toxicology

- Analytical toxicology is defined as the "detection, identification and measurement of foreign compounds (xenobiotics) in biological and other specimens."

- Analytical methods are existing for a multitude of compounds like:
  - Chemicals
  - Pesticides
  - Pharmaceuticals
  - Drugs of abuse
  - Natural toxins
- Analytical toxicology can aid in the diagnosis, management, prognosis, and prevention of poisonings.
- The roles of analytical toxicology service include:
  - Emergency qualitative and/or quantitative assays for certain common poisons
  - Screening for cases where the cause of illness is unknown but may involve a poison
  - Analyses to monitor the effectiveness of particular treatment or elimination techniques (e.g., hemoperfusion and hemodialysis)
  - Analyses for the biological monitoring of populations exposed to toxic chemicals occupationally or environmentally
  - Advice on the collection, storage, and transport of specimens and on the interpretation of results of analyses
  - Research into toxicokinetics and mechanisms of toxicity
- The ideal location for an analytical toxicology laboratory is within, or nearby, hospitals where the poisoned patients are treated which reduces the time required for transport of samples and facilitates consultations between clinicians and analysts.
- Quality assurance – analytical data given by laboratory services must be dependable, and this can be confirmed by using internal and external quality assurance procedures.

27.4 Toxicovigilance

- The WHO document entitled *Guidelines for poison control* provides recommendations on toxicovigilance activities.
- According to the WHO, toxicovigilance is defined as "the active process of identifying and evaluating the toxic risks existing in a community and evaluating the measures taken to reduce or eliminate them."
- It involves active detection, validation, and follow-up of clinical adverse events related to toxic exposures through household, occupational, or environmental chemicals and products.
- Toxicovigilance activities include the following:
  - **Surveillance**

    The sources of information on poisoning incidents of public health significance are obtained from doctors' reports, hospital databases of poisoning cases admitted in intensive care units and emergency medical services, and poison information centers.

It also involves detection and validation of clinical adverse events happening among more vulnerable subgroups like in children.

Proactive surveillance programs, e.g., systematic measurement of blood lead levels in urban children with subsequent evaluation of affected children, are also being undertaken.

– **Investigation**

Data validation – completeness and precision of available data should be ensured.

Aggregation of toxicological case reports from different sources can be used to generate signals which need to be further evaluated.

The causal relationship between the adverse clinical event and toxic exposure needs to be determined.

The available clinical, analytical, and/or biological data are carefully matched with other possible medical diagnoses, exposure characteristics of the patient, and toxicological plausibility.

Thus, the governmental departments and other healthcare agencies should undertake investigation and identify the type of poison and its diagnosis, management, and prognosis and assess its impact on society.

– **Risk communication**

The healthcare professionals and the public are to be appraised with the toxicological information. Electronic publications like *Poisoning watch* and *poisoning.com* contain articles on poisoning agents, case reports, and surveillance statistics for educating the healthcare professionals.

Public is alerted to the various poisoning risks by distribution of pamphlets, fact sheets, posters, and health talks.

- The International Programme on Chemical Safety (IPCS) was established in 1980 as a collaborative program of the International Labour Organization (ILO), the United Nations Environment Programme (UNEP), and the World Health Organization (WHO) as a joint venture to evaluate the risks to human health and the environment posed by chemicals, enabling all countries throughout the world to develop their own chemical safety measures.
- Poison information centers are one of the most key sources of poisoning information. The enquires coming to the centers are analyzed for the following:
  - Identification of specific settings or definitive agents giving rise to poisoning
  - Identification of particular sets of populations experiencing a higher incidence of poisoning
- Toxicovigilance also helps in the identification of emerging toxicological concerns like problems due to the following:
  - Reformulation of a product or a change to its packaging or labelling
  - Advent of a new drug of abuse
  - Identification of an environmental contamination
- One of the examples of hazard identification and risk assessment by toxicovigilance is the identification of lung toxicity of leather waterproofing sprays. Use of waterproof sprays on leather clothes or shoes in a closed room was found to be associated with increased incidence of acute alveolitis by poison information

centers. On investigation, it was found that aerosolized fluoroalcene resin (a component of waterproof sprays) was the causative agent. Following the ban of 1,1,1-trichloroethane to protect the ozone layer, the fluoroalcane waterproofing resins were replaced by the fluoroalcene resin because of their increased solubility in isoalcane solvents. Unlike the fluoroalcane waterproofing resin, the fluoroalcene resin formed smaller particles which were inhaled and induce adverse reactions in the lungs of the workers. This example illustrates the sentinel role of poison information center in toxicovigilance.

27.5 Poison Information Centers

- Poison information services should be available in every country.
- Poison information center refers to a specialized division providing information on poisoning to healthcare professionals and the community.
- It should be located at centers with emergency and intensive care services, medical library, and an analytical laboratory. Preferred locations are university teaching hospitals or in toxicological or public health institutions.
- The poison information center should operate 24 h a day, all around the year. Hence, they should be able to provide round-the-clock services.

27.5.1 Resources Required for Establishment of Poison Information Centers

- **Human resources**
  - It requires a multidisciplinary team of poison information specialists which include physicians, nurses, analysts, pharmacists, veterinarians, and other scientists representing a wide variety of disciplines including biology, chemistry, medicine, and pharmacology.
  - They generate and offer expert information and guidance about preventing and treating poisoning.
  - The members of the team answering the queries must be an expert in toxicology and should also be in regular contact with analytical and treatment facilities.
  - At least two poison information specialists are recommended to be on duty to answer queries.
- **Equipment and facilities** – office furniture and facilities are required for the following:
  - Answering telephone enquiries (through reserved telephone lines)
  - Consultation with patients
  - Preparation of documents
  - Staff meetings
  - Secretarial and administrative work
  - Storage of confidential data

- **Library facilities** – it should contain:
  - Indexes, guides, and listings concerned with medicines and other agricultural and chemical products available in the local market plus the local pharmacopeia
  - Books or other publications related to the animal and plant toxins of the region
  - Standard textbooks of medicine, chemistry, pharmacology, and analytical toxicology
  - Journals of medicine and toxicology
  - Dictionaries relating to the main areas covered by the documentation in the center
- **Facilities for treatment units**
  - Establishment of clinical toxicology units which deal exclusively with the management of patients with poisoning.
  - These units require:
    Presence of devices and areas for the resuscitation, decontamination, and initial management of poisoning cases
    Validated and tested protocols for the treatment of common acute poisoning cases
    Availability of antidotes
    Availability of emergency transport facilities for the patient
    A disaster management plan for dealing with untoward emergencies and major chemical accidents
    Adequate and well-trained staff
- **Facilities for laboratory services –** it includes:
  - Basic equipment including digital balances, centrifuges, vortex mixer, water bath, refrigerator, freezer, and fume cupboard
  - Basic equipment for techniques such as colorimetry, photometry, spectrophotometry, and thin layer chromatography
  - Sophisticated analytical techniques such as immunoassay, gas chromatography, mass spectrometry, radioimmunoassay, high-performance liquid chromatography, and atomic absorption spectrophotometry
  - Well-trained staff – minimum one experienced toxicological analyst, laboratory technician, administrative staff, and possibly a documentalist

27.5.2 Main Functions of Poison Information Center

- Provision of toxicological information and advice
  - Provide information and guidance on:
    Diagnosis, prognosis, treatment, and prevention of poisoning
    Toxicity of chemicals and the risks they pose
  - Information should be provided to all who will benefit from it, including medical and paramedical professionals, media, and public.

  - Communication channels include telephone (most common), computer networks, written responses to enquiries, publications, and direct consultations.
  - All information and guidance should be adapted to the specific settings of the suspected poisoning.
- Management of patients with poisoning
  - The poison information centers are usually associated with its own clinical toxicology unit or treatment facilities for management of poisoned patients
  - Also, these units provide information to the first person in contact with the poisoned patient, appropriate to the enquirer's level of understanding regarding the further course of action.
- Provision of laboratory analytical services
  - These services are essential for the diagnosis, assessment, and treatment of certain types of poisoning.
  - They aid in the identification, description, and estimation of toxic substances in both biological and non-biological samples.
  - They also help to define the kinetics of the toxin including its absorption, distribution, metabolism, and elimination.
  - Analyses that are directly related with the treatment of poisoned patients should be available all through the day.
  - The analytical laboratory should preferably be located in the same place as poison information center and treatment facility for adequate interdisciplinary collaboration.
- Toxicovigilance activities and research
  - Analysis of enquiries received by these centers helps in identification of toxic risks in the community. This information should be shared with appropriate healthcare authorities in order to undertake preventive and regulatory measures.
- Education and continuous training in the prevention and treatment of poisoning
  - The poison information centers have educational responsibilities and need to train the healthcare professionals and the public regarding the prevention and treatment of poisoning.

27.5.3 Establishment of Poison Information Centers Worldwide and in India

- Poison information centers were first established in the Netherlands in 1949.
- Later in 1961 and 1963, these centers were established in England and the United States, respectively.
- In India, the first poison information center was established by the Department of Pharmacology at the All India Institute of Medical Sciences, New Delhi, in December 1995.
- Subsequently, other poison information centers have been established in India, some of which are enlisted below:
  - National Institute of Occupational Health, Ahmedabad
  - Amrita Institute of Medical Sciences, Cochin

- P. D. Hinduja National Hospital and Medical Research Centre, Mumbai
- JSS Hospital, Mysore

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Part V

Molecular Biology in Pharmacology

Regulation of Gene Expression

28

Neel Jayesh Shah

Abstract

Our genetic machinery is regulated extensively and is the reason why each organ has cells that express a unique set of genes, even though all cells in our body have the same chromosomes. Regulation starts at the epigenetic level, controlling the unwinding of chromosomes from histones by methylation and acetylation. Transcription of mRNA is immensely regulated by the promoter regions on the DNA. mRNA can also be regulated posttranscriptionally by miRNA and siRNA. Translation of proteins and their modification by ubiquitination after synthesis represents another level of regulation. The application of this science in the treatment of cancer is explained in this chapter along with the mechanisms by which drugs like vorinostat, romidepsin, miravirsen, steroids, and bortezomib act.

Keywords

Gene regulation · Epigenetics · MicroRNA (miRNA) · Small interfering RNA (siRNA)

28.1 Introduction

- Just as it is necessary to control a nuclear fission reaction to prevent an explosion, our genetic machinery is exquisitely regulated by mechanisms, perhaps more complex than the expression of genes themselves.
- Be it a hepatocyte, lymphocyte, osteocyte, or a glomerular cell, all contain identical chromosomes. It is the regulation of their genes' expression that essentially is responsible for their differentiation.
- Looking at organisms from a broader perspective, regulation of genetic expression gives them the power to adapt to different environments such as the response

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of melanocytes to the sun or the neurons to learning, involving switching on or off the synthesis of various proteins or enzymes.
- Regulation can occur at all steps, starting from the unwinding of nucleosomes, DNA transcription to posttranslational modification of proteins. Regulation which happens more upstream implies a larger control over expression.

28.2 Epigenetic Control of Regulation

- Epigenetics represents the control of gene expression that occurs without any change in the DNA sequence. This phenomenon does not involve regulation of translation but works even before DNA gets transcribed into mRNA. Cellular differentiation is an example of epigenetics at work.
- Densely spiralled chromosomes wound around histone proteins (nucleosomes) which represent the sites for regulating the amount of DNA that gets into contact with transcription factors. This tightly wound chromatin structure can be opened up by chemical modification of DNA or of histones.
- Acetylation leads to dissociation of histones from DNA and allows transcription to proceed. On the other hand, methylation of DNA (when it occurs in the promoter region) prevents transcription by causing coiling of DNA. Thus, DNA methylation and histone deacetylation work together to bring about gene silencing.
- The enzymes involved are histone methylase (HMT), DNA methyl transferase (DNMT), histone acetylase (HA), and histone deacetylase (HDAC). Any error in epigenetic control can lead to excess or reduced DNA transcription which, in case of tumor suppressor genes, can lead to cancers.
- Quite expectedly, there are many anticancer drugs that work by epigenetic mechanisms. HDAC inhibitors such as vorinostat, belinostat, and romidepsin allow acetylation of histones and thereby promote the transcription of tumor suppressor genes. They are used in solid and hematological malignancies such as lymphoma.
- In cancers, many tumor suppressor genes are silenced by methylation and are not allowed to express. Drugs such as azacitidine and decitabine inhibit DNA methyltransferase apart from their cytotoxic action at higher doses.

28.3 Regulation of Transcription

This next phase of regulation of gene expression is the most commonly used mechanism and involves regulation of production of mRNA from DNA.

- The LAC operon is one of the earliest known examples of regulation of mRNA synthesis in prokaryotes. Here, the two *transcription factors* which control transcription are lactose and glucose. The presence of lactose and the absence of glucose leads the promoter region to transcribe lactase enzyme mRNA.

- Promoter regions are regions present near 5′ transcription start sites in the DNA which the transcription machinery identifies to start transcription. They also act as binding regions for repressors.
- Repressor is a substance that binds to promoter and prevents the transcription machinery (RNA polymerase) to move ahead. In case of LAC operon, lactose prevents repressor from binding to the promoter region, and thus, the transcription machinery identifies the free promoter region to get going. In eukaryotes, TATA box is a similar example.

28.3.1 Eukaryotes

- When it comes to transcription of larger regions in eukaryotes, this kind of regulation is not enough. In larger systems like that of humans, the promoter (called as regulatory elements) can be present even far away from the coding regions. Transcription factors are signals that bind to regulatory elements in the DNA (and are not as simple such as lactose or glucose, but something more regulated). They can be present in the cytosol or in the nucleus. They are usually activated by phosphorylation or dephosphorylation. Important examples of transcription factors in eukaryotes are:
  - **CREB**
    cAMP response elements binding transcription factor (CREB) controls the transcription of several genes. CREB is itself regulated by many second messengers such as growth factors, cAMP, and calcium calmodulin system. At the basic level, phosphorylation and dephosphorylation modulate CREB. An example is differentiation of neurons by brain-derived neurotrophic factor (BDNF) that phosphorylates CREB.
  - **AP-1**
    AP-1 set of transcription factors regulate the transcription of neuropeptides and neurotransmitter receptors.
  - **Steroid hormone receptor superfamily**
    This set of transcription factors are present in the nucleus and directly bind to DNA. They are regulated by hormones such as glucocorticoids, estrogen, testosterone, and thyroid hormones and vitamins such as retinoids and vitamin D3.

28.4 Posttranscriptional Modification

- This step of regulation occurs after the mRNA is synthesized and controls how much should be the final amount of protein that needs to be formed.
- Regions of the mRNA molecule which are not translated into protein but regulate mRNA function include the 5′ cap, 5′ untranslated region, 3′ untranslated region, and the poly(A) tail. Normally, mRNA undergoes activation by poly-A tail addition and capping of 5′ end.

- Of special importance is the 3′ untranslated region because it is the site for regulation of gene expression. The following are the ways of posttranscriptional regulation.

28.4.1 miRNA

- MicroRNA (miRNA), first discovered in 1993, bind to the 3′ untranslated region of the mRNA and prevent their translation. One miRNA can affect the stability of hundreds of mRNA which reflects non-specificity.
- miRNAs, upon binding to the mRNA, downregulate expression by inhibiting translation or by causing endonuclease-mediated cleavage of the mRNA itself. However, miRNAs can even cause upregulation of translation, an example of which is the miRNA: tumor necrosis factor-alpha. Miravirsen is a miRNA-based drug still in clinical trials for use in hepatitis C.

28.4.2 siRNA

- Small interfering RNA (siRNA), first discovered in 1998, is similar to miRNA, both being 21–26 nucleotides long, but siRNA is synthesized from double-stranded RNA, whereas miRNA is made from stem-loop type transcripts.
- Another important difference is that siRNA inhibits more specifically the mRNA which it intends to silence. Due to this specificity, siRNA are good candidates for drug development.
- Numerous techniques have been explored to transport this small sequence of nucleotides into the nucleus. These include delivery by retroviruses, liposomes, or electroporation. The challenge is transporting them into each and every cell.
- Examples of siRNA that have entered clinical trials are against diseases such as amyloidosis, RSV infection in lung transplant patients, and breast cancer. More than 6500 patents have been filed in the USA itself which are related to the use of siRNA in cancer. This reflects the intense research and potential of siRNA in therapeutics.

28.5 Regulation of Translation

- Eukaryotic initiation factors (such as eIF-2) help in initiation of translation by binding to ribosomes (subunit 40S). This binding is controlled by dephosphorylation, which activates eIF-2 and thus initiates translation of the protein.
- On the other hand, when the cell is in stress, kinases (four types) of the eIF get activated and prevent translation. Another mechanism to control the rate of translation initiation involves the recognition of mRNA 5′-cap by an eIF: eIF4F.

28.6 Modification of Proteins

- Activation of digestive enzymes/zymogens such as pepsinogen and lipase in the pancreas is a classic example of why protein activation needs to be regulated. Even insulin is regulated by conversion of preproinsulin and proinsulin into insulin.
- Ubiquitination adds an ubiquitin group to the proteins that need to be degraded. Ubiquitination is also known as recycling of proteins. An example of drug that targets ubiquitin pathway is bortezomib. This drug prevents the degradation of pro-apoptotic factors.

28.7 Conclusion

Regulation of gene expression is under extensive control. It can be permanent, temporary, or even dynamic. It gives the flexibility to adapt to the environment. Regulation can occur at all steps from exposing DNA to transcription machinery, actual transcription process, mRNA modifications, translation, and finally post-translational modification of proteins. Each step has been extensively studied, and drugs have been discovered that can modulate them. Regulation of gene expression is particularly disturbed in diseases such as cancer and is a hot topic for research.

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Proteomics

29

Neel Jayesh Shah

Abstract

Proteomics is the systemic evaluation of protein levels in the body and correlation with pathogenic and physiologic states. They can be analyzed individually or as a pattern, the latter being accomplished by algorithms run by computers. Proteomics can be used to identify biomarkers of early disease like in cancer as well as to identify drug targets, efficacy markers, and toxicity markers of drugs. Its advantage over genomics lies in the measurement of real-life changes in gene expression. Genomics and proteomics, when correlated together, help in understanding *systems biology*. While microarrays and immunoassays detect proteins based on antigenic property, mass spectrometry relies on charge and mass of proteins.

Keywords

Proteomics · Microarrays

29.1 What Is Proteomics?

Proteomics is the systemic evaluation of protein content in the body and correlating them with the physiological or pathological state of the body. The term "proteomics" was coined in 1997, barely a couple of decades back. The goals of proteomics are as follows:

- To characterize metabolic pathways.
- Interconnect them with gene expression.
- Explain the cause or consequence of diseases.
- *Identify biomarkers*.
- *Identify targets where drugs can act to modify the pathways*.

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29.2 Clinical Proteomics

Clinical proteomics refers to application of proteomics in disease states. The following are the applications.

29.2.1 Early Diagnosis of Diseases

- Diseases like cancer are detected when it is too late and metastasis has occurred. Serum, saliva, and urine are easily accessible and act as potential fluids where proteomics offers early chance of biomarker detection.
- A pattern of proteome can be used for diagnosis instead of a specific protein and can complement histopathology.

29.2.2 Personalized Therapy

- Choosing drugs based on the patient's unique protein expression pattern.
- Structural or 3-D analysis of the causative proteins enables designing of effective drugs.
- Administering multiple drugs which block a pathological pathway at multiple levels.
- Identifying efficacy and toxicity in the real time.

29.3 Challenges in Proteomics

- Ever changing and heterogeneity of the body's metabolism. Protein signatures have intra- as well as interbody difference in healthy state as well as diseased state.
- A vast number of proteins are expressed in the human body, so it is difficult to link specific protein to specific disease.

29.4 Advantages over Genomics

- A gene may not necessarily be expressed, but proteomics measures the levels of protein circulating in the body in real life.
- Many transcripts may give rise to same protein, and in proteomics we measure the end product.

29.5 Methods of Analyzing Proteins

29.5.1 Microarrays and Immunoassays

- Characterize proteins based on their antigenic nature and affinity to antibodies and subsequent identification by chemiluminescence, e.g., ELISA and Western blot.

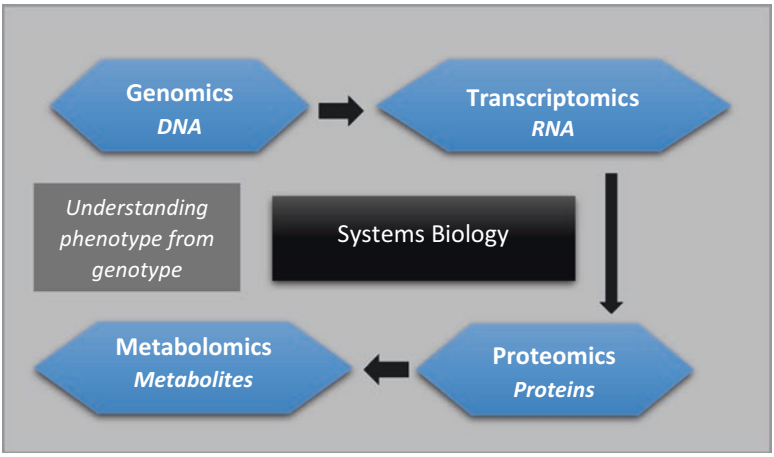

Fig. 29.1 A flow chart depicting how various "omics" lead to understanding of the functioning of the biological system and a holistic approach

29.5.2 Mass Spectrometry

- Characterizes molecules by their mass and charge and thereby the time they take to fly over to the electrode, smaller ones reaching faster. Molecules or sets of proteins have a unique time-of-flight signature, calculated by algorithms, e.g., surface-enhanced laser desorption ionization-time-of-flight (SELDI-TOF).

29.6 Conclusion

- Proteomics is still evolving and its full potential is yet to be tapped.
- Artificial intelligence is used for protein pattern analysis which learns and adapts with increasing protein data. This branch is called "bioinformatics for proteomics" (Fig. 29.1).

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Techniques Involved in Studying Receptor Dynamics

30

Neel Jayesh Shah

Abstract

Receptor population is dynamic and is exemplified by their rapidly changing expression in neuronal cells, receptor internalization with steroid ligands and growth hormones, and their upregulation and downregulation as in β_2 receptors. Fluorophore tagging to recombinant receptors and their visualization in cultured cells represent a way by which movement of receptors can be traced. However, the linking molecules between receptor and fluorophore are bulky. "Single molecule" tagging has been made possible by creating mutated receptors using unnatural amino acids that preserve function as well as they act as handles where luciferase enzyme can bind. Apart from tracing fluorescent activity, the surface receptor density in neurons can be estimated as being proportional to the electrical activity measured ("functional" tagging). These methods have been crucial in understanding how receptors are cycled in their lifetime.

Keywords

Receptor dynamics · Upregulation · Downregulation · Fluorophore tagging · Functional tagging

30.1 Introduction

- Receptor population inside or at the surface of the cell is dynamic; it changes with the environment and the ligands and is under constant control of the cell. Examples of receptor dynamics include upregulation and downregulation.
- Neurons produce new receptors (e.g., $GABA_A$, AMPA, NMDA, and Gly receptors) and send them toward the dendrites in vesicles by the help of microtubules and actin and myosin filaments. This system can adapt rapidly.
- Another example of receptor dynamics is when certain G-protein-coupled receptors (e.g., epidermal growth factor receptor (EGFR)) get coupled (hetero-

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dimerization) and translocated in response to ligands such as the epidermal growth factor.

30.2 Methods for Studying the Number or Cycling of Receptors

30.2.1 Fluorophore Tagging and Optical Mapping

- This method brings about live recording of the receptor dynamics with the following steps.
  - **Step 1:** *Creating a recombinant gene* in vitro – a receptor DNA and a fluorescent protein DNA are inserted into a plasmid.
  - **Step 2:** *Inserting into a cell line of interest* – this plasmid will self-replicate inside the host cell line and express the receptor protein and the fluorescent protein that has the inherent property of tagging to the receptor and thus creating a conjugated receptor.
  - **Step 3:** *Exposing this setup to a ligand*, *agonist*, *or antagonist* – this allows us to study receptor dynamics about how the receptor localizes or gets cycled for repeated exposure to ligand.
  - **Step 4:** *Real-time visualization* by a fluorescence microscope with specialized lenses (confocal microscope). Based on the site of fluorescence emitted, we can predict where the receptor has translocated.
- For example, in an experiment to study receptor dynamics of the androgen receptor (AR), the AR gene was made to express in a plasmid along with *Aequorea* green fluorescent protein (GFP) in a HeLa cell line (a human cell line derived from cervical cancer). On adding the ligand, dihydrotestosterone (DHT), the receptor migrated rapidly to an intranuclear compartment (Fig. 30.1).

30.2.2 Inserting Antibody to the Epitope That Binds to the Receptor (Immunostaining)

- The culture of cells having the receptor of interest can be treated with the corresponding antibody to the receptor (e.g., sheep antibody to her-2/neu receptor). This antibody should have been conjugated with a fluorescent dye. Hence, after

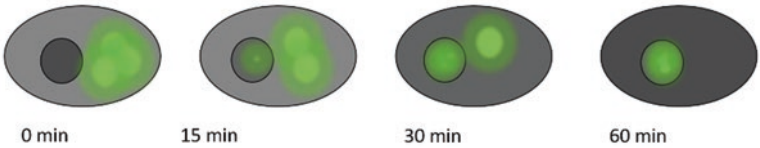

Fig. 30.1 Localization of hormonal receptors from the cytoplasm to the nucleus after exposure to ligand, as visualized by fluorescence microscopy

exposure to a ligand, the fluorescence can be mapped to detect the movement of receptors.

30.2.3 Inserting Amino Acids into the Protein

- The limitation of the fluorescent method described above is that the molecules are often bulky and a minimum of two molecules are needed, namely, a binder and an emitter fluorophore pair. This may alter the ligand binding and receptor functioning. On the other hand, the method of *inserting a reactive amino acid into the receptor* has the advantage of "single-molecule" labeling.
- This method has been used to study G-protein-coupled receptors (GPCRs) in vitro and consists of the following steps:
  - Creating a GPCR DNA construct from human DNA. In experiments, rhodopsin and CCR5 GPCRs have been used.
  - Inserting this construct into plasmid meant to infect *E. coli* cells.
  - Creating constructs for tRNA that can insert mutated amino acids in the plasmid. Examples of unnatural amino acids are *p*-acetyl-*L*-phenylalanine and *p*-benzoyl-*L*-phenylalanine.
  - Creating constructs for suppressor tRNA that suppress insertion of the normal amino acid and inserting this suppressor tRNA into the plasmid.
  - Inserting "luciferase" DNA into the plasmid.
  - Hence, we have a plasmid that contains the GPCR DNA, DNA for tRNA that inserts mutated amino acid, DNA for suppressor tRNA that would prevent insertion of the normal amino acid, and DNA for luciferase enzyme.
  - Now this plasmid after insertion into *E. coli* would create mutated GPCRs that contain unnatural amino acids (and preserved function) and the luciferase enzyme. These unnatural amino acids act as "handles" for the luciferase enzyme.
  - Now, with the help of a microscope, the conformational changes happening in the GPCR can be visualized on exposure to a ligand.
- A challenge of this mutagenesis approach is that it may alter the functioning of the receptor.

30.2.4 Functional Tagging

- Functional tagging is useful for neuronal receptors such as acetylcholine R, AMPA R, and GABA R ("R" stands for receptor). It is dependent on the biophysical and the pharmacological properties of the receptor.
- Neuronal activity can be measured by electrophysiological recordings when ligands bind to these receptors and cause depolarization by ion flow. Thus, electrical current is directly proportional to the magnitude of receptor binding by the ligands such as ACh.

– Selecting the tag: The "tag" is an innate molecule that can irreversibly bind to the active pool of receptors present in the postsynaptic cell membrane facing the synaptic cleft. It should not bind to the inactive pool present in the cytoplasm.
– When tag inactivates the receptors, the current generated also drops proportionately.
– Hence, an increase in electrical activity would be as a result of new receptors getting localized to the postsynaptic cell membrane.

30.3 Applications of Understanding Receptor Dynamics

- To know the lifetime and cycling of receptors
- To know the binding sites of receptors

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Polymerase Chain Reaction

31

Neel Jayesh Shah

Abstract

Polymerase chain reaction or PCR is a method of multiplying DNA copies and was invented in 1983. This process depends on denaturation of DNA at high temperatures, annealing of primers to the DNA, and elongation and synthesis of new DNA strands by the heat-resistant *Thermophilus aquaticus* polymerase. It utilizes nucleotides and magnesium chloride as a cofactor. PCR can generate more than 30 billion copies of DNA in a couple of hours. Its advanced method, real-time PCR (RT-PCR), can quantify simultaneously while multiplying the DNA and is, hence, also known as quantitative PCR (qPCR). It uses fluorescence-emitting dyes such as SYBR green and TaqMan to quantify DNA multiplication. PCR is used in forensics to multiply DNA evidence, in diagnosing genetic diseases and mutations from body fluids like saliva and blood, and in genetic engineering.

Keywords

Polymerase chain reaction (PCR) · Real-time PCR · qPCR · SYBR green · TaqMan

31.1 Introduction

Polymerase chain reaction (PCR) or the process of multiplying DNA copies was invented by Dr. Kary Mullis in 1983. The DNA of interest may come from any source available such as blood, saliva, fossil, or a crime scene.

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31.2 Utility of PCR

- To generate a high quantity of the desired DNA which can be used for forensic testing.
- To detect mutations in the amplified DNA copies by further downstream processes like electrophoresis.
- To diagnose diseases such as sickle cell anemia.
- To utilize the DNA in gene cloning or genetic engineering.

31.3 Materials Required for a PCR Reaction

- Thermocycler: A thermal device which rapidly changes temperature as per the settings.
- *Thermophilus aquaticus* (Taq) polymerase.
- Water.
- Primers are oligonucleotides which bind to the complementary sequence of single-stranded DNA and act as starting points.
- Nucleotides which are the building blocks of DNA (dATP, dGTP, dCTP, dTTP).
- Magnesium chloride acts as a cofactor and catalyzes the PCR.
- The number of copies generated can be calculated by the formula $2^n - 2n$. For example, if the PCR runs 35 times in a couple of hours, it generates $2^{35} - 70$ copies of the desired gene, i.e. 34,359,738,368 – 70 = 34,359,738,298 copies (Table 31.1).

31.4 Real-Time PCR

- Real-time PCR (RT-PCR) was developed in 1992 by Higuchi et al. Real-time PCR, as the name suggests, amplifies as well as analyzes the DNA simultaneously, i.e., in real time.
- In conventional PCR, to analyze the product, a separate process has to be run such as gel electrophoresis. Electrophoresis separates the products as per their size by making the DNA run toward a positive pole inside a gel, DNA being inherently negatively charged.

Table 31.1 Steps of polymerase chain reaction

Sequence	Step name	Temperature	Process
1	Denaturation	96 °C	DNA strands separate
2	Annealing	56 °C	Primers bind to the corresponding site on both strands of DNA
3	Elongation	72 °C	Taq polymerase utilizes nucleotides to synthesize new DNA
1, 2, 3	Repeat for around 35 cycles		

Taq *Thermophilus aquaticus*-derived DNA polymerase which is heat resistant

31.4.1 Principles of RT-PCR

- Dye such as SYBR green is included in the PCR mixture. This dye binds to the amplified DNA.
- Fluorescence emitted by the dye is measured, and its intensity lets us know the quantity (if any). In case the desired DNA has not been amplified, no fluorescence is detected.
- Therefore, the amount of product synthesized is proportional to the fluorescence emitted. For this reason, RT-PCR is also known as *quantitative PCR (qPCR)*.
- RT-PCR estimates the quantity of DNA produced as well, whereas conventional PCR-electrophoresis only estimates quality, i.e., tells us if desired DNA is present or not.

31.5 Labels Used to Quantify DNA Multiplication

- **SYBR green**
  - Fluorophores with intrinsically strong fluorescence such as SYBR green, which emit fluorescence on binding to the minor groove of DNA. The fluorescence is 1000 times more upon binding than in the resting phase. There are chances of less sensitivity due to binding additionally to primer-dimer products. SYBR green is cost-effective.
- **Hydrolysis-based labels such as TaqMan**
  - These are oligonucleotides and have a quencher at one end and detector at the other end. The detector emits fluorescence even at baseline, but this is absorbed by the quencher.
  - When the Taq polymerase elongates sequences in each cycle of PCR, Taqman probe utilizes the endonuclease activity of Taq polymerase. When the oligonucleotide breaks, the quencher and detector separate, and now the fluorescence is no longer "quenched" but detected in proportion to the amount of DNA produced.

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Southern, Western and Northern Blotting

32

Neel Jayesh Shah

Abstract

Southern blotting, discovered in 1975 by E.M. Southern, represents a technique to detect a gene of interest in the DNA sample. The steps involved are isolation of DNA, its separation by electrophoresis, transfer to a suitable medium, hybridization to probes and visualization of the gene if it is present. Western blotting is the counterpart which is used to detect proteins. The difference lies in the visualization process. In Western blotting, this is made possible by primary and secondary antibodies, whereas in Southern blotting, a radiolabeled (fluorescent) probe or dye that binds to the DNA is used. Application of Western blotting includes identifying HIV antigens or Hepatitis B surface antigen in blood. Northern blotting is used to detect mRNA of interest, where after separation by electrophoresis, cDNA is used as a probe that binds to the RNA strand; the application includes finding alternate transcript size. The term 'blotting' in all the three techniques represents the transfer of material after separation to nitrocellulose paper by means of diffusion.

Keywords

Northern blotting · Southern blotting · Western blotting

32.1 Southern Blotting

Southern blotting was developed by E.M. Southern in 1975. The term 'blotting' refers to the transfer of proteins, DNA or RNA from a gel to a membrane and its subsequent detection. Southern blotting allows visualization of a specific gene of interest out of the whole DNA. It encompasses the following steps.

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32.1.1 Isolation

- DNA can be obtained from blood or even saliva. Cell membranes need to be broken down by detergents, and proteins have to be removed by proteinase enzymes.
- Pure DNA is obtained by alcohol precipitation. DNA extraction is a distinct process which needs separate elaboration and is outside the scope of this chapter.

32.1.2 Cleavage

- The isolated DNA is cleaved at multiple places with the help of restriction endonuclease enzymes.
- An enzyme like Eco R1 is obtained from *E. coli*, and the nucleic acid recognition sequence where the enzyme cuts is G/AATTC. Thus, fragments of different lengths are obtained.
- Choosing the restriction enzyme is very important as it is based on the DNA of interest. It should be able to cleave the sequence that we want to detect.
- On the other hand, any restriction enzyme can be used for the sole purpose of breaking DNA into smaller fragments. We can identify the DNA of interest by specific probe (discussed below).

32.1.3 Separation

- These small pieces of DNA are separated by gel electrophoresis, using either PAGE or agarose gel. DNA fragments get separated based on size. Higher base pair DNAs remain on the upper part, whereas lower base pair DNAs migrate farther.

32.1.4 Denaturation

- The separated DNA is denatured by adding alkaline solution such as NaOH.

32.1.5 Transfer

- The gel is transferred onto a sturdier filter, such as nitrocellulose paper. The blot obtained on the nitrocellulose paper is made permanent by drying at 80 °C or exposing to UV rays.

32.1.6 Hybridization

- The paper is then exposed to a hybridization probe – a single-stranded DNA fragment with a known sequence whose complementary presence in the target DNA is to be determined.
- The probe DNA is labelled so that it can be detected, usually by incorporating radioactivity or tagging the molecule with a fluorescent or chromogenic dye.

32.1.7 Visualization

- To make it visible to the naked eye, a dye is usually added to the DNA before loading into the well. For example, ethidium bromide dye binds to DNA and can be seen under UV light.

32.1.8 Application of Southern Blotting

- Southern blotting is used to identify if a person has CYP2C9 enzyme mutation or not. If the enzyme is mutated and cannot function properly, then that person must not be prescribed certain drugs which rely on CYP2C9 for their breakdown such as warfarin and phenytoin.
- **Step 1:** For this, we need to know the exact polymorphism which we are looking for. For instance, if the mutated sequence is GAATAC instead of GACTAC, then we must choose a restriction enzyme that can identify the normal wild gene sequence, i.e. GACTAC. This enzyme will cut the DNA only if it is wild.
- **Step 2:** When the separated DNA is exposed to a radiolabeled sequence and subsequently to an X-ray film, the gene of the person having the mutation will be highlighted on the X-ray film in a different manner than that of a normal person (wild gene). The third category apart from wild and mutated is the heterozygous mutant, which carries one wild allele and one mutated allele (Figs. 32.1 and 32.2).

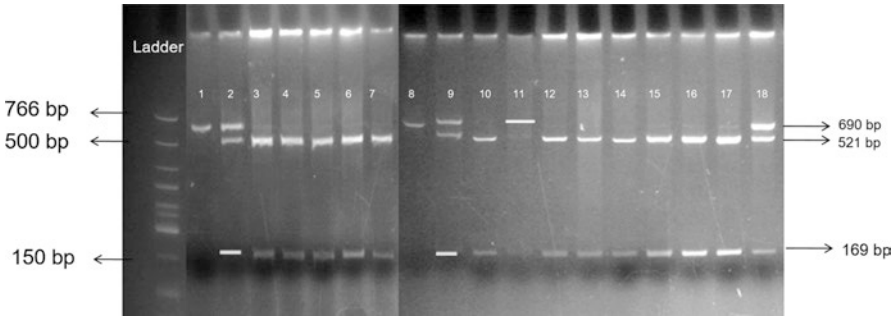

Fig. 32.1 An actual agarose gel electrophoresis seen under UV light, which was loaded with DNA samples from 18 people. Person 1, 8 and 11 are homozygous mutant. Those with three bands are heterozygous, and those with two bands have wild alleles for the *CYP2C9* gene

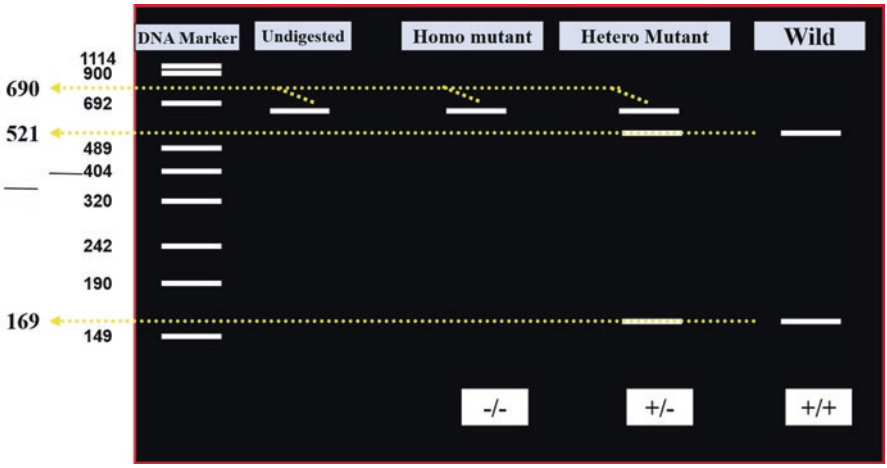

Fig. 32.2 A simplified figure to understand the bands obtained. Note the commercially available DNA ladder added on the left side which gives us reference values for identifying our products

- **Wild gene**: The restriction enzyme Ava II (obtained from *Anabaena variabilis*) has cut only the wild gene. Therefore the wild gene has 2 products at 521 base pair level and 169 base pair level.
- **Heterozygous gene:** In case of heterozygous genotype, the Ava II enzyme cuts the wild allele, but the one mutated allele was not cut. Therefore, we get 1 band at 690 base pair level (uncut product) and 2 bands at 521 and 169 levels (the 2 products of the wild allele).
- **Homozygous mutant:** In case of homozygous mutant, there was no action of the restriction enzyme, and hence, there is just 1 band at the undigested 690 base pair level.

32.2 Western Blotting

Western blotting refers to immune detection of proteins post-electrophoresis; it is also known as *immunoblotting*. It was introduced by Towbin and his colleagues in 1979. 'Western' is just a simile that complements the term 'Southern', which actually refers to a scientist as discussed above. Western blotting can be divided into four parts.

32.2.1 Protein Separation

- Protein separation is by using physical or chemical methods that disrupt the cell membrane. The lysate is obtained using centrifugation and is used downstream.

32.2.2 Gel Electrophoresis

- Polyacrylamide gel electrophoresis or PAGE is a commonly used technique for characterization of proteins. PAGE is a mesh-like network of cross-linked polymers in which proteins can travel inside the pores. The solution of acrylamide is poured in between cassettes which act as a mould to cast the gel, and a comb is placed at the top which forms the well.
  - Sodium dodecyl sulphate (SDS), a detergent, is mixed with the protein sample so as to denature or unfold the protein. SDS also adds a negative charge to the protein.
  - Dye like bromophenol blue is also mixed with the proteins so as to make the dye front visible.
- **Protein separation in PAGE is based on the following:**
  - **Size:** Larger proteins will travel less, whereas at the same time, a smaller protein would have traversed a greater distance.
  - **Charge:** If a protein has high charge, it will be attracted more towards the opposite pole compared to a less charged protein. Separating on basis of protein charge makes it mandatory to use non-denaturing PAGE.
  - **Pore size of the gel:** If higher concentration of acrylamide is used, the pores will be smaller, and the proteins will travel less (Fig. 32.3).

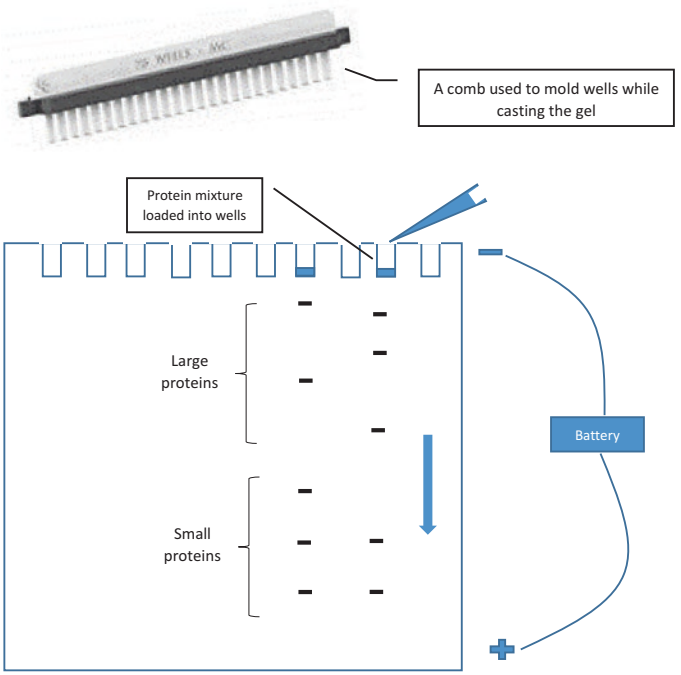

Fig. 32.3 A polyacrylamide gel electrophoresis process showing separation of proteins

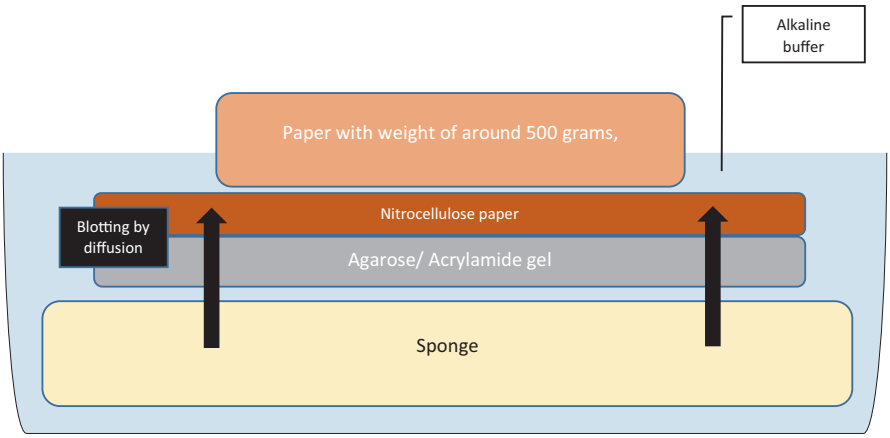

Fig. 32.4 Blotting by method of diffusion or capillary action

32.2.3 Blotting and Transfer

- In 1979, the process of transferring electrophoresed gel to a nitrocellulose membrane was discovered. This can be made possible through simple diffusion (Fig. 32.4), vacuum-assisted solvent flow or electrophoretic elution.

32.2.4 Visualization

- The blots obtained can be highlighted by antibody binding. A radiolabeled antibody is used so that the protein of interest can be detected radiologically.
- Two types of antibodies are used: one specific to the protein of interest (primary antibody) and another specific to the host species of the primary antibody (secondary antibody). The secondary antibody is usually conjugated with an enzyme which produces a detectable signal.
- Although it may be sufficient to use only primary antibody which is conjugated with an enzyme such as horseradish peroxidase, this is expensive, and not all varieties of antibodies are available in this conjugated form. Secondary antibody has the potential to bind to a number of primary antibodies and, hence, is more cost-effective to produce.

32.3 Northern Blotting

Northern blotting was discovered in 1979 and remains the foremost technique in mRNA detection, of which we already know the sequence. The following are the steps involved in it.

32.3.1 RNA Separation

- The cell is lysed, and other products like proteins and DNA are removed by proteinase and DNAase.

32.3.2 Designing Complementary DNA

- The complementary DNA (cDNA) sequence to the mRNA of interest is designed, and the complementary sequence is labelled with radiologically and enzymatically luminescence active probe. Even RNA probes can be used.

32.3.3 Separation of RNA

- The agarose gel electrophoresis is used for separation of the RNA. The electrophoresis is of denaturing nature (made possible by the addition of formaldehyde) so that secondary structure of RNA is broken down into primary structure or linear strands. RNA is separated based on its length.

32.3.4 RNA Transfer

- The RNA is transferred into aminobenzoxymethyl filter paper (nitrocellulose does not bind well with RNA) by blotting.

32.3.5 Addition of a Predesigned Probe

- A predesigned probe is added into the separated RNA. An RNA-DNA hybrid is formed if the RNA of interest is present.

32.3.6 Washing

- The unhybridized probe is removed by washing in several changes of buffer.

32.3.7 Detection

- The light or radioactivity signal is detected, and the band which contains the RNA of interest is thus identified. In case of a radioactive probe, the filter is exposed to an X-ray film.

Table 32.1 Characteristics of the blotting techniques

Characteristics	Southern blotting	Northern blotting	Western blotting
Molecule to be detected	DNA	RNA	Protein
Extraction from body fluids	Alcohol precipitation	Cellulose chromatography	Differential centrifugation
Gel used for separation	Agarose/polyacrylamide, denatured by NaOH	Agarose, denatured by formaldehyde	Polyacrylamide, denatured by SDS
Blotting method	Capillary	Capillary	Electroblotting
Blotting paper	Nitrocellulose or nylon	Nylon or diazobenzoxymethyl (DBM)	Nitrocellulose or polyvinylidene difluoride (PVDF)
Probe used for hybridization	Complementary single-stranded DNA	Complementary single-stranded DNA	Primary and secondary antibodies
Detection	Radiography	Colorimetric/ radiography	Colorimetric
Application	DNA mutations, pharmacogenomics, forensics	Gene expression studies	Diagnosis of pathological antigens such as HBsAg

SDS sodium dodecyl sulphate, *HBsAg* hepatitis B surface antigen

32.3.8 Applications of Northern Blotting

- Determining transcript size and for detecting alternatively spliced transcripts.

Table 32.1 highlights the differences between the three blotting techniques.

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Antisense Oligonucleotides

33

Neel Jayesh Shah

Abstract

Antisense oligonucleotides or "ASO" are short-chained DNA sequences which are used to "turn off" genes. They achieve this by mechanisms such as activating an enzyme RNase which cleaves mRNA, by creating steric hindrance to the binding of mRNA to ribosomes, or by disrupting ribosome machinery. They can also be in the form of a silencing RNA when modified appropriately. Apart from a classification based on mechanism of action, they can also be classified by chemical types. ASOs are transported inside cells by receptors, endocytosis, and pinocytosis. Vectors are used only in in vitro experiments and not in in vivo. Challenges to their use include rapid degeneration, non-specific effects, and non-oral administration. Successful examples include fomivirsen (used in CMV retinitis), which targets the mRNA of cytomegalovirus, and mipomersen (used in hypercholesterolemia), which targets ApoB gene.

Keywords

Antisense oligonucleotides (ASO) · Fomivirsen · Mipomersen

33.1 Definition

Antisense oligonucleotides or ASOs are short-chained DNA sequences complementary to a target mRNA whose *translation into proteins or conversion into a mature mRNA needs to be inhibited*.

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33.2 Characteristics

- ASOs are single-stranded DNA sequences. They are 13–25 nucleotides long and may be unmodified or chemically modified.
- ASOs have high molecular weight (>7000 Da), are negatively charged, and have challenged the dogma that drugs need to be nonpolar and small molecules (<500 Da).
- They are usually administered intravenously and subcutaneously and through intrathecal or intravitreal routes.
- ASOs are meant to be used when a particular gene is implicated in disease, and they are supposed to "turn off" the gene. Apart from being used as therapeutic agents, these are also used to study gene function.

33.3 Different Mechanisms of Action

33.3.1 Degrading the mRNA

- RNase H-dependent oligonucleotides: Some ASOs bind to a small region of the mRNA and, thereafter, activate an enzyme RNase which degrades the mRNA. They can be designed to bind to any region of the mRNA. These ASOs are compatible with phosphorothioate chemical structure (explained below).

33.3.2 Altering the Splicing of Pre-mRNA

- Steric-blocking oligonucleotides will physically prevent or inhibit the progression of splicing (splicing is the process of maturation of mRNA). These ASOs have to be designed to target the initiation codon at the 5′ end of the mRNA.
- For this reason, even though 70% of ASOs currently work in this manner, they are becoming less popular than RNase H-dependent oligonucleotides, e.g., peptide nucleic acid-based ASOs.

33.3.3 Inhibiting Translation

- Second-generation ASOs can inhibit elongation of the protein or can work by disrupting the ribosomes

33.3.4 Silencing or si-RNA

- The silencing or si-RNA can also be used as ASOs, instead of DNA, when suitably modified.

33.4 Chemical Types of ASOs

33.4.1 First-Generation ASOs

- **Methylphosphonates**
  - The oxygen atom of the phosphate linkage is replaced by a methyl group.
  - They are stable to nucleases but do not permeate the cell easily.
- **Phosphorothioates**
  - The oxygen atom of the phosphate linkage is replaced by a sulfur atom.
  - These are highly soluble and act by a unique RNase H-dependent mechanism. They are more resistant to nucleases, e.g., phosphorothioate DNA (PS).
- **Phosphoramidates**
  - In this case also, The oxygen atom of the phosphate linkage is replaced by a sulfur atom.

33.4.2 Second-Generation ASOs

The second-generation ASOs are more resistant to nucleases, have better penetrating property through the lipid bilayer, and exert less non-specific effects.

- **RNA 2′-*O*-methyl ASOs (OMe)**
  - The 2′ ribose position in the RNA is substituted with a methyl group.
  - They act by steric hindrance and are less potent in activating the RNAse H activity.
- **RNA 2′-*O*-methoxy-ethyl ASOs (MOE)**
  - The 2′ ribose position in the RNA is substituted with a methoxy-ethyl group.
  - They also act by steric hindrance and are less potent in activating the RNAse H activity.
- **Chimeric**
  - A first-generation ASO is joined with a second-generation ASO.
  - Hence, a chimeric ASO combines the advantage of a phosphorothioate ASO (first generation) with the high-specificity of RNA 2′-*O*-methyl ASO (second generation).

33.4.3 Third-Generation ASOs

The third-generation ASOs are developed by the chemical modifications in the furanose ring of the nucleotide. The zwitter ions present in their chemical structure make them more resistant to the nuclease enzyme and enhance the binding specificity. However, the RNAse H activity is almost completely abolished.

- **Morpholino or locked nucleic acids (LNAs)**
  - These ASOs work at the level of pre-mRNA to mRNA maturation by inhibiting intron excision or splicing from the pre-mRNA. They hybridize to the 5′ and 3′ regions involved in splicing in the processing of mRNA.
- **Peptide nucleic acids (PNAs)**
- **Hexitol nucleic acids (HNAs)**
  - Both act by steric hindrance.

33.5 Challenges in Using ASOs

- *Rapid degradation* by endo- as well as exonucleases.
- *Toxicity of the degradation products* to the growth of the cell.
- Efficacy remains low since binding to a specific site in mRNA is not always possible due to hindrances from the *secondary or tertiary structure* of mRNA. Hence, the secondary structure of mRNA needs to be predicted first by using algorithms, and then a favorable site has to be chosen on which the ASO can bind easily.
- *Off-target effects of ASOs*
  - Since ASOs are oligonucleotides on their own, the efficacy of an ASO needs to be confirmed as occurring due to its actual antisense mechanism or if occurring due to other non-specific effects.
  - In general, thrombocytopenia is a concern in the development of all ASOs, and many ASOs failed trials due to this side effect.
- *Checking efficacy*
  - For the same reason mentioned above, biological end points need not be measured of efficacy.
  - Even a northern blot to check for reduced RNA expression may not be accurate since some ASOs work by inhibiting protein synthesis. Hence, only a western blot which if demonstrates a reduced amount of protein being formed gives an idea of the efficacy.

33.6 Delivery of ASOs to Cell

- Transport of ASOs inside the cells takes place through active transport and depends on the type of cell being targeted, the structure, and concentration of ASO.
- At low concentrations, transport occurs by membrane-bound receptors. At higher concentrations, once the receptors become saturated, endocytosis (adsorptive endocytosis) and pinocytosis (fluid-phase pinocytosis) transport the ASOs inside the cell.
- Advantages of using vectors:
  - It is essential that ASO is transported to the nucleosome for its activity; vectors have been used for the same.
  - Protect the ASO from nucleases and, thus, improve stability.

  - Prevent the ASO from being restricted to a lysosome, where they are rendered useless.
  - Permit usage of lesser concentrations of ASO.
- Types of vectors used
  - **Liposomes**: Bilayers of phospholipids and cholesterol, e.g., the cationic liposomes. Nucleic acids can be either carried in the aqueous interior or tagged to the lipid membrane.
  - **Peptides**: ASOs can be covalently coupled to peptides. The peptides modulate membrane integrity and do not need any receptor or transporter.
  - **Creation of pores**: Chemically, mechanically, or by electroporation. However, these are not useful in vivo.
- Vectors are not used in clinical trials, and it seems that ASOs do not depend on vectors for nuclear localization. Vectors have been used as support mainly in lab experiments.

33.7 ASOs in the Market and in Development

33.7.1 US-FDA-Approved ASOs

- Fomivirsen (Vitravene®) for the treatment for cytomegalovirus retinitis (1998) (targets mRNA of cytomegalovirus), mipomersen (Kynamro®) for homozygous familial hypercholesterolemia (decreases expression of ApoB protein), eteplirsen (Exondys 51™) for Duchenne muscular dystrophy, and nusinersen (Spinraza™) for spinal muscular atrophy are some of the approved ASOs.
- Fomivirsen, the first US-FDA-approved antisense therapy, also acts by a non-specific mechanism of inhibiting virus binding to cells. It is given by intravitreal injection in doses of 330 μg every week.

33.7.2 ASOs in Clinical Trials

- A phosphorothioate ASO (oblimersen) is in clinical trial that targets the mRNA of bcl-2, an apoptosis-regulating gene implicated in melanoma and chronic lymphocytic lymphoma. Another ASO targets protein kinase-C and is being developed to be used in non-small cell lung cancer.

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Recombinant DNA Technology

34

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Abstract

Recombinant DNA or rDNA technology refers to the creation of a hybrid or chimeric DNA by inserting a foreign sequence into the DNA of another species. It starts with extracting the gene of interest by using an appropriate restriction endonuclease enzyme and incorporating this gene into a suitable self-replicating vector such as a plasmid. This is made possible by cleaving the vector's DNA using the same restriction endonuclease and sealing it back using DNA ligase. Finally, the vector is inserted into the host such as *E. coli* or *Saccharomyces cerevisiae*. Now the plasmid will multiply and the translation machinery of the E. coli will synthesize proteins from this plasmid. rDNA technology has been used to synthesize hormones, vaccines, drugs like alpha-interferon, and genetically modified food, and in gene therapy. Two similarly manufactured recombinant DNA technology products are termed *biosimilars* as opposed to generic drugs. In 2012, the draft guidelines were launched on "Similar Biologics: Regulatory Requirements for Marketing Authorization in India."

Keywords

Recombinant DNA (rDNA) technology · Genetic engineering · DNA restriction enzymes

34.1 Introduction

- Recombinant DNA or rDNA technology refers to the creation of a hybrid or chimeric DNA by inserting a foreign sequence into the DNA of another species.

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- Foreign DNA can be inserted into the pre-existing genome of an organism (gene therapy), or the recombinant DNA can be created ex vivo and then inserted inside the host to synthesize proteins of interest.
- The products obtained by rDNA technology are termed as *biologics*, and their generic versions are termed as *biosimilars*. Biologic is a broad term which includes any product obtained from a living system. Other biologics include monoclonal antibodies.

34.2 Steps in the Synthesis of an rDNA (Fig. 34.1)

- **Choosing a DNA of interest**
  - This depends on the protein end product, for example, insulin can be synthesized from the human gene which codes for insulin.
- Getting multiple copies of this DNA using the *polymerase chain reaction (PCR)*
- *Extracting this gene* by using an appropriate *restriction endonuclease enzyme*
- **Choosing a suitable vector such as a plasmid or bacteriophage virus**
  - This vector should be *self-multiplying* after it reaches the host cell. Hence, the vector should have genes like initiation codon and promoter regions.
  - The vector should also have a genetic marker for selection (antibiotic resistance genes) containing restriction sites and minimum nonessential DNA.
- **Cleaving the vector's DNA using the same restriction endonuclease**
  - The sticky ends thus created should be able to accept the previously isolated DNA of interest. The vector's DNA can be sealed back using *DNA ligase*.

34.3 Steps in Synthesis of Protein from the Generated rDNA

- **Inserting the vector into the host**
  - The host can be *E. coli* or *Saccharomyces cerevisiae* as their genome has been studied enormously. *Bacteria are preferred as they are easy to grow and maintain, they multiply easily, and bacterial plasmids can be manipulated easily*.
  - The vector can be inserted using techniques such as transduction (if the vector is a bacteriophage) or conjugation (for plasmid vectors).
- Now the plasmid will multiply, and the translation machinery of the *E. coli* will synthesize proteins from this plasmid. Hence, we will get human insulin from *E. coli*. This will be called as recombinant insulin.

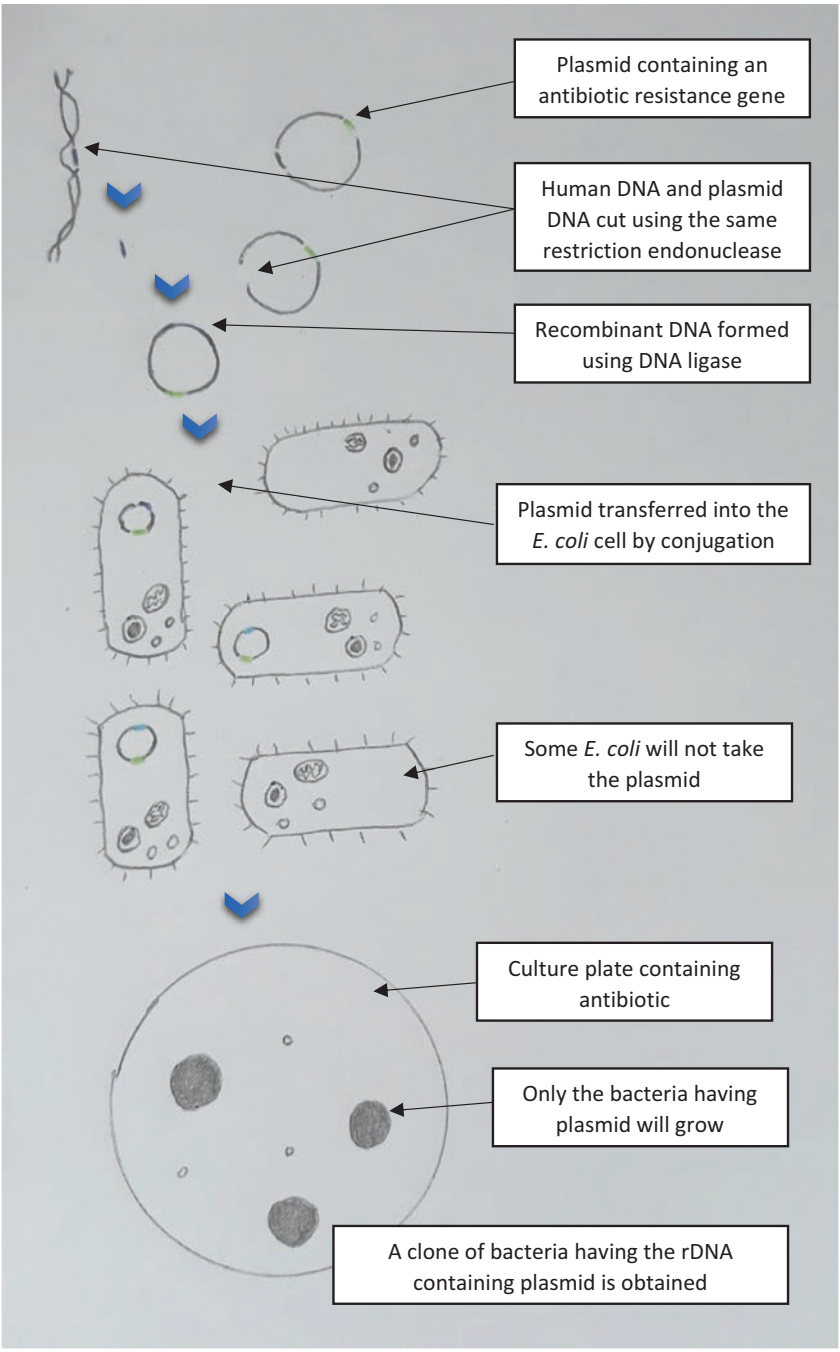

Fig. 34.1 Steps in obtaining bacteria with rDNA plasmid

34.4 Challenges in Recombinant DNA Technology

34.4.1 Manufacturing

- Only some *E. coli* cells will accept the plasmid. The challenge is to identify which ones have accepted. For this, *a plasmid containing antibiotic resistance genes is used*.
- When the *E. coli* cultures are exposed to antibiotics, only those which have incorporated the plasmid survive.

34.4.2 Safety

- *Unexpected adverse events* are more likely to happen with recombinant products, due to chances of impurities in the manufacturing process. For example, minor change in the packaging process of erythropoietin caused pure red cell aplasia.

34.4.3 Dispensing

- Noninterchangeable: *biotechnology products must be prescribed by brand name* and cannot be substituted like other generics.

34.5 Uses of Recombinant DNA Technology

- *Synthesis of hormones* such as insulin, erythropoietin, and growth hormone
  - Recombinant technology allows mass production of safe products, free from foreign antigens, and in case of insulin, recombinant insulins have replaced older products like bovine insulin.
- *Vaccines* such as hepatitis B
- Therapeutic *drugs* like alpha interferon, blood clotting factor VIII, and streptokinase.
- *Genetically modified food* (GM food), which may increase yield, impart resistance to pests or modify quality, e.g., BT cotton which produces a pesticide.
- *Gene therapy*: replacement of disease-causing gene with a normal gene such as cystic fibrosis, hemophilia, muscular dystrophy, and sickle cell anemia (Refer to Chap. 23 on Gene therapy).

34.6 Development of Regulatory Guidelines

Guidelines that control recombinant DNA technology have been developed due to concerns about the following:

- Use of plasmids with various combinations of *antibiotic resistance genes*. The product from the antibiotic resistance genes can enter into humans along with the protein of interest.
- Moving DNA among various species is a *departure from the natural evolution* and selection of species.
- *Health effects* of genetically modified food.
- Regulations are comparatively recently formed: "Draft Guidelines on Similar Biologics: Regulatory Requirements for Marketing Authorization in India" were announced in 2012, by the Department of Biotechnology (DBT) to address the pre-marketing and post-marketing regulatory requirement.
- Getting marketing approval for generic versions of recombinant products is not the same as other drugs. They are considered as new products and therefore require the permission of the Drug Control Authority for both import and local production for marketing purposes.
  - As opposed to simple chemical drugs, where bioequivalence studies can be done to prove similarity, in case of biosimilars, *the safety, potency, and efficacy have to be proven again as no two biosimilar products are exactly similar.* This is because of the complexity in manufacturing these products.

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