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Proflavine an acridine DNA intercalating agent and strong antimicrobial possessing potential properties of carcinogen

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Abstract

Proflavine finds a wide array of applications in clinical, therapeutic, industrial and cutting edge research. This study investigates properties of proflavine and brings out its current status based on overall merits and demerits. Review was carried out starting from late 1800s to 2017 about all aspects of proflavine. We have accessed digital libraries in University of Cambridge, Oxford University, Harvard University and Massachusetts Institute of Technology through their institutional repository software. Popular open source solutions like DSpace, EPrints, Digital Commons, Fedora Commons, Islandora and Hydra were included. Proflavine finds many applications including as an anti microbial agent and often used as a topical agent. This compound denatures bacterial DNA leading to lysis of bacteria. Due to its intercalating property it affects host DNA, which has potential chances to induce skin cancer and other malignancies. Reactive oxygen species (ROS) released by proflavine play a crucial role in denaturing host DNA. Proflavine can penetrate beyond epidermal and dermal structures and accumulate in cell nuclei. In human cell culture proflavine is known to be taken up by many kinds of cells and is concentrated in the nuclei. Our studies revealed that despite its oncogenic potential, proflavine currently finds its applications in therapeutic, diagnostic and in research not alone in developing countries but also in developed countries. Proflavine exhibited wide potent activity against various groups of microorganisms. After exposure in human skin proflavine alters the structure of epidermal DNA strands leading to mutation. Many industrial workers, researchers, health care professionals are exposed to proflavine in enormous amounts daily through oral, respiratory and cutaneous routes. We conclude that even though proflavine has strong antimicrobial and other uses due to its carcinogenic nature, we recommend that it is time to rethink the use of this compound and to search for good alternative replacement.

Keywords: Proflavine; DNA damage; Free radical; Antimicrobial; Carcinogen

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1. Introduction

Cancer plays a crucial role in causing severe morbidity and enormous mortality throughout the world. Many types of these cancers are induced by toxic chemicals, biological compounds and microorganisms. The major mechanism of oncogenesis in human body is due to alteration or insertion of genes in the normal host DNA, thus leading to mutation of genetic material. There are so many cutaneous diseases caused by microorganisms like Gram positive, Gram negative bacteria, fungi, parasites and some viruses. For therapeutic use proflavine is often used as topical antiseptic agent as well as a disinfectant. In other sectors like industries, laboratories workers are exposed to proflavine by various ways.

Proflavine, an acridine dye is a known DNA intercalating agent. Proflavine, an acridine dye with a flavine nucleus can penetrate the epidermal and dermal structures in the in vivo stained cells and accumulate in the cell nuclei, only cells of the central nervous system did not absorb any proflavine [1]. In human cell culture also, proflavine is known to be taken up by many kinds of cells and is concentrated in the nuclei [2]. Proflavine is a strong DNA intercalating agent. The generation of reactive oxygen species (ROS) by photo excited proflavine is reported in presence of a macromolecule in the reaction [3]. Proflavine when excited with visible light can induce DNA strand cleavage [4]. Piette et al. have shown the production of ROS from proflavine [5] that causes base modification and strand breaks in DNA [6]. Replication of DNA at this stage leads to mutation or apoptosis [7].

Riboflavin (vitamin B_2) is structurally similar to proflavine; proflavine has an amino group while riboflavin has a ribityl group in the side chain structure. Earlier studies from our laboratory have shown that riboflavin generates superoxide anion in visible light and the rate of formation of superoxide anions is stimulated in the presence of double stranded DNA [8]. Another study has also shown that riboflavin causes breakage of calf thymus and super coiled plasmid DNA [9] and hemolysis of red blood cells [10]. Photoilluminated riboflavin also causes protein degradation. This degradation is enhanced when a transition-metal ion, such as Cu (II), is present in the reaction with riboflavin [11]. This protein degradation is preceded by the binding of riboflavin to the protein at or around tryptophan residues. Photo illuminated proflavine, like riboflavin, can lead to production of ROS in the human body.

In the present scenario, proflavine is widely employed in various circumstances including biomedical applications. Even though proflavine has potential antimicrobial properties on the other hand it exhibited toxic effects on protein, DNA and many enzymes. This study is extremely vital and its findings may be applied in the understanding of morphogenesis of the cell, sub cellular degeneration, cellular DNA auto degradation, cellular aging, anti-aging process, cellular oncogenesis and mechanism of action of potential carcinogens. So these findings of this study directly or indirectly help oncologists, cellular biologists, biochemists, pathologists and embryologists in understanding the biological effects, biochemical impacts and its protein degradation of oxygen free radicals generated by proflavine.

2. Methods

Detailed study with review of literature was carried out starting from the year late 1800s from the discovery of proflavine until the recent developments on proflavine in February 2017. All aspects of proflavine were included for this study. Particular emphasis was given to know about: a) Nature of proflavine, b) Mechanism of action of proflavine, c) Properties of proflavine, d) Action of proflavine on microorganisms, e) Action of proflavine on DNA and enzymes, f) Proflavine and its carcinogenic potential.

We have accessed digital libraries in University of Cambridge, Oxford University, Harvard University and Massachusetts Institute of Technology through their institutional repository software. Data searching was carried out from archived and organized contents of the respective libraries. Popular open source solutions like DSpace, EPrints, Digital Commons, Fedora Commons, Islandora and Hydra were included. 500 potential set of literature were selected and 100 appropriate articles were identified for this study based on convenience sampling.

3. Results

3.1. Interaction with nucleic acids

The nature of the interaction of acridine derivatives, especially aminoacridines, with nucleic acids has attracted increasing attention since their earliest use as cellular stains. The widespread biological effects of acridine derivatives gradually came to be connected principally with their ability to interact with nucleic acids, but interest in this interaction was heightened

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when the mechanism of the binding processes involved was understood. The binding ability to nucleic acid depends on the basicity of the acridine which will cause a marked increase in intrinsic viscosity and a decrease in sedimentation rate of DNA, and this indicates an increase in counter length of the DNA double helix [12]. Hence this study strongly reveals that proflavine interacts with DNA thus leading to damage of nucleotides.

3.2. Mutation of DNA due to proflavine

Several investigators studied the binding of proflavine and other acridines such as acriflavine on native denaturated unhydrolyzed DNA in solution. Proflavine was found to bind best to alternating purine–pyrimidine sequences regardless of their nature, the drug is thought to exert its biological action mainly by binding to DNA. In neutral aqueous solutions, proflavine exhibits a strong absorption maximum at 444 nm, and upon addition of DNA, a pronounced red shift in the proflavine absorption maximum to 460 nm occurs, that is indicative of the intercalation of proflavine molecules. Studies using CD spectroscopy showed that proflavine relax the superhelical organization of DNA, leading to the formation of a B-like structure without further structural changes as compared to other minor groove-binding drugs such as Hoechst-33258 which is reported to cause other structural changes [13]. The introduction of this simple intercalator affects both the conformational features and dynamic properties of the oligonucleotide double helix, as both major and minor grooves became wider with the addition of the intercalating drug. Water uptake accompanying the complex formation indicates the importance of water as thermodynamic participant [14].

Association of proflavine with DNA has been reported to give specific effect, like frameshift mutation during in vitro DNA replication of single-stranded DNA template by the Klenow fragment of *Escherichia coli* DNA polymerase I. A novel inhibition of polymerization was found opposite to all pyrimidines in the template when proflavine template complexes were exposed for 10 s in white light, such frameshift is based on the hypothesis that the polymerase passes by a template base without copying it leading to deletion. Thus, supporting the proposed mutagenic mechanism for proflavine-induced mutations in which frameshift is produced as a consequence of exonuclease or DNA polymerase activity at the 3′ ends of nicks in the DNA is observed in experiment with thymidylate synthase gene of bacteriophage T4 [15]. Moreover, proflavine caused inhibition of transcription process, and did not cause displacement of the enzyme RNA polymerase from the promoter. Considerable interest has been focused recently on the role that some host transcriptional factors may play in the initial activation of human immunodeficiency virus (HIV-1) gene expression by interaction with the long terminal repeat of the integrated provirus [16], and proflavine was found to do both classical and threading intercalation with the TAR RNA of HIV-1. Indirectly proflavine may cause gene expression of HIV-1 intercalated proflavine is known to oxidize the DNA guanine upon irradiation with visible light. This review found out that proflavine certainly interacts with the components of DNA resulting to mutation.

3.3. Action on enzymes

Proflavine certainly interferes in the function of enzymes. It has different mechanisms of action on enzymes [15,16]. The main mode of its action on enzymes is by degeneration. It also prevents binding of enzyme substrate complex [16]. Interest in the biological activity of the acridines has centered on their antibacterial, antimalarial and mutagenic properties. Apart from a few isolated instances, little interest has been taken in their action on enzymes until the last 20 years or so. The interest in its action on enzymes was generated due to our increased understanding of cellular metabolism, as reaction of acridines with enzyme system usually resulted in the inhibition of the enzyme. The inhibition of RNA synthesis in rat liver mitochondria by low concentration of acriflavine revealed that mitochondrial DNA is involved in the process [17]. This study clearly brings out the toxic effect of proflavine on enzymes.

3.4. Antibacterial action of proflavine

An interest in the effect of acridines as wound disinfectants was developed since its antibacterial effect was stronger, in contrast to many other substances and was shown to be retained in the presence of body fluids and pus. Both proflavine and 3,6-diamino-10-methylacridinium chloride became widely used for this purpose. Some pathogen was found to be resistant to acridines [18]. This study observed that proflavine has potential broad antimicrobial properties.

3.5. Clinical/therapeutic application of proflavine

The application of not more than 0.5 g of powdered proflavine at a time was recommended, a treatment that

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gave good results in a number of intractable mixed infections [19].

Proflavine, for umbilical cord care in the umbilical cord separation time is still in use [20].

3.6. Diagnostic uses of proflavine

The acridine dyes proflavine and acriflavine were applied as fluorochromes, by several investigators in the past because of their high quantum efficiency, which made them especially useful for automated cell analysis. It can be used routinely in clinical laboratories because this single stain could help in differentiation of leukocytes as well as counting of reticulocytes without altering the cells morphology [21]. The binding of proflavine to human adult hemoglobin in ferrous state will cause the X-band appeared in EPR spectrum to displays the characteristics of T-state of the ligated tetramer. In parallel, oxygen affinity for the deoxygenated derivative of ferrous human adult hemoglobin decreases in the presence of proflavine. Recently the investigation made on acridines, especially proflavine, is its action on DNA and proteins [22]. This study discovered that proflavine is employed in various clinical applications.

3.7. Mechanism of generation of reactive oxygen species (ROS)

The probable mechanism for the generation of various ROS from photo excited proflavine is developed by our team. (Fig. 1) [23]. Proflavine upon photo illumination is excited to singlet state which gives rise to triplet state through inter system crossing. When H_2O and O_2 are present in the reaction photo excited proflavine can then give rise to $^3O_2$ and $^1O_2$ through direct energy transfer (pathway (I)). These $^3O_2$ and $^1O_2$ can participate in the protein degradation reaction. Through an alternative pathway (II), the photo excited proflavine can accept electron from molecular oxygen and give rise to cationic radical which further reacts with molecular oxygen and give peroxide radical. This peroxide radical can in the presence of H_2O give $^{\cdot}OH$ or $^{\cdot}OOH$ and in the process Cu(II) may be reduced to Cu(I) if present in the reaction, and proflavine then returns to the ground state. The $^{\cdot}OH$ and $^{\cdot}OOH$, in addition to $^3O_2$ and $^1O_2$, are also available in the reaction to attack the target molecule and cause further damage. This study clearly found the main mode of action of proflavine by the release of toxic free radicals and other ROS.

3.8. Role of reactive oxygen species (ROS) on DNA damage

Acridine dyes, specifically proflavine, are photodynamic agents that are known to target DNA as well as other biomolecules. Although the exact mechanism of photodamage to DNA initiated by proflavine is not well characterized. Whether its electronically excited state reacts directly with DNA via electron transfer and/or hydrogen atom abstraction mechanisms is not clearly understood. Using 2′-deoxynucleotide-proflavine model systems, only guanosine-5′-monophosphate gives rise to a substantial quenching of the fluorescence. In contrast, all of the other nucleotides slightly enhanced the fluorescence of proflavine. Furthermore, an enhancement in the proflavine fluorescence decay kinetics is correlated with the G-C content of the DNA [24]. These results indicate that guanine residues are responsible for the quenching of the fluorescence of proflavine when this molecule forms complexes with DNA. Furthermore, because guanine is the most easily oxidizable nucleic acid base, electron transfer from guanine to electronically excited proflavine molecule is likely to constitute the first step in the complex series of reactions that lead to DNA damage by strand cleavage.

The induction of free radical from proflavine bound to DNA molecule can cause a single strand scission upon irradiation with visible light at high flounce rate as shown by agarose gel electrophoresis. This is consistent with the hypothesis that a free electron is ejected during the excitation of bound proflavine by visible light. Both superoxide dismutase and ceruloplasmin decrease the e.p.r. signal observed in the reaction system suggesting that proflavine produces superoxide anions when complexed to DNA. Superoxide anion may be formed either by direct reaction between the electron ejected by excited proflavine and molecular oxygen, or by decomposition of the peroxide radical formed by the combination of a DNA base and molecular oxygen [25]. Studies have implicated the formation of reactive metabolites in the mechanism of hepatotoxicity. This study definitely observed that ROS released by proflavine damages DNA.

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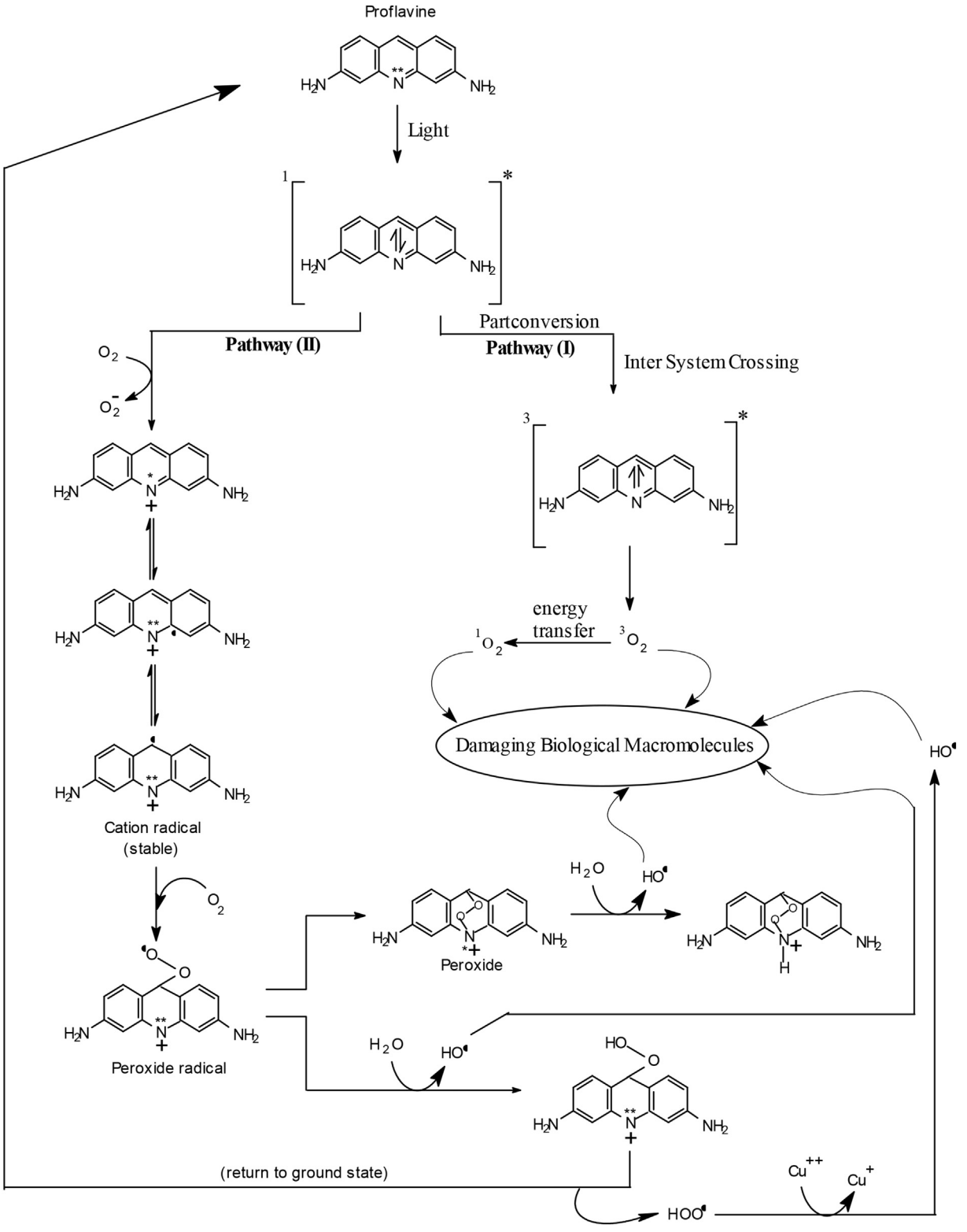

Fig. 1. Scheme for photo excitation of proflavine and generation of ROS.

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4. Conclusions

Based on our results we observed that proflavine interacts with nucleic acids. Furthermore our study confirmed that, proflavine induces mutation of human DNA. Our study also supported earlier studies that proflavine affects the functioning of enzymes. We also found that the prime mechanism of action of proflavine is by generation of ROS. Proflavine is a acridine DNA intercalating agent. It possesses potent antibacterial, antifungal, antiparasitic and antiviral properties. Proflavine destroys microorganisms by denaturation of DNA. Due to its abundant antimicrobial properties proflavine is conventionally employed as antimicrobial agent in various forms even in developed countries. Due to its intercalating property it also affects human DNA after exposure. Proflavine can be contracted by the human body by many ways like cutaneous, respiratory and oral routes. It may result in frameshift mutation and leads to various types of malignancies and tumors. The key mechanism of action of proflavine is by generating reactive oxygen species (ROS). Generation of ROS leading to release of free radicals is further induced by photo illumination/sunlight. We conclude that even though proflavine has strong antimicrobial and other uses, due to its carcinogenic nature, we recommend that it is time to rethink the use of this compound and to search for good, alternative, non toxic replacement. We further suggest World Health Organization (WHO), international and national health policy makers to restrict the use of proflavine especially in clinical and therapeutic applications. We also conclude that more scientific knowledge and studies should be brought forward on beneficial applications of proflavine. Further studies are also needed to nullify the toxic consequences of proflavine.

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Conflict of interest

Authors declare that there is no conflict of interest.

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