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Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction

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Animal models are commonly used in the preclinical development of new drugs to predict the metabolic behaviour of new compounds in humans. It is, however, important to realise that humans differ from animals with regards to isoform composition, expression and catalytic activities of drug-metabolising enzymes. In this review the authors describe similarities and differences in this respect among the different species, including man. This may be helpful for drug researchers to choose the most relevant animal species in which the metabolism of a compound can be studied for extrapolating the results to humans. The authors focus on CYPs, which are the main enzymes involved in numerous oxidative reactions and often play a critical role in the metabolism and pharmacokinetics of xenobiotics. In addition, induction and inhibition of CYPs are compared among species. The authors conclude that CYP2E1 shows no large differences between species, and extrapolation between species appears to hold quite well. In contrast, the species-specific isoforms of CYP1A, -2C, -2D and -3A show appreciable interspecies differences in terms of catalytic activity and some caution should be applied when extrapolating metabolism data from animal models to humans.

Keywords: animal model, CYP, cytochrome P450, drug metabolism, non-rodent, rodent

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1. Introduction

Relevant pharmacotoxicological properties of new chemical entities have to be extensively studied in laboratory animals before human administration. Although the validity of animal testing to predict efficacy and safety in human has been questioned, it is generally believed that pharmacokinetic (PK) data can be extrapolated to humans reasonably well, using the appropriate PK principles. In general, rodents are used because of their short lifespan, allowing for the growth of a large number of animals in a short period of time and, consequently, the feasibility of many studies. In contrast, large animals live longer, thus allowing for longitudinal studies, and they are more similar in size to humans providing an opportunity to address issues related to scaling up to human therapy [1]. Body size and weight have always been considered important covariables for describing the major PK parameters of xenobiotics across species. This was formalised in the concept of allometric scaling, which states that anatomical, physiological and biochemical variables in mammals (such as tissue volumes, blood flow and process rates) can be scaled across species as a power function of the body weight [2]. The methodology was applied to the prediction of plasma concentration–time profiles [3] and the main PK parameters (distribution volumes and clearances) [4], and a number of modifications were subsequently

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proposed for improving the accuracy of these predictions [5,6]. Despite the fact that the allometric approach is empirical, it reflects, to some extent, observations on the relationships of some anatomical and physiological properties with body weight, such as liver weight as a percentage of body weight. As a consequence, the relative amount of hepatic enzymes, such as CYP/gram body weight [7], is higher in small animals than in humans. All this points out that, in general, small animals tend to eliminate drugs more rapidly than human beings when compared on a weight-normalised basis. Other physiological parameters, such as body temperature (36 – 38°C), haematocrit (40 – 45%) and serum albumin concentration (30 – 40 g/l) are relatively conserved among animals and are independent of animal size [8].

The most important drug-metabolising enzyme family, CYP, is one of the conserved entities among species. CYP appears to be derived from a single ancestral gene from ~ 1.36 billion years ago [9]. Although all members of this superfamily possess highly conserved regions of amino acid residues, there are relatively small differences in the primary amino acid sequences of the CYPs across species. However, even small changes in the amino acid sequences can give rise to profound differences in substrate specificity and catalytic activity. Thus, differences in CYP isoforms between species are a major cause of species differences in drug metabolism. In contrast, for drugs that are not or only partly metabolised, species differences seem smaller and cross-species PKs can be predicted very well by allometric scaling. Therefore, this introduction is focused on a description of the main CYP isoforms involved in drug metabolism and on comparison of the different isoforms among animals and man. This review is organised into sections according to CYP subfamily. For each subfamily, isoform composition and expression is briefly described in the different animal species (mouse, rat, dog, monkey and human), as well as their induction and inhibition properties. Each section is concluded by a subsection summarising the most relevant similarities and differences across species.

2. CYP

CYP is a group of haemoproteins that play a central role in the oxidative metabolism (phase I) of clinically used drugs and other xenobiotics. In general, CYP enzymes bind two atoms of oxygen, resulting in the formation of a water molecule together with the production of a metabolite, which is generally more polar than the parent drug. Often hydroxylation, dealkylation or oxidation occurs, but ring-opening and reduction can also take place. The CYP superfamily is divided into families (e.g., CYP1, -2, -3, etc.), between which the primary structure is > 40% identical, and into subfamilies (labelled with letters A, B, C, etc.) between which the primary structure is > 55% identical [10,11], and finally by an Arabic number, representing the individual enzyme. In man, > 50 isoforms have been isolated and ~ 35 CYP isoenzymes are of clinical relevance. The CYP families 1, 2 and 3 appear to be responsible for the metabolism of drugs and other xenobiotics, but they are also involved in metabolic conversion of a variety of endogenous compounds such as vitamins, bile acids and hormones. The CYP isoenzymes from the other families are generally involved in endogenous processes, particularly hormone biosynthesis. In animals and in man, CYPs can be found in virtually all organs, notably the liver, small intestine, skin, nasal epithelia, lung and kidney, but also in testis, brain and other organs. However, the liver (300 pmol of total CYPs/mg microsomal protein) and the intestinal epithelia (~ 20 pmol of total CYPs/mg microsomal protein) are the predominant sites for CYP-mediated drug elimination, whereas the other tissues contribute to drug elimination to a much smaller extent [12,13].

3. Variability in CYP-mediated metabolism: induction, inhibition and polymorphism

Drug–drug interactions may occur as a result of induction of the expression of metabolising enzymes, or as a result of inhibition of enzyme activity or expression. One of the intriguing aspects of the CYP family is that some, but not all, of the enzymes are inducible. Human CYP1A1, -1A2, -2B6, -2C8, -2C9, -2C19 and -3A4 are known to be inducible, whereas CYP2D6 is not. This induction is due to transcriptional activation, which results in increased mRNA and subsequent protein synthesis. In contrast, CYP2E1 is induced by protein stabilisation. Induction results in increased metabolism of the inducing xenobiotic itself (autoinduction), or of concomitantly administered substrates/drugs, resulting in increased clearance and altered PKs.

Transcriptional activation is mainly mediated by nuclear receptors (pregnane X receptor [PXR], constitutive androstane receptor [CAR], G protein-coupled receptor and vitamin D_3 receptor) for the induction of CYP3A and -2B isoforms, whereas the cytosolic receptor aryl hydrocarbon receptor (AhR) is involved in CYP1A induction. In general, induction in enzyme expression results in induced clearance of xenobiotics, and may be considered beneficial or harmful, depending on the case. For example, the induction of CYP1A isoforms by β-naphthoflavone (βNF) reduces the carcinogenicity of 7,12-dimethylbenz[a]anthracene in rodents [14]. In contrast, CYP1A isoforms can also activate some compounds, such as benzo[a]pyrene to their carcinogenic metabolite [15], and the induction of these isoforms increases the risk of carcinogenicity. Induction of metabolism usually needs synthesis of new enzyme, and, consequently, takes days to develop. However, unlike induction that compromises the efficacy of the drug in a time-dependent manner, CYP inhibition is an immediate response (or in the case of time-dependent inhibition, within hours), and may result in undesirable elevations in plasma concentrations of co-administered drugs, with therapeutic and toxicological consequences. The mechanism of inhibition can be reversible, which is the most common form, or irreversible (mechanism-based inhibitors or suicide inhibitors), leading to the formation of reactive metabolites and causing the permanent loss of enzyme activity

Table 1. CYP enzymes of the major drug-metabolising CYP family in humans, rat, mouse, dog and monkey.

Family	Subfamily	Human	Mouse	Rat	Dog	Monkey
CYP1	A	1A1, 1A2	1A1, 1A2	1A1, 1A2	1A1, 1A2	1A1, 1A2
	B	1B1	1B1	1B1	1B1	1B1
CYP2	A	2A6, 2A7, 2A13	2A4, 2A5, 2A12, 2A22	2A1, 2A2, 2A3	2A13, 2A25	2A23, 2A24
	B	2B6, 2B7	2B9, 2B10	2B1, 2B2, 2B3	2B11	2B17
	C	2C8, 2C9, 2C18, 2C19	2C29, 2C37, 2C38, 2C39, 2C40, 2C44, 2C50, 2C54, 2C55	2C6, 2C7*, 2C11*, 2C12*, 2C13*, 2C22, 2C23	2C21, 2C41	2C20, 2C43
	D	2D6, 2D7, 2D8	2D9, 2D10, 2D11, 2D12, 2D13, 2D22, 2D26, 2D34, 2D40	2D1, 2D2, 2D3, 2D4, 2D5, 2D18	2D15	2D17‡, 2D19‡, 2D29‡, 2D30‡
	E	2E1	2E1	2E1	2E1	2E1
CYP3	A	3A4, 3A5, 3A7, 3A43	3A11, 3A13, 3A16, 3A25, 3A41, 3A44	3A1/3A23, 3A2*, 3A9*, 3A18*, 3A62	3A12, 3A26	3A8

*Gender difference.
‡Strain specific.

until new enzymes are synthesised. The inhibitors may be substrates which are metabolised by the same P450 enzyme (e.g., ketoconazole for CYP3A4) or substances that are merely inhibitors, but not substrates, of CYPs (e.g., quinidine for CYP2D6). In addition to induction and inhibition, genetic polymorphisms can also result in interindividual differences in metabolic activity. A polymorphism is usually defined as a genetically determined difference, affecting ≥ 2% of the population under investigation. Polymorphism means heritable DNA changes that lead to lack of production of CYP isoforms, lack of inducibility or synthesis of a form of CYP with altered catalytic activity. In humans, several isoforms, such as CYP2C9 [16], -2C19 [16], -1A1 [17], -2B6, -2D6 [18], -3A4 [19] and -3A5 [20], have been demonstrated to be polymorphic. Polymorphisms have been shown to have clinical consequences, resulting in toxicity of some drugs, and may alter efficacy of other drugs in the affected individuals.

4. CYP1A

The CYP1A subfamily consists of two members, CYP1A1 and -1A2 (**Table 1**), in mouse, rat, dog, monkey and man. CYP1A shows a strong conservation among species [21] with an identity to human > 80% in rat (83 and 80%, respectively, for CYP1A1 and -1A2), mouse (83 and 80%, respectively for CYP1A1 and -1A2), dog (84% for CYP1A2) and monkey (95% for both CYP1A1 and -1A2). Both have been studied extensively because of their roles in the metabolism of two important classes of environmental carcinogens, polycyclic aromatic hydrocarbons (PAH) [22] and arylamines [23].

4.1 CYP1A1

CYP1A1 is expressed only at very low levels in mouse, rat and human liver and it is essentially an extrahepatic enzyme that is present predominantly in the small intestine [24-26], lung [27], placenta [28] and kidney [29]. In monkey and dog, CYP1A1 is present only at low levels in the livers of untreated animals [30,31]. In contrast to rats, in which CYP1A1 is the predominant form expressed in rat small intestine [32], CYP1A1 is only weakly detected in mouse small intestine [33]. There are no reports on CYP1A1 and -1A2 activity in monkey and dog small intestine. The expression levels of CYP1A1 in human small intestine are reported to be variable. According to McDonnell *et al.* [34] the CYP1A1 catalytic activity varied considerably in human small intestine and microsomal preparations, and this high interindividual variability was confirmed by several other laboratories, leading to the suggestion that CYP1A1 expression may not be constitutively expressed, but only expressed after induction [35]. In fact, higher levels of CYP1A1 are often associated with increased smoking, physical exercise and ingestion of chargrilled meats. However, Paine *et al.* [36] concluded that the high variability of CYP1A1 in human liver microsomal preparations could not be accounted for by smoking habits alone, but that diet may be significantly involved.

CYP1A1 is able to oxidise benzo[a]pyrene [37] and other PAHs to their toxic derivatives. For example, dibenzo[a]pyrene, which is considered to be the most potent carcinogen among all PAHs, is oxidised almost exclusively by CYP1A1 in humans to highly mutagenic diol-epoxides [38].

4.2 CYP1A2

CYP1A2 is expressed mainly in the liver and is not, or weakly, expressed in extrahepatic tissues in human [39], rat and mouse [29]. In human liver, CYP1A2 accounts for 13% of the total CYP content [13,40] and is involved in the metabolism of ~ 4% of drugs on the market [41]. In contrast, in monkey and dog, CYP1A2 is expressed at low levels in the liver of untreated animals [42,43], even though a strong similarity in

amino acid sequence to human CYP1A2 has been demonstrated (95% in monkey). In monkey, the CYP1A enzymes may differ in their activities between strains of the same species [44], and CYP1A2 is less expressed in cynomolgus monkey than in the marmoset [45]. In human, CYP1A2 metabolises several drugs, including phenacetin, tacrine, ropinirole, acetaminophen, riluzole, theophylline and caffeine [46].

4.3 Induction of CYP1A

Both CYP1A1 and -1A2 are under the transcriptional regulation of the Ah locus, involving the interaction of AhR/AhR nuclear translocator heterodimeric complexes with upstream enhancer elements, and the transmission of the induction signal from the enhancer to the promoter. This is followed by subsequent transcription of the appropriate mRNA and translation of the corresponding proteins [47]. Both isoforms are inducible, not only by food or cigarette smoke, but also by drugs, and their profiles of induction are quite similar among species. The ingestion of the PAHs, such as 3-methylcholanthrene or the treatment with βNF in rat [48,49], mouse, monkey [30] and dog [43], leads to an increase of the CYP1A protein level in numerous tissues, such as small intestine, liver and lung, as well as in cells in culture. In man [50], but not in mouse [51] or rat [52], the antiulcer drug omeprazole has been reported to induce CYP1A2 in the liver [53]. The effect of omeprazole on CYP1A2 is thus an example of species-dependent gene expression regulation, which is also observed for CYP3A regulation (see Section 11.6). In man, slight induction of CYP1A2 by rifampicin was suggested by a 15% increase in metabolism of caffeine to paraxanthine in healthy subjects [54]. The induction of CYP1A1 by PAHs mediated by the AhR results in the formation of mutagenic/carcinogenic diol-epoxides in target tissues, including liver [55]. The levels of CYP1A1 can be induced by smoking, although the response varies considerably. However, attempts to correlate the inducibility of the enzyme with the incidence of smoking-induced lung cancer have been inconclusive.

4.4 Inhibition of CYP1A

Besides enzyme induction, enzyme inhibition is even more clinically relevant and has been described both for CYP1A1 and -1A2 isoforms. Examples include the coadministration of enoxacin, a quinolone antibiotic that is able to inhibit CYP1A2, resulting in a decrease in the clearance of *(R)*-warfarin, a CYP1A2 substrate [56]. In general, furafylline is considered as a selective, noncompetitive, mechanism-based inhibitor of CYP1A2 [57,58], whereas α-naphthoflavone is an inhibitor of both CYP1A1 and -1A2 [59]. Similar to humans, furafylline also selectively inhibits CYP1A2, relative to CYP1A1, in rats. However, furafylline inhibits rat CYP1A2 only at a 1000-fold higher concentration than is required to inhibit the human isoenzymes, suggesting a major difference in the active site geometry between human and rat orthologues of CYP1A2 [60]. In addition, furafylline inhibits CYP1A2 activity in mouse and dog to a lower extent when compared with human, whereas no inhibition was observed towards CYP1A2 in monkey [61]. Interestingly, ketoconazole, a well-known human CYP3A4 inhibitor, has been reported to also be a potent inhibitor of the CYP1A1 enzyme in man [36] and rat [62], thus indicating a possible crossreactivity with the more abundant CYP3A isoenzymes.

4.5 Conclusion

CYP1A1 and -1A2 show strong conservation among species. CYP1A1 is expressed at very low levels in the liver of all species, whereas its expression in extrahepatic tissue, such as small intestine, is variable. Similarly, CYP1A2, which is highly expressed in the liver, is more variable depending on the species. In general, CYP1A is inducible in rodents and non-rodents, but the variable effect of some inducers, such as omeprazole, is an example of species difference in gene expression regulation. In addition, furafylline inhibits CYP1A2 activity in human, mouse, rat and dog to a different extent, whereas no inhibition was observed towards CYP1A2 in monkey.

5. CYP1B

5.1 CYP1B in animal species and man

In humans, CYP1B was discovered when it was found to be transcriptionally induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin within a human keratinocyte cell line [63]. Following this, extensive research was focused on the inducibility of CYP1B1, especially given that it is differentially expressed within the tumour microenvironment of several human cancers [64,65]. CYP1B1 is constitutively expressed in normal tissues, such as the heart, brain, placenta, lung, liver, kidney and prostata [63], but it is expressed at much higher levels in tumour cells compared with the surrounding normal tissue [64,66]. Thus, CYP1B1 induction is an important factor in determining risk associated with hormone-mediated cancers. In addition, CYP1B1 is involved in the metabolism of some clinically relevant anticancer agents used in the treatment of hormone-mediated cancer. Human CYP1B1 also catalyses estrogens to yield active 4-hydroxylated derivatives that may cause breast cancer. In rat, CYP1B1 is expressed in liver and lung, at least at the mRNA level [67]. In mouse, CYP1B1 has been detected in several tissues such as the testis, kidney, skeletal muscle, lung, spleen, brain and heart, but not in liver [29,68].

In human and rodent species, CYP1B1 can bioactivate carcinogenic PAHs, such as benzo[a]pyrene, to DNA-reactive species associated with toxicity, mutagenesis and carcinogenesis [67,69]. Furthermore, benzo[a]pyrene can induce expression of CYP1B1 by means of the AhR [67].

5.2 Conclusion

In human and animal species, CYP1B1 is the only gene product of the CYP1B subfamily. In human, CYP1B1 is constitutively expressed in normal tissues, and is expressed at much higher levels in tumour cells compared with the surrounding

normal tissue. However, little is known about CYP1B1 in rodent and non-rodent species and, therefore, it is difficult to make a species comparison.

6. CYP2A

6.1 CYP2A in animal species and man

In human, the CYP2A family includes CYP2A6, -2A7 and -2A13 (**Table 1**). CYP2A6 is expressed in human liver and accounts for ~ 4% of total hepatic CYP, whereas other human P450 subfamily forms (2A7 and 2A13) appear to be expressed at even lower levels. Human CYP2A6 shows different substrate specificity in comparison with CYP2A enzymes in animal species. In contrast to rodents, in which CYP2A enzymes have steroid 7α- and 15α-hydroxylation activities, CYP2A6 is not involved in the hydroxylation of steroids [70]. CYP2A6 is engaged in the metabolism of xenobiotics; for example, *O*-deethylation of 7-ethoxycoumarin, 7-hydroxylation of coumarin (a marker reaction), oxidation of nicotine, cyclophosphamide, ifosfamide, fadrozole and aflatoxin B1 [24,70]. In addition, CYP2A6 seems to have overlapping catalytic specificity with CYP2E1 in the activation of nitrosamines [71]. CYP2A13 is an enzyme predominantly expressed in the human respiratory tract and significantly involved in the activation of aflatoxin B1 to carcinogenic derivatives [72] and in nicotine metabolism [73].

In rat, the CYP2A family includes CYP2A1, -2A2 and -2A3. Rat CYP2A1 (female dominant) and -2A2 (male dominant) are expressed in the liver (2%) [74]. In contrast, CYP2A3 is not expressed in the rat liver [75,76], and is constitutively expressed in the oesophagus, lung and nasal epithelium, but not in small intestine, liver or kidney. Rat CYP2A1/2 show ~ 60% homology in amino acid sequence to human CYP2A6. In contrast to human, rat endogenous steroids are CYP2A substrates: CYP2A1 catalyses 7α-hydroxylation of testosterone, whereas CYP2A2 is responsible for 15α- and 7α-hydroxylation of testosterone.

In mouse the CYP2A family comprises CYP2A4, -2A5, -2A12 and -2A22. CYP2A5 and -2A4 show high sequence similarity and differ in only 11 amino acids [24]. CYP2A5 is expressed mainly in the liver, olfactory mucosa, kidney, lung, brain and small intestine, but not in the heart or spleen [77]. CYP2A5 resembles the human orthologue in catalysing 7-hydroxylation of coumarin [78]. CYP2A4 is a female-predominant form in liver in several inbred mouse strains, and its gene is transcriptionally repressed by growth hormone in males [79]. CYP2A4 was also detected in the kidneys and, at very low levels, in the olfactory mucosa.

In dog, the CYP2A family comprises CYP2A13 and -2A25, and in monkey, the family comprises CYP2A23 and -2A24. As reported by Bogaards *et al.* [61], dog and monkey microsomes catalyse coumarin 7-hydroxylation.

Human CYP2A6 antibody showed moderate to strong inhibition of coumarin 7-hydroxylase activities compared with monkey, dog, human and mouse [61]. In human, CYP2A6 is inhibited by diethyldithiocarbamate *in vitro* [80].

CYP2A isoforms are inducible. In humans, CYP2A6 is induced by phenobarbital, rifampicin, dexamethasone and nicotine [13,70]. In rats, CYP2A3 mRNA was increased by treatment with 3-methylcholanthrene and pyrazole in the oesophagus, kidneys and the distal part of the small intestine [75]. The mechanism of CYP2A gene induction is not well understood, but recent studies concerning the murine CYP2A5 indicate the role of CAR, PXR and PPAR in transcriptional activation of CYP2A5 [77]. Moreover, human CYP2A6 may be regulated post-transcriptionally by interaction of the nuclear ribonucleoprotein A1 with CYP2A6 mRNA.

6.2 Conclusion

In humans and rodents, CYP2A is expressed in liver and extrahepatic tissues. The substrate specificity of human CYP2A6 is considerably different from CYP2A enzymes in animal species.

7. CYP2B

Several CYP2B isoforms have been identified in a number of mammalian species (**Table 1**). These isoforms were among the first microsomal CYPs purified and show the most dramatic induction by barbiturates.

7.1 CYP2B in human

In humans, the CYP2B family includes CYP2B6 and -2B7. CYP2B6 is expressed in the liver and in some extrahepatic tissues, whereas CYP2B7 mRNA expression was detected in lung tissue [81]. Although historically human CYP2B6 was thought to play only a minor role in drug metabolism, more recent estimates suggest that CYP2B6 is involved in the metabolism of nearly 25% of drugs on the market today [82], such as the anticancer drugs cyclophosphamide and tamoxifen [83], the anaesthetics ketamine and propofol [84] and procarcinogens, such as the environmental contaminants aflatoxin B1 and dibenzanthracene [85]. In contrast to previous studies that detected CYP2B6 only at 0.2% of the total human liver CYP content [13,40], recent studies [86,87] using more selective and specific immunochemical detection methods have demonstrated that the average relative abundance of CYP2B6 in human liver is in the range of 2 – 10% of the total CYP content. In human small intestine, the mRNA level of CYP2B6 was not detected by reverse transcriptase polymerase chain reaction [68]. In addition, significant interindividual differences in hepatic CYP2B6 expression, which varies in some studies from 25- to 250-fold, have been reported [88]. These large differences may be due to both polymorphism and induction. This finding of CYP2B6 variability suggests that there are significant interindividual differences in the systemic exposure to a variety of drugs that are metabolised by CYP2B6, with the consequent variation in therapeutic and toxic responses [89]. In particular, recent studies have reported that liver tissue of females express significantly higher amounts of CYP2B6 than male liver tissues, and that CYP2B6 activity was 3.6- to 5.0-fold higher in Hispanic

females than in Caucasian or African-American females [90]. This CYP2B6 variability may be explained by a combination of single nucleotide polymorphisms that differ in each ethnic group and/or by different expression levels of other gene products, such as nuclear receptors (e.g., the relative CAR, mRNA is higher in females than in males) or by hormonal influences (e.g., sex hormones) [90].

7.2 CYP2B in mouse

In mouse, among several CYP2B isoenzymes, CYP2B9 and -2B10 are the major CYP2B isoenzymes expressed constitutively. CY2B10 mRNA is detected in the liver and small intestine, and its expression seems to be higher in the duodenum than in the liver [25]. However, data on metabolic activity are lacking. As in rats, the CYP2B family is sexually dimorphic, but female mice express more CYP2B9 isoenzymes than males. In contrast, CYP2B10 was equally expressed in both sexes [91].

7.3 CYP2B in rat

Rats express three CYP2B isoenzymes, CYP2B1, -2B2 and -2B3. CYP2B1 and -2B2 are structurally related isoenzymes (97% identical) with very similar substrate specificities [92]. However, CYP2B1 is generally much more catalytically active than CYP2B2. Both are expressed constitutively in the liver and extrahepatic tissues such as small intestine and lungs [26,49]. The mRNA expression of CYP2B1 [26] and the pentoxyresorufin *O*-dealkylase activity (correlated to CYP2B1/2) seems to be as high in the small intestine as in the liver, according to several reports [26,93], with the highest levels in the duodenum [32,93]. Their constitutive expression in liver is sexually dimorphic, with male rats expressing higher CYP2B levels than females [94]. This sexual dimorphism may be explained by a sex-dependent secretion of pituitary growth hormone, which suppresses CYP2B expression more in female rats than in males [95].

7.4 CYP2B in dog

In dog, the main 2B isoform is CYP2B11, which has a 75% homology, with respect to amino acid sequence, to that of rat CYP2B1 [96]. Remarkably, CYP2B11 catalyses the *N*-demethylation of dextromethorphan (mediated in human by CYP3A) and the 4′-hydroxylation of mephenytoin (a drug-metabolising step that is mediated in human by CYP2C19), and, together with CYP3A12, dog CYP2B11 also contributes to *(S)*-warfarin–hydroxylation (mediated in human by CYP2C9) [97]. Interestingly, the dog is the only mammalian species able to metabolise PAHs through its CYP2B isoenzyme [24].

7.5 CYP2B in monkey

In monkey, so far, only one CYP2B isoform, referred as CYP2B17, has been purified and characterised from liver microsomes from cynomolgus monkeys. The N-terminal amino acid sequence of the protein (the first 34 residues) closely resembles that of the protein encoded by the 2B6 cDNA from human (94%), and its content, as estimated by immunoblot analysis, was 70 pmol/mg (~ 5% of total CYP) [98].

7.6 Induction of CYP2B

The CYP2B family can be strongly induced in man and in animals, both rodent and non-rodent species. Phenobarbital is a potent inducer of CYP2B in many different species [30,43,48,50]. In human and mouse, phenobarbital upregulates CYP2B gene by activation of CAR [99]. In addition, PXR ligands, such as rifampicin in human [100] and dexamethasone in rat [48] and mouse [100], induce CYP2B, demonstrating a crossregulation of this drug-metabolising enzyme in which both CAR and PXR may be involved.

7.7 Inhibition of CYP2B

2-Isopropenyl-2-methyladamantane and 3-isopropenyl-3-methyldiamantane are among the most potent human CYP2B6-selective inhibitors discovered so far [101]. Both compounds also inhibited reactions catalysed by rat CYP2B2 [101]. *N*-(α-methylbenzyl-)-1-aminobenzotriazole was identified as an inhibitor of CYP2B in both mouse and dog microsomes [102]. In rat, the benzodiazepine, clonazepam, has been proved to be a potent noncompetitive or 'mixed type'-competitive inhibitor of catalytic activities mediated by CYP2B. Remarkably, a commercially available antibody to rat CYP2B was found to crossreact with CYP2B family of mouse, dog and human, but not with monkey CYP2B, with respect to the inhibition of 7-ethoxy-4-trifuoromethyl-coumarin *O*-dealkylation [61].

7.8 Conclusion

CYP2B was detected in the liver of all species. In contrast, CYP2B was not detected in the human small intestine, but was highly expressed in the intestine of rat and mouse. Different isoforms are found in the species of interest for ADME studies, and have different substrate specificities. CYP2B is strongly induced by phenobarbital in both rodent and non-rodent species. Interestingly, CYP2B is sexually dimorphic in human, rat and mouse, but this is not described for dog and monkey.

8. CYP2C

The CYP2C subfamily is the most complex subfamily of the CYPs found in human and animal species with several different isoforms.

8.1 CYP2C in human

In human, the CYP2C family (**Table 1**) is involved in the metabolism of ~ 16% of drugs on the market at present [103]. CYP2C8 and -2C9 are the major forms, accounting for 35 and 60%, respectively, of total human CYP2C, whereas CYP2C18 (4%) and -2C19 (1%) are the minor expressed CYP2C isoforms [104]. CYP2C8, -2C9 and -2C19 proteins are primarily located in the liver, where they account for ~ 20% of total CYP [13]. However, other expression levels

were also reported, and the expression appears to show race-related differences and genetic polymorphism [16].

CYP2C18 is not expressed in the liver and is most abundantly expressed in human epidermis [105]. CYP2C8 is expressed mainly in the liver, but its mRNA was also detected in the kidney, adrenal glands, brain, uterus, mammary glands, ovary and duodenum [106]. CYP2C8 is involved in the metabolism of retinol and retinoic acid, arachidonic acid, and benzo[a]pyrene, and in the oxidation of the anticancer drug paclitaxel [107]. In addition to liver, CYP2C9 mRNA is also detected in the kidney, testes, adrenal gland, prostate, ovary and duodenum [106]. CYP2C9 metabolises many clinically important drugs, including the diabetic agents tolbutamide, the anticonvulsant phenytoin, the *S*-enantiomer of the anticoagulant warfarin and numerous anti-inflammatory drugs such as ibuprofen, diclofenac, piroxicam, tenoxicam, mefenamic acid [108], the antihypertensive losartan [109], the antidiabetic glipizide and the diuretic torasemide [110]. CYP2C19 has been detected in the liver and duodenum [106,111]. CYP2C19 has also been shown to metabolise several drugs such as *(S)*-mephenytoin, omeprazole and other important proton pump inhibitors [112], certain tricyclic antidepressants such as imipramine [113], the anxiolytic agent diazepam, some barbiturates [114] and the antimalarial drug proguanil [16]. CYP2C19 is highly polymorphic. Poor metabolisers (PMs) of CYP2C19 represent ~ 3 – 5% of Caucasians and African-Americans and 12 – 100% of Asian groups [16]. Toxic effects can occur in PMs exposed to diazepam, and the efficacy of some proton-pump inhibitors may be greater in PMs than in extensive metabolisers at low doses of these drugs. In humans, no differences in CYP2C isoforms between male and female have been reported [115].

8.2 CYP2C in mouse

The mouse CYP2C family is larger and more complex than its human counterpart, with > 10 members published so far, including CYP2C29, -2C37, -2C38, -2C39, -2C40, -2C44, -2C50, -2C54 and -2C55 [116-118], plus several unpublished new members [118]. Like human and rat, the mouse CYP2C has an important physiological role through the oxidation of arachidonic acid into regio- and stereospecific epoxyeicosatrienoic acids and hydroxyeicosatetraenoic acids. The expression of different CYP2Cs is organ selective [119]. CYP2C29 is expressed in liver as well as in extrahepatic tissues including the brain, kidney, heart, small intestine, lungs, adrenal, aorta, testicular and ovarian [118,120]. Among the CYP2Cs expressed in murine lung, CYP2C29 is most abundant [119]. CYP2C37 is most abundant in the liver, white blood cells and female adrenals [118], whereas CYP2C38 and -2C40 were found in the liver, brain, kidney, lungs, heart and small intestine. In particular, CYP2C40 is the major CYP2C found in both the kidney and small intestine, and it is the only enzyme found to produce the anti-inflammatory mediator 16-HETE [121], whereas in human 16-HETE is mainly produced by polymorphonuclear leukocytes [122]. CYP2C44, a new member of CYP2C family, has been detected mainly in the liver, kidney and adrenals [117]. CYP2C44 has the lowest homology with other known mouse CYP2Cs (50 – 60% identical at the amino acid level). CYP2C44 does not metabolise the common CYP2C substrate tolbutamide and thus differs from CYP2C29, -2C38 and -2C39 isoforms. Midazolam has been reported to be metabolised by CYP2C in addition to CYP3A [123], resulting in the formation of α-OH midazolam.

8.3 CYP2C in rat

In rats, the CYP2C family includes several isoforms, such as CYP2C6, -2C7, -2C11, -2C12, -2C13, -2C22 and -2C23. The CYP2C family is the most abundant CYP2C isoform in rat liver and is involved in the oxidation of dihydropyridines and aflatoxin B1, and in the hydroxylation of steroids [124]. There are sex-dependent differences in the expression of the CYP2C family in rats, which are developmentally regulated and manifest in adult animals. Immunological data have shown that CYP2C12 is more highly expressed in the livers of female adult rats than in male, but those differences are not present in immature and old rats [21]. The CYP2C7 isoform, which catalyses retinoic acid 4-hydroxylation and steroid 5α-reduction, is female predominant [125]. In contrast, CYP2C11, the major male-specific androgen 2α- and 16α-hydroxylase of adult liver, is not expressed in immature rats and is induced dramatically at puberty (beginning 4 – 5 weeks of age) in male rats, but not in female [126]. CYP2C11 is the predominant isoform in male rat liver, comprising up to 50% of the total CYP content [126], and is also expressed in extrahepatic tissues such as kidney and small intestine at lower levels [32,127]. Therefore, the suppression of this isoform in the liver helps to explain the decline in drug-metabolising capacity [125]. CYP2C13 is also male specific, and is expressed not only in liver, but also in extrahepatic tissue, such as in the rat brain [128]. In contrast, CYP2C6 is expressed gender independently [125], and is detected in the liver and at lower levels in the small intestine [32]. CYP2C23 is highly expressed in rat kidney and has been suggested to be important in producing compensatory renal artery vasodilatation in response to salt loading. The gender-dependent expression of the CYP2C family in rat has been demonstrated to be regulated at the level of the hypothalamic–pituitary axis [21] by the secretion of a growth hormone that regulates the expression of uniquely male versus uniquely female CYP isoforms. In addition, there is evidence in the literature that some anticancer drugs, such as cisplatin [129] and cyclophosphamide [130,131], suppress the expression of CYP2C isoenzymes in liver and in other tissues, and their action is in part related to the hormonal perturbation of testosterone and estradiol that these cytotoxic agents induce. Drugs such as phenobarbital [132], dexamethasone, as well as other foreign chemicals, such as ethanol, have been shown to suppress CYP2C11 expression in liver, probably due to their influence on testosterone serum levels.

8.4 CYP2C in dog

Despite dog being the most commonly used non-rodent species in safety evaluation, which are required for any new drug prior to use in man, knowledge concerning the canine CYP system, and in particular the CYP2C family, is limited. Two canine CYP2C isoenzymes have been isolated so far, CYP2C21 and -2C41. These two canine CYP2C isoforms exhibit 70% nucleotide and amino acid identity. Moreover, they exhibit 74 – 83% nucleotide and 67 – 76% amino acid identity with the human CYP2Cs. In particular, canine CYP2C41 is more homologous to the human CYP2Cs than CYP2C21. Both isoenzymes were found in dog liver, but the expression is highly variable; CYP2C41 was present in only one of the nine dogs tested. In addition, the CYP2C41 gene was found only in 4 out of 28 dogs investigated [133]. Therefore, this strong polymorphism in the CYP2C41 subfamily may be an important source of variability in the metabolic clearance of xenobiotics that are metabolised by CYP2C41 in dogs [133]. In addition, the metabolism of specific human CYP2C substrates, such as tolbutamide, warfarin and *(S)*-mephenytoin, is impaired in dog compared with human liver, illustrating an important species difference between dog and human drug metabolism [97].

8.5 CYP2C in monkey

In monkey, the CYP2C family consists of two isoforms, CYP2C20 and -2C43. These isoforms are both expressed in the liver, and show an identity of 83 and 77% for the nucleotide and amino acid sequences, respectively. Among the CYP2C isoenzymes in human, CYP2C43 shows the highest identity with CYP2C9 (95 and 92% in nucleotide and amino acid sequences, respectively), followed by CYP2C19 (93 and 89%), CYP2C18 (86 and 80%) and CYP2C8 (84 and 78%). CYP2C43, but not CYP2C20, was able to metabolise *(S)*-mephenytoin, a probe substrate of CYP2C19 in human. In contrast, CYP2C43 was not able to metabolise tolbutamide, a probe substrate of CYP2C9 in human. Therefore, monkey CYP2C43 appears to be functionally related to human CYPC19, but not to human CYP2C9, although the N-terminal sequence (first 18 residues) was identical for CYP2C43 and -2C9 [134].

8.6 CYP2C induction

Human CYP2C8, -2C9 and -2C19 are inducible isoforms. Compounds known to activate PXR, such as rifampicin and dexamethasone, or CAR, such as phenobarbital, induce CYP2C8, CYP2C9, and to a less extent CYP2C19 [135], although the precise mechanism of induction by xenobiotics has not yet been elucidated [136]. For example, it has been reported that in human rifampicin enhances the clearance of the CYP2C9 probe drugs tolbutamide and *(S)*-warfarin, as well as the metabolism of the CYP2C19 probe *(S)*-mephenytoin [137].

8.7 CYP2C inhibition

Sulfaphenazole is perhaps the most potent and selective inhibitor of CYP2C9 [46]. The mode of inhibition is through ligation to the haem iron of CYP2C9. In dog and monkey, sulfaphenazole shows a similar inhibition profile, even though to a lesser extent in comparison to human [61]. In contrast, no inhibition of diclofenac metabolism by sulfaphenazole was found in rat liver microsomes, thus indicating a difference between the active sites of human CYP2C9- and rat CYP2C9-related protein [61]. In addition, the azole antifungal fluconazole [138], the 5-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors [139] and fluvastatin [139] are inhibitors of CYP2C9. The most relevant inhibitors of CYP2C19 are the selective serotonin re-uptake inhibitors, such as fluoxetine and fluvoxamine [140], whereas some antifungal drugs, such as miconazole, voriconazole and fluconazole, are inhibitors of both CYP2C9 and -2C19 isoforms [141]. Inhibitors of CYP2C8 have been identified from a wide variety of therapeutic classes, such as montelukast, salmeterol, ritonavir, ketoconazole, tamoxifen, quercetin, simvastatin and lovastatin [142].

8.8 Conclusion

CYP2C is the largest and most complicated subfamily in several species including human, rat and mouse. CYP2C is detected in the liver of rodent and non-rodent species, and its expression in extrahepatic tissue is isoform specific. Remarkably, the expression of CYP2C is sex dependent in adult rats. Substrate specificities are largely different between human and animal isoforms, and particularly CYP2C-mediated metabolism in the dog poorly represents human metabolism. In addition, CYP2C is not expressed in all dogs, making prediction hazardous. CYP2C is inducible in man, but, so far, relatively little information is available on the mechanism of CYP2C regulation.

9. CYP2D

CYP2D isoforms have been identified in several mammalian species (**Table 1**), and are involved in the monooxygenation of various chemicals, including antidepressants (e.g., desipramine), β-blockers (e.g., propanolol), antiarrhythmics (e.g., sparteine) and others, such as dextromethorphan and methadone [143]. CYP2D was the first isoform shown to be polymorphic. Induction of CYP2D has not been reported so far.

9.1 CYP2D in human

Although CYP2D6 is expressed at a low level in human liver, accounting for ~ 4% of total CYP (12.8 pmol/mg microsomal protein) [41,144], this enzyme is involved in the biotransformation of 30% of drugs on the market at present [41]. In human, only one isoform, CYP2D6, is expressed in various tissues including the liver, kidney, placenta, brain, breast, lungs and small intestine [145]. CYP2D7 and -2D8 are inactive pseudogenes. Following its discovery, CYP2D6 has been the most studied human genetic polymorphism in drug metabolism, with > 80 identified alleles within most human populations and racial groups. Problems in drug metabolism related to polymorphism became evident when sparteine [146]

and debrisoquine [147] were found to be metabolised at different rates among individuals. Approximately 7 – 10% of the Caucasian population inherits mutant *CYP2D6* alleles as an autosomal recessive trait [147], leading to individual variation in response to many drugs that are cleared by CYP2D. Another polymorphism stratifies the population depending on the copy number of wild-type alleles between PMs (zero copies), intermediate metabolisers (one copy), extensive metabolisers (two copies), and ultrarapid metabolisers (multiple copies). In addition, this genetic variation in CYP2D6 is associated with a heightened risk for diseases and cancer, for example Parkinson's disease, lung cancer, liver cancer and melanoma [148]. In the small intestine, CYP2D6 is expressed in the duodenum and jejunum [93] and not in the ileum and colon. Like CYP3A4, it is localised within the mucosal enterocytes. The mean specific enzyme content of jejunal microsomes was reported to be < 8% of hepatic CYP2D6 microsomal content (0.85 versus 12.8 pmol/mg), and there is extensive interindividual variability in protein content of both tissues [144].

9.2 CYP2D in mouse

Few studies have been performed to characterise the CYP2D family in mouse. There are at least nine mouse CYP2D genes (CYP2D9, -2D10, -2D11, -2D12, -2D13, -2D22, -2D26, -2D34 and -2D40), but some of the isoenzymes have not been characterised for expression and function [149]. One isoenzyme, CYP2D22, has been suggested to be the orthologue of human CYP2D6 [150], and it has been detected abundantly in liver, whereas intermediate levels of expression are seen in the adrenal, ovary and mammary glands.

9.3 CYP2D in rat

In rats, six CYP2D isoforms (CYP2D1, -2D2, -2D3, -2D4, -2D5 and -2D18) have been identified by genomic analysis [151]. The rat and human CYP2D isoforms share a high sequence identity (> 70%) [152]. Among these six, CYP2D5 and -2D18 have > 95% similarity in amino acid sequence to CYP2D1 and -2D4, respectively [153]. Like human CYP2D6, the six isoforms are expressed in various tissues such as the liver, kidney and brain [154]. The mRNA of each isoform shows a specific tissue distribution [155]. CYP2D2 and -2D3 mRNAs are mainly expressed in the liver, kidney, and small intestinal mucosa. In contrast, CYP2D1/5 mRNAs are expressed in various tissues. CYP2D4/18 mRNAs are expressed in the brain, adrenal glands, ovary and testis, in addition to the liver, kidney and small intestinal mucosa [143]. CYP2D4 has also been identified in rat breast [156]. Therefore, the specific tissue distribution of rat CYP2D isoforms suggest that each isoform has specific catalytic properties and plays specific roles in various tissues [143]. For example, *(R)*-mianserin was N-oxidated only by CYP2D1, whereas 8-hydroxylation was performed by all isoforms [157]. Among the six isoforms, CYP2D1 is the rat orthologue of human CYP2D6.

9.4 CYP2D in dog

CYP2D15 is the major CYP2D in dog, with enzymatic activities similar to human CYP2D6 [41]. CYP2D15 is predominantly expressed in the liver, with lower, but detectable, levels in several other tissues. CYP2D15 is polymorphic and three different CYP2D15 cDNA clones have been identified [158,159]. Two clones correspond to variant full-length CYP2D15 cDNA (termed CYP2D15 WT2 and CYP2D15 V1), whereas the third was identified as a slicing variant missing exon 3 (termed CYP2D15 V2). Bogaards *et al.* [61] reported that dog and human liver microsomes showed similar enzyme kinetics with respect to the 1′-hydroxylation of bufuralol. In addition, the quinidine inhibition profiles obtained from dog and human microsomes show strong similarities [61]. Therefore, because of the similar enzyme kinetics and quinidine inhibition profiles, dogs seem to be the most similar species to man with respect to CYP2D inhibition [41,61].

9.5 CYP2D in monkey

In monkey, the expression of different isoforms of CYP2D is strain related. In cynomolgus monkey, a full-length cDNA (called CYP2D17) encodes a 497-amino acid protein that is 93% identical to human CYP2D6 [160]. The recombinant CYP2D17 catalyses the oxidation of bufuralol to 1′-hydroxybufuralol and dextromethorphan to dextrophan, reactions shown to be mediated by CYP2D6 in human, and strongly inhibited by quinidine. In Rhesus monkey, CYP2D42 was detected and is probably an orthologue of human CYP2D6. In marmoset monkeys, two isoforms, CYP2D30 and -2D19, have been isolated in two different female marmosets bred in different laboratories. Even though both isoforms have shown strong homologies in their nucleotide and amino acid sequences, respectively [161], some differences in their catalytic activities have been revealed. Marmoset CYP2D30, similar to human CYP2D6, exhibited high debrisoquine 4-hydroxylase activity and relatively low debrisoquine 5-, 6-, 7- and 8-hydroxylase activities, whereas CYP2D19 exhibited opposite catalytic activity. In addition, the two marmoset recombinant enzymes are involved in the bufuralol metabolism. In particular the 1′S-OH bufuralol metabolite was produced in higher quantity by CYP2D30, whereas the 1′R-OH bufuralol metabolite was produced in higher quantity by CYP2C19 [161]. Like in humans, quinidine exhibited an inhibitory effect towards bufuralol 1′-hydroxylation activities. In Japanese monkey (*Macaca fuscata*), a full-length cDNA encoded a 497-amino acid protein (designated CYP2D29) that is 96, 91 and 88% homologous to human CYP2D6, cynomolgus monkey CYP2D17 and marmoset monkey CYP2D19, respectively. Like human CYP2D6, this isoform catalyses the metabolism of debrisoquine and bufuralol [162].

9.6 CYP2D inhibition

CYP2D6 is inhibited by very low concentrations of quinidine. Although not metabolised by CYP2D6, but by CYP3A4, quinidine conforms closely to the structural

requirements for a substrate for the enzyme [163]. In addition to quinidine, which is one of the most potent CYP2D6 inhibitors, some other compounds, such as the serotonin re-uptake inhibitors and the HIV-I protease inhibitor ritonavir, have a strong inhibitory interaction with CYP2D6 [164]. With regards to the other species, Bogaards *et al.* [61] reported that the quinidine inhibition profiles obtained from dog and human microsomes show strong similarities. In contrast, its quinine isomer shows moderate inhibition in dog, but not in human [61]. In rat, monkey and mouse, the inhibition profile was different in comparison with human. Bogaards *et al.* [61] reported negligible (in rat and mouse) or low (in monkey) inhibitory effects by quinidine towards bufuralol 1′-hydroxylase catalytic activity, whereas the quinine isomer weakly inhibited CYP2D in monkey and rat [61,124,165], but not in mouse [61].

9.7 Conclusion

The CYP2D family shows genetic polymorphism resulting in variation in functional activity in drug metabolism in man. As in other species, such as rat [166] and dog [158], polymorphism was observed. Remarkably, the inhibition profile was different among species; quinidine inhibits CYP2D in man, dog and monkey, but not in rat and mouse. In contrast, the isoform quinine has inhibitory effect towards CYP2D in rat, dog and monkey, but not in man. CYP2D is thought to be noninducible.

10. CYP2E1

10.1 CYP2E1 in animal species and man

CYP2E1 shows a strong conservation among species (**Table 1**) with an identity to human CYP2E1 of 80% for rat, mouse and dog and of 96% for monkey. CYP2E1 is the only gene of this subfamily. In human, CYP2E1 accounts for ~ 6% of total CYP in the liver and is involved in the metabolism of 2% of the drugs on the market at present [41]. CYP2E1 appears to have a dual physiological role, namely roles in detoxification and nutritional support. CYP2E1 is expressed in many tissues, such as the nose, the oropharynx (exposed to airborne xenobiotics), the lungs and the liver. The inducibility and adaptive responsiveness to xenobiotics is suggestive of a protective role. Regarding xenobiotics such as ethanol, CYP2E1 plays a detoxification role, preventing ethanol from reaching excessive levels. Its inducibility by ethanol was shown, not only in experimental animals [167], but also in man [168]. In terms of its nutritional role, the upregulation of CYP2E1 plays a useful physiological role when starvation and/or low carbohydrate diet prevail because of its contribution to the metabolism of fatty acids and its capacity to convert ketones to glucose [169]. However, like many other useful adaptive systems, when the adaptation ceases to be homeostatic and becomes excessive, adverse consequences prevail. CYP2E1-mediated metabolism generates oxygen radicals and, when this exceeds the cellular detoxification systems, it results in oxidative stress with its various pathological consequences. This is true not only when excess alcohol has to be metabolised, but also when CYP2E1 is confronted with an excess of ketones and fatty acids associated with diabetes and/or obesity [170]. A few drugs are metabolised by CYP2E1, such as acetaminophen, caffeine and chlorzoxazone, the latter being considered a marker of CYP2E1 activity [171]. Although relatively few drugs are oxidised by CYP2E1, the list of carcinogens that can be activated by CYP2E1 is quite extensive and includes benzene, styrene, acrylonitrile and nitrosoamines. CYP2E1 may generate reactive oxygen intermediates, such as superoxide radicals [172], which play a key role in liver injury because of the interaction with cellular proteins or DNA [173]. Other examples of organic compounds that show selective injurious action in the liver, as well as in other tissues of alcoholics, include industrial solvents, such as bromobenzene [174] and vinylidene chloride [175], as well as anaesthetics such as enflurane [176]. Enhanced metabolism and toxicity pertains also to a variety of prescribed drugs, including isoniazid, phenylbutazone and acetaminophen. CYP2E1 activity is inducible by ethanol and acetone in both rodents and non-rodents. The regulation of CYP2E1 expression is complex, and involves transcriptional, post-transcriptional, translational and post-translational mechanisms [177]. CYP2E1 is transcriptionally activated in the first hour after birth. Xenobiotic inducers elevate CYP2E1 protein levels through both increased translational efficiency and stabilisation of the protein from degradation, which appears to occur primarily through ubiquitylation and proteasomal degradation [177].

Like human, many substrates, such as organic solvents, nitrosamines and drugs such as paracetamol, are metabolised by rodent CYP2E1. Therefore, rodents may be an appropriate model to study CYP2E1-dependent metabolism in man [41]. However, in dogs and monkeys some discrepancies have been found. In dog microsomes, the antibody against human CYP1A was shown to influence the 6-hydroxylation of chlorzoxazone, a typical activity of CYP2E1 in man [61]. In monkeys, CYP2E1 activities in liver microsomes seem to be similar to human CYP2E1 [44], however, inducibility of this enzyme by 3-methylcholantrene (another typical inducer of the CYP1A) indicates significant differences in the mechanism of induction of CYP2E1 [178]. Disulfiram and diethyldithiocarbamate are mechanism-based inhibitors of CYP2E1 in man. Moreover, 3,4- and 3,5-dichlorophenyl derivates have recently been demonstrated to be potent inhibitors of human CYP2E1 [179]. In addition, diethyldithiocarbamate is a potent mechanism-based inhibitor of 6-OH-chlorzoxazone formation in microsomes of rodent and non-rodent species [180], indicating a species-conserved mechanism for the oxidative biotransformation of chlorzoxazone among species.

10.2 Conclusion

CYP2E1 is expressed in the liver and in many extrahepatic tissues of several animal species. CYP2E1 plays a physiological

role and is involved in the metabolism of few drugs. Like human, CYP2E1 is inducible by ethanol and acetone in rodents and non-rodents. In spite of some discrepancies, CYP2E1 is well conserved, and, therefore, the extrapolation between species appears to be hold quite well. The rat seems to be the best model for human in this respect.

11. Cytochrome P4503A

The CYP3A subfamily (**Table 1**) plays a very important role in the metabolism of xenobiotics, and has a very broad substrate specificity. It is highly inducible and can be inhibited by numerous drugs. Therefore, large interindividual differences in CYP3A-mediated metabolism have been reported.

11.1 CYP3A in human

The CYP3A subfamily is the most important of all human drug-metabolising enzymes because this subfamily is involved in the biotransformation of ~ 50% of therapeutic drugs on the market at present [41], although its content in the liver is only 30% of total P450. Some examples of drugs metabolised by CYP3A are terfenadine, the benzodiazepines midazolam and triazolam, quinidine, lidocaine, carbamazepine, nifedipine, tacrolimus, dapsone, erythromycin and dextromethorphan [41,181]. In addition to drugs, CYP3A is involved in the oxidation of a variety of endogenous substrates, such as steroids, bile acids and retinoic acid [182]. Humans express four CYP3A enzymes, CYP3A4, -3A5, -3A7 and -3A43. CYP3A4 and its related -3A5 are the most abundant CYP isoforms in human liver, and are involved in the biotransformation of the majority of drugs [183]. CYP3A4 and -3A5 are expressed in the liver, stomach, lungs, small intestine and renal tissue. The level of CYP3A4 content is highest in the liver, with a median value of 70 pmol/mg microsomal protein, but it is also expressed in the human duodenum, jejunum and ileum (31, 23 and 17 pmol/mg microsomal protein, respectively) [184]. It is located at the apex of the enterocytes [185] and plays a major role in the first-pass metabolism of xenobiotics. CYP3A protein levels and catalytic activity decrease longitudinally along the small intestine. Although the levels of CYP3A in the small intestine expressed per mg microsomal protein are generally 10 – 50% lower than those found in the liver, in some individuals CYP3A concentration is equal to, or even higher than, those in the liver [184]. This together with its strategic localisation at the tip of the villus suggests that intestinal CYP3A plays a major role in drug metabolism. In addition, P-glycoprotein can influence the metabolism process by recycling drugs between enterocytes and lumen, thereby increasing drug exposure to intestinal metabolic enzymes [186]. Thus, the amount of an orally administered drug that reaches the systemic circulation can be reduced by both intestinal and hepatic metabolism. In addition to CYP3A4, CYP3A5 has recently been demonstrated to play a major role in adults. Initial data suggested that CYP3A5 accounted for only a small proportion of the total hepatic CYP3A content in only ~ 20% of samples [187], and when expressed it accounts for a third of CYP3A4 [188]. However, recent evidence indicates that CYP3A5 may represent > 50% of the total CYP3A in some individuals [189]. In addition, within mucosa of the colon and the stomach, CYP3A5 protein and mRNA appear to be more prominent than the corresponding CYP3A4 protein and mRNA [190]. Furthermore, CYP3A5 is expressed in a third of Caucasian livers and more than half of African-American livers examined [189]. CYP3A7 and -3A43 isoenzymes seem to play a minor role in the metabolism of drugs. In fact, CYP3A7 is expressed in fetal liver only, whereas CYP3A43, which is expressed in liver, appears to be very restricted, both in terms of its activity and expression (0.2 – 5% compared with CYP3A4) [191].

11.2 CYP3A in mouse

In mouse, there are six CYP3A isoforms identified so far. CYP3A11 and -3A13 show a maximal level of expression at 4 – 8 weeks of age, but the levels of CYP3A13 detected in the liver were much (5- to 10-fold) lower compared with CYP3A11 [192]. Of the mouse CYP3A isoforms, CYP3A11 is the isoform most similar to human CYP3A4, having 76% amino acid homology [193]. In addition, CYP3A11 is also expressed in the small intestine [194] like CYP3A4 in man. CYP3A16 has been identified as a fetal form, which diminishes rapidly after birth [195]. CYP3A25 appears primarily in the liver and small intestine of newborn and adult mice, with no evidence of gender bias in expression [196]. CYP3A41 [197] and -3A44 [198] were cloned and reported to have a female-specific expression pattern. The catalytic activity of the mouse CYP3A form towards clinically active drugs has not been extensively tested, but it has been shown that compounds such as aflatoxin B1 and ethylmorphine are metabolised by mouse CYP3A isoforms [199] similar to human [200,201]. No strain-related differences between nude and CD-1 mouse have been revealed in CYP3A11 expression [25].

11.3 CYP3A in rat

In rats, CYP3A1, -3A2, -3A9, -3A18, -3A23 and -3A62 have been reported as CYP3A forms [202-206]. CYP3A23 has been identified to be identical to CYP3A1 by analysis of the *CYP3A1* gene [207]. These CYP3A forms appear to be expressed in a sex-specific manner in rats. For example, CYP3A2 [208] and -3A18 [209] are male-specific forms, whereas CYP3A9 is a female dominant form [209]. Recent studies from Matsubara *et al.* [210] have identified the new rat CYP3A62 form, and its expression profile is similar to that of human CYP3A4 and rat CYP3A9. CYP3A62 is the predominant form in the intestinal tract, whereas CYP3A1 and -3A2 were detected only in the liver. In addition, CYP3A9 and -3A18 were detected in the liver and in the small intestinal tract [210]. The rat is not a good model for the human situation to study CYP3A4 induction because CYP3A1 (the main CYP3A form in rat liver) is not induced by rifampicin,

a typical human CYP3A inducer [52]. Moreover, some discrepancies in metabolism have also been revealed between rats and human; for example, many prototypical substrates of human CYP3A enzymes, such as dydropyridine calcium-channel blockers (e.g., nifedipine), are not metabolised by rat CYP3A1 [211,212].

11.4 CYP3A in dog

In dog, the CYP3A family comprises two isoforms: CYP3A12 and -3A26. Both have been detected in the liver. Several distinctions in catalytic activity have been identified between these two enzymes. The major differences in steroid hydroxylases identified clearly demonstrate that CYP3A26 is less active than CYP3A12 [213]. The human isoforms CYP3A4 and -3A5 have been shown to exhibit some parallels when compared with canine CYP3A12 and -3A26 [213].

11.5 CYP3A in monkey

In cynomolgus monkey CYP3A8 represents ~ 20% of the CYPs in monkey liver [214], and it is 93% similar to the human CYP3A4 protein [178]. Taking into account the higher total CYP levels/mg liver microsomal protein in monkeys compared with human, this represents four- to five-times higher levels of CYP3A8 compared with CYP3A4 per unit of liver.

In addition, the human 3A marker, midazolam 1′-hydroxylase, showed five-fold higher CYP3A activity in cynomolgus monkeys than in man. Estimated kinetic parameters indicate that the affinity for midazolam is lower in monkey microsomes (higher estimated K_m), but capacity is higher (higher estimated V_{max}) than in human microsomes, consistent with the higher total CYP3A levels in monkeys. However, in another CYP3A assay, erythromycin *N*-demethylation, monkeys exhibit a 19-fold higher activity than humans, and this cannot be completely accounted for by the higher levels of CYP3A protein in monkey microsomes. Therefore, differences are evident in affinity and enzymatic rates for CYP3A substrates [44].

11.6 CYP3A induction

The induction of the CYP3A family is extensively studied because of its importance on drug metabolism in man. The CYP3A protein is highly inducible by drug exposure, mainly through transcriptional activation. The major part of CYP3A4 transcriptional activation is mediated by PXR [215]; hence, the activation of PXR by xenobiotics is a good indicator of induction of the *CYP3A4* gene. In human and dogs, rifampicin is a strong inducer of CYP3A [43,100], but not in rat and mouse, whereas dexamethasone and pregnenolone 16α-carbonitrile are strong PXR activators and/or inducers of CYP3A [48,100]. In addition, dexamethasone induces CYP3A in human, but not in dog [52]. Those differences of induction among species is explained by discrepancies in the ligand-binding domain of PXR of the order of 70 – 75%, implying that their ligand specificities may differ dramatically between species. Therefore, extrapolation of animal data with respect to the inducibility of the CYP3A subfamily in human can be problematic [216].

11.7 CYP3A inhibition

Ketoconazole is a potent and well-studied inhibitor of CYP3A in human and animals and is often used *in vitro* and *in vivo* as a diagnostic inhibitor. The drug is not only an inhibitor of CYP3A (K_i < 1 μM), but it is also a CYP3A substrate, being metabolised into the imidazole ring, which is also the site of its ligation to the haem [217]. Not surprisingly, oral ketoconazole is contraindicated with many CYP3A substrates and can cause drug–drug interactions. In addition, ketoconazole has been reported as a potent inhibitor of the CYP1A1 enzyme in man [36] and in rat [62], thus indicating a possible cross-reactivity with the more abundant CYP3A4 isoenzymes. In particular, in rat, ketoconazole inhibited the activities of CYP1A2 and -2C6 in addition to CYP3A1/2 isoforms. Moreover, ketoconazole showed inhibition of diclofenac 4′-hydroxylase activity in mouse, rat and monkey, which was not found in human and dog [61]. Other azole antifungals, such as itraconazole, have CYP3A inhibitory effect in man [218]. In addition, there are several examples of inhibitors acting as mechanism-based or suicide inhibitors. The macrolide antibiotic, erythromycin [219], is one example of a mechanism-based inhibitor. Due to the large number of drugs metabolised by CYP3A4, being a potent inhibitor can have a detrimental effect on the marketability of a compound. This is exemplified by mibefradil, which was withdrawn from the market during its first year of sales due to its extensive CYP3A4 drug–drug interactions [220].

11.8 Conclusion

CYP3A is the most important isoform involved in the metabolism of xenobiotics in all species. However, the various CYP3A isoforms expressed in different species show different substrate specificities, making the extrapolation from animal to man quite hazardous. In addition, CYP3A is inducible in rodents and non-rodents, but the variable effect of some inducers, such as rifampicin, pregnenolone 16α-carbonitrile and dexamethasone is an example of species-dependent gene expression regulation. This high inducibility is the cause of large interindividual variations in metabolism in individual patients, which may vary 20- to 50-fold. In addition, prominent interspecies differences in inhibition have been reported.

12. Expert opinion

For the development of new drugs, the investigation of drug metabolism mediated by CYP and the evaluation of potential drug–drug interaction is essential. The experimental approach is based on animal drug-metabolising systems, and is used to predict kinetics and toxicity in man. However, this interspecies comparison suffers from certain limitations because specific isoforms are expressed in different species,

and even when a high degree of sequence identity in the amino acid sequences exists between the isoforms, this does not automatically mean similar catalytic specificity [24]. None of the animal species are completely similar to man with respect to all CYP enzymes activities. However, similarities can be found for some specific CYP isoforms. For example CYP2E1, of which only one isoform is known, shows no appreciable differences with respect to expression and catalytic activity between species according to several authors [24,61], and extrapolation between species appears to hold quite well. Regarding CYP1A, all species seem to express the two isoforms CYP1A1 and -1A2, albeit to a different extent, and different catalytic activity has been observed. Therefore some caution is required in extrapolation. CYP2C, -2D and -3A show substantial differences in terms of isoforms, expression, organ specificity and catalytic activity. According to Bogaards *et al.* [61] and Zuber *et al.* [41], compared to man, dog seems to be more similar for CYP2D activity, monkey for CYP2C activity, mouse for CYP1A activity, and mouse and male rat for CYP3A activity. However, the selection of the best animal species to be used during the development of a new drug is difficult, considering that different animal models might be needed depending on the particular study type (e.g., metabolism, induction or inhibition). Relevant *in vitro* studies with liver microsomes, hepatocytes, liver slices and recombinant enzymes are very valuable to make this selection because they are usually the only source of information for the human situation.

In the future, much effort should be devoted to increase predictivity for the human situation. For example, incubation with specific isoenzymes expressed in cell systems is necessary to determine which CYP isoform is involved in metabolism of a new chemical entity and to allow comparison among species. All important human CYPs are now available as single recombinant-expressed isoforms. For rat, only some (but not all) recombinant expressed single CYPs are commercially available, whereas isoforms from mouse, monkey and dog are scarcely available. The assessment of the absolute amount of CYP isoforms in different animals and in different organs, such as liver and small intestine, will help to identify and understand species differences in terms of organ specificity and catalytic activity, and to predict metabolic clearance in man. In addition, future research should focus on the investigation of the transporters involved in drug clearance in different animal species in order to explain potential metabolic differences among animals.

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