Species and strain differences in drug metabolism in liver and intestine
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Chapter 1

Species differences between mouse, rat, dog, monkey and human cytochrome P450-mediated drug metabolism

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Abstract

Animal models are commonly used in the preclinical development of new drugs to predict the metabolic behaviour of new compounds in human. It is however important to realize that humans differ from animals with regard to isoform composition, expression and catalytic activities of drug metabolizing enzymes. In this review we aim to 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 these results to human. We focus on cytochrome P450s (CYPs) that 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. We 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, CYP2C, CYP2D, and CYP3A show appreciable interspecies differences in terms of catalytic activity and some caution should be applied when extrapolating metabolism data from animal models to human.

§ 1. Introduction

Relevant pharmaco-toxicological 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 data can be extrapolated to human reasonably well, using the appropriate pharmacokinetic principles. Generally, rodents are used because of their short lifespan, allowing 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-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 pharmacokinetic parameters of xenobiotics across species. This was formalized 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) [4,5], and a number of modifications were subsequently proposed for improving the accuracy of these predictions [6,7]. 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, for example, liver weight as a percentage of body weight. As a consequence, the relative amount of hepatic enzymes, such as cytochrome P450/gram body weight is higher in small animals than in human [8,9]. All this points out that, in general, small animals tend to eliminate drugs more rapidly than human beings when compared on a weight-normalized basis. Other
physiological parameters, such as body temperature (36°-38°C) hematocrit (40-45%) and serum albumin concentration (3-4 g/dl) are relatively conserved among animals and are independent of animal size [10].

The most important drug metabolizing enzyme family, cytochrome P450 (CYP), is one of the conserved entities among species. Cytochrome P450 appears to be derived from a single ancestral gene, ~1.36 billion years ago [11]. 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 cytochrome P450s 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 metabolized, species differences seem smaller and cross-species pharmacokinetics 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. The review will be organized in sections by CYP subfamily. For each subfamily isoform composition and expression will be briefly described in the different animal species (mouse, rat, dog monkey and human) as well as their induction and inhibition properties. Each section will be concluded by a subsection summarizing the most relevant similarities and differences across species.

§ 2. Cytochrome P450

Cytochrome P450 (CYP) is a group of hemoproteins that play a central role in the oxidative metabolism (phase I) of clinically-used drugs and other xenobiotics. Generally, CYP enzymes bind two atoms of oxygen resulting in the formation of a water molecule together with the production of a metabolite, generally more polar than the parent drug. Often hydroxylation, dealkylation or oxidation occurs, but also ring-opening, and reduction can take place.

The P450 superfamily is divided into families (e.g. CYP1, CYP2, CYP3, etc.) where the primary structure is more than 40% identical, and in subfamilies (labeled with letters A, B, C, etc.) where the primary structure is more than 55% identical [12,13], and finally by an Arabic number, representing the individual enzyme. In man, more than 50 isoforms have been isolated and ~ 35 CYP isoenzymes are of clinical relevance, although mainly the P450s 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, intestine, skin, nasal epithelia, lung and kidney, but also in testis, brain etc. 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 P450-mediated drug elimination, while the other tissues contribute to a much smaller extent to drug elimination [14,15].
§ 3. Variability in CYP-mediated metabolism: induction, inhibition and polymorphism

Drug-drug interactions may occur as a result of a) induction of the expression of metabolising enzymes or, b) 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, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP3A4 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 stabilization. Induction results in increased metabolism of the inducing xenobiotic itself (autoinduction), or of concomitantly administered substrates/drugs, resulting in increased clearance and altered pharmacokinetics.

Transcriptional activation is mainly mediated via nuclear receptors (PXR, CAR, GR and VDR) for the induction of CYP3A and CYP2B isoforms, whereas the AhR receptor 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 [16]. In contrast, CYP1A isoforms can also activate some compounds, such as benzo[a]pyrene to their carcinogenic metabolite [17] 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 until new enzymes are synthetised. The inhibitors may be substrates which are metabolized 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 human, several isoforms, such as CYP2C9 [18], CYP2C19 [18], CYP1A1 [19], CYP2B6 and CYP2D6 [20], CYP3A4 [21] and CYP3A5 [22] have 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

This P450 subfamily consists of two members, CYP1A1 and CYP1A2 (Table 1) in mouse, rat, dog, monkey and human. CYP1A shows a quite strong conservation among species with an identity to human higher that 80% in rat (83 and 80%, respectively for CYP1A1 and CYP1A2), mouse (83 and 80%, respectively for CYP1A1 and CYP1A2), dog (84% for CYP1A2) and monkey (95% for both CYP1A1 and CYP1A2). Both have been studied extensively because of their roles in the metabolism of two important classes of environmental carcinogens, polycyclic aromatic hydrocarbons [24] and arylamines [25].

§ 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 intestine [26-28], lung [29-31], placenta [32] and kidney [33]. Also in monkey and dog CYP1A1 is present only at low levels in livers of untreated animals [34,35]. In contrast to rats, where CYP1A1 is the predominant form expressed in rat small intestine [36], CYP1A1 is only weakly detected in mouse intestine [37]. There are no reports on CYP1A1 and CYP1A2 activity in monkey and dog intestine. The expression levels of CYP1A1 in human intestine are reported to be variable. According to McDonnell et al. [38], the CYP1A1 catalytic activity varied considerably in human intestine and microsomal preparations and this high interindividual variabilty was confirmed by several other laboratories, leading to the suggestion that CYP1A1 expression may not be constitutively expressed but only expressed after induction [39]. In fact, higher levels of CYP1A1 are often associated with increased smoking, physical exercise and ingestion of chargrilled meats. However, Paine et al. [40] concluded that the high variability of CYP1A1 in human liver microsomal preparations could not be accounted for by smoking habits, but that diet may be significantly involved in the variability of CYP1A1.

CYP1A1 is able to oxidize benzo[a]pyrene [41] and other polycyclic aromatic hydrocarbons to their toxic derivatives. For example, dibenzo[a]pyrene, considered to be the most potent carcinogen among all polycyclic aromatic hydrocarbons, is oxidized almost exclusively by CYP1A1 in human to highly mutagenic diol-epoxides [42].

§ 4.2. CYP1A2

CYP1A2 is expressed mainly in the liver and is not or weakly expressed in extrahepatic tissues in human [43], rat and mouse [33]. In human liver CYP1A2 accounts for 13% of the total CYP content [15,44] and is involved in the metabolism of ~ 4% of drugs on the market [45]. In contrast, in monkey and dog, CYP1A2 is expressed at low levels in the liver of untreated animals [46,47], even though a quite 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 [48] and CYP1A2 is less expressed in cynomolgus monkey than in the marmoset [49]. In human, CYP1A2 metabolizes several drugs, including phenacetin, tacrine, ropinirole, acetaminophen, riluzole, theophylline and caffeine [50].
§ 4.3. Induction of CYP1A

Both CYP1A1 and CYP1A2 are under the transcriptional regulation of the Ah (aryl hydrocarbon) locus, involving the interaction of Ah receptor/ARNT (aryl hydrocarbon receptor 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 [51]. Both isoforms are inducible not only by food or by cigarette smoke, but also by drugs and their profiles of induction are quite similar among species. The ingestion of the polycyclic aromatic hydrocarbons (PAHs), such as 3-methylcholanthrene (3-MC) or the treatment with β-NF in rat [52,53], mouse, monkey [34] and dog [47], leads to an increase of the CYP1A protein level in numerous tissues, such as intestine, liver and lung and also in cells in culture. In man [54], but not in mouse [55] and in rat [56], the antiulcer drug omeprazole has been reported to induce CYP1A2 in the liver [57]. The effect of omeprazole on CYP1A2 is thus an example of species dependent gene expression regulation, which is also observed for CYP3A regulation (see paragraph 11.6). In man, slight induction of CYP1A2 by rifampicin was suggested by a 15% increased metabolism of caffeine to paraxanthine in healthy subjects [58].

The induction of CYP1A1 by polycyclic aromatic hydrocarbons mediated by the Ah receptor results in the formation of mutagenic/carcinogenic diol-epoxides in target tissues, including liver [59]. 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 the smoking-induced lung cancer incidence 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 CYP1A2 isoforms. Examples include the co-administration of enoxacin, a quinolone antibiotic, which is able to inhibit CYP1A2, resulting in a decrease of the clearance of R-warfarin, a CYP1A2 substrate [60]. In general, furafylline is considered as a selective, non-competitive, mechanism based inhibitor of CYP1A2 [61,62], whereas α-naphthoflavone is an inhibitor of both CYP1A1 and of CYP1A2 [63-65]. Similar to human, also in rats furafylline selectively inhibits CYP1A2, relative to CYP1A1. However, furafylline inhibits rat CYP1A2 only at a 1000 fold higher concentration than was required to inhibit the human isoenzymes, suggesting a major difference in the active site geometry between the human and rat orthologues of CYP1A2 [66]. In addition, furafylline inhibits CYP1A2 activity in mouse and in dog to a lower extent when compared to human, whereas no inhibition was observed towards CYP1A2 in monkey [67]. Interestingly, ketoconazole, a well-known human CYP3A4 inhibitor has been reported to be also a potent inhibitor of the CYP1A1 enzyme in man [40] and in rat [68], thus indicating a possible cross-reactivity with the more abundant CYP3A isoenzymes.
§ 4.5. Conclusion

CYP1A1 and CYP1A2 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 intestine, is variable. Similarly, CYP1A2, which is highly expressed in the liver, is more variable depending on the species. Generally, 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 human, 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 [69]. Since then, extensive research was focused on the inducibility of CYP1B1, especially given that it is differentially expressed within the tumor microenvironment of several human cancers [70,71]. CYP1B1 is constitutively expressed in normal tissues, such as heart, brain, placenta, lung, liver, kidney, prostat [69], but it is expressed at much higher levels in tumor cells compared with the surrounding normal tissue [70,72]. 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 catalyzes estrogens to active 4-hydroxylated derivatives that may cause breast cancer. In rat, CYP1B1 is expressed in liver and lung, at least at the mRNA level [73]. In mouse, CYP1B1 has been detected in several tissues such as testis, kidney, skeletal muscle, lung, spleen, brain, liver and heart, but not in liver [33,74]. In human and rodents species, CYP1B1 can bioactivate carcinogenic polycyclic aromatic hydrocarbons, such as benzo[a]pyrene to DNA-reactive species associated with toxicity, mutagenesis and carcinogenesis [73,75]. Furthermore benzo[a]pyrene can induce expression of CYP1B1 via the aryl hydrocarbon receptor [73].

§ 5.2. Conclusion

In human and animal species, CYP1B1 is the only gene of this sub-family. In human, CYP1B1 is constitutively expressed in normal tissues, and is expressed at much higher levels in tumor cells compared with the surrounding normal tissue.

§ 6. CYP2A

§ 6.1 CYP2A in animal species and man

In human, the CYP2A family includes CYP2A6, CYP2A7 and CYP2A13 (Table 1). CYP2A6 is expressed in human liver and accounts for about 4% of total hepatic P450, whereas
other human P450 subfamily forms (2A7 and 2A13) appear to be expressed at even lower levels. Human CYP2A6 shows a different substrate specificity, in comparison to CYP2A enzymes in animal species. In contrast to rodents where CYP2A enzymes have steroid 7α- and 15α-hydroxylation activities, CYP2A6 is not involved in the hydroxylation of steroids [76]. CYP2A6 is engaged in the metabolism of xenobiotics, e.g. O-deethylation of 7-ethoxycoumarin, 7-hydroxylation of coumarin (a marker reaction), oxidation of nicotine, cyclophosphamide, iphosphamide, fadrozole and aflatoxin B1 [26,76]. In addition, CYP2A6 seems to have overlapping catalytic specificity with CYP2E1 in the activation of nitrosamines [77]. CYP2A13 is an enzyme predominantly expressed in human respiratory tract and significantly involved in the activation of aflatoxin B1 to carcinogenic derivatives [78] and in the nicotine metabolism [79].

In rat, the CYP2A family includes CYP2A1, CYP2A2 and CYP2A3. Rat CYP2A1 (female dominant) and CYP2A2 (male dominant) are expressed in the liver (2%) [80]. In contrast, CYP2A3 is not expressed in the rat liver [81,82] and is constitutively expressed in the esophagus, lung and nasal epithelium, but not in intestine, liver and kidney. The rat CYP2A1/2 show about 60% homology in amino acid sequence to human CYP2A6. In contrast to human, in the rat endogenous steroids are CYP2A substrates: CYP2A1 catalyses 7α-hydroxylation of testosterone, while CYP2A2 is responsible for 15α- and 7α-hydroxylation of testosterone.

In mouse CYP2A4, CYP2A5, CYP2A12 and CYP2A22 belong to CYP2A family. CYP2A5 and CYP2A4 show high sequence similarity and differ in only 11 aminoacids [26]. CYP2A5 is mainly expressed in liver, olfactory mucosa, kidney, lung, brain and small intestine, but not in heart or spleen [83]. CYP2A5 resembles the human orthologue in catalyzing 7-hydroxylation of coumarin [84]. CYP2A4 is a female-predominant form in liver in several inbred mouse strains, and its gene is transcriptionally repressed by growth hormone in males [85]. CYP2A4 was also detected in kidney and, at very low level, in the olfactory mucosa.

In dog, CYP2A13 and CYP2A25 and in monkey, CYP2A23 and CYP2A24, are members of CYP2A family. As reported by Bogaards et al. [67] dog and monkey microsomes catalyse coumarin 7-hydroxylase.

The human CYP2A6 antibody showed moderate to strong inhibition of coumarin 7-hydroxylase activities towards monkey, dog, human and mouse [67]. In human, CYP2A6 is inhibited by diethyldithiocarbamate in vitro [86].

CYP2A isoforms are inducible. In human, CYP2A6 is induced by phenobarbital, rifampicin, dexamethasone and nicotine [76,15]. In rats, CYP2A3 mRNA was increased by treatment with 3-methylcholanthrene and pyrazole in the esophagus, in the kidney and in the distal part of the intestine [81]. The mechanism of CYP2A gene induction is not well understood, but recent studies concerning the murine CYP2A5 indicate the role of constitutive androstane receptor (CAR), pregnane X receptor (PXR) and peroxisome proliferators-activated receptor (PPAR) in transcriptional activation of CYP2A5 [83]. Moreover, human CYP2A6 may be regulated post-transcriptionally via interaction of the nuclear ribonucleoprotein A1 with CYP2A6 mRNA.
§ 6.2. Conclusion

In human 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 several mammalian species (Table 1). These isoforms were among the first microsomal cytochrome P450s purified and show the most dramatic induction by barbiturates.

§ 7.1. CYP2B in human

In human, the CYP2B family includes CYP2B6 and CYP2B7. CYP2B6 is expressed in liver and in some extrahepatic tissues, whereas CYP2B7 mRNA expression was detected in lung tissue [87]. 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 [88], such as the anticancer drugs cyclophosphamide and tamoxifen [89], the anesthetics ketamine and propofol [90,91] and procarcinogens such as the environmental contaminants aflatoxin B1 and dibenzanthracene [92]. In contrast to previous studies that detected CYP2B6 only at 0.2% of the total human liver CYP content [15,44], recent studies [93,94] using more selective and specific immunochemical detection methods have demonstrated that the average relative abundance of CYP2B6 in human liver ranges from 2 to 10% of the total CYP content. In human intestine, the mRNA level of CYP2B6 was not detected [74] by RT-PCR. In addition, significant interindividual differences in hepatic CYP2B6 expression, which varies in some studies from 25- to 250- fold, have been reported [95]. 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 [96]. In particular, recent studies have reported that liver tissue of females express significantly higher amounts of CYP2B6 than do male liver tissues and that CYP2B6 activity was 3.6-5.0 fold higher in Hispanic females than in Caucasian or African-American females [97]. This CYP2B6 variability may be explained by a combination of SNPs (single nucleotide polymorphism) 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, constitutive androstane receptor, mRNA is higher in females than in males) or by hormonal influences (e.g. sex hormones) [97].

§ 7.2. CYP2B in mouse

In mouse, among several CYP2B isoenzymes, CYP2B9 and CYP2B10 are the major CYP2B isoenzymes expressed constitutively. CYP2B10 mRNA is detected in the liver and in the intestine and its expression seems to be higher in the duodenum than in the liver.
However data on metabolic activity are lacking. Similar to rats, the CYP2B family is sexual dimorphic, but female mice express more CYP2B9 isoenzymes than males. In contrast CYP2B10 was equally expressed in both sexes [98].

§ 7.3. CYP2B in rat

Rats express three CYP2B isoenzymes, CYP2B1, CYP2B2 and CYP2B3. CYP2B1 and CYP2B2 are structurally related isoenzymes (97% identical) with very similar substrate specificities [99-101]. However, CYP2B1 is generally much more catalytically active than CYP2B2. Both are expressed constitutively in liver and extrahepatic tissues such as intestine and lung [28,53].

In particular, the mRNA expression of CYP2B1 [28] 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 [28,102] with the highest levels in the duodenum [36,102]. Their constitutive expression in liver is sexually dimorphic, with male expressing higher CYP2B levels than female rats [103]. This sexual dimorphism may be explained by a sex-dependent secretion of pituitary growth hormone which suppress CYP2B expression more in female than in male rats [104].

§ 7.4. CYP2B in dogs

In dog, the main 2B isoform is CYP2B11 which has an 75% identity with respect to aminoacid sequence to that of rat CYP2B1 [105]. Remarkably, CYP2B11 catalyses the N-demethylation of dextromethorphan (mediated in human by CYP3A) and the 4’-hydroxylation of mephenytoin (a drug metabolizing step that is mediated in human by CYP2C19), and, together to CYP3A12, dog CYP2B11 also contributes to S-warfarin–hydroxylation (mediated in human by CYP2C9) [106]. Interestingly, the dog is the only mammalian species able to metabolize polycyclic aromatic hydrocarbons through its CYP2B isoenzyme [26].

§ 7.5. CYP2B in monkey

In monkey, so far, only one CYP2B isoform, referred as CYP2B17, was purified and characterized from liver microsomes from cynomolgus monkeys. The amino-terminal amino acid sequence of the protein (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 (about 5% of total P450) [107,108].

§ 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 [34,47,52,54]. In human and mouse, phenobarbital up-regulates CYP2B gene by activation of CAR [109]. Also PXR ligands, such as rifampicin in human [110] and dexamethasone in rat [52] and mouse [110] induce CYP2B, demonstrating a cross-
regulation 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-methyladamantane belong among the most potent human CYP2B6-selective inhibitors discovered to date [111]. Both compounds also inhibited reactions catalysed by rat CYP2B2 [111]. N-(alpha-methylbenzyl)-1-aminobenzotriazole was identified as inhibitor of CYP2B in both mouse and dog microsomes [112]. In rat, the benzodiazepine, clonazepam has been proven to be a potent non-competitive or "mixed type" competitive inhibitor of catalytic activities mediated by CYP2B. Remarkably, a commercially available antibody to rat CYP2B was found to cross-react with CYP2B family of mouse, dog and human, but not with monkey CYP2B with respect to the inhibition of 7-ethoxy-4-trifluoromethyl-coumarin O-dealkylation [67].

§ 7.8. Conclusion

CYP2B was detected in the liver of all species. In contrast, CYP2B was not detected in the human intestine, but it was highly expressed in the intestine of rat and mouse. Different isoforms are found in the species of interest for ADME-studies, that have different substrate specificities and CYP2B is strongly induced by phenobarbital in both rodent and non-rodent species. Interestingly, CYP2B is sexual 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 P450s 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 about 16% of drugs currently on the market [113]. CYP2C8 and CYP2C9 are the major forms, accounting for 35% and 60%, respectively, of total human CYP2C, while CYP2C18 (4%) and CYP2C19 (1%) are the minor expressed CYP2C isoforms [114]. CYP2C8, CYP2C9 and CYP2C19 proteins are primarily located in the liver where they account for approximately 20% of total cytochrome P450 [15]. However, also other expression levels were reported and the expression appears to show race-related differences and genetic polymorphism [18].

CYP2C18 is not expressed in liver and is most abundantly expressed in human epidermis [115].

CYP2C8 is expressed mainly in the liver, but its mRNA was also detected in kidney, adrenal glands, brain, uterus, mammary glands, ovary and duodenum [116]. CYP2C8 is involved in the metabolism of retinol and retinoic acid, arachidonic acid, benzo[a]pyrene and in the oxidation of the anticancer drug paclitaxel [117].
In addition to liver, CYP2C9 mRNA is also detected in the kidney, testes, adrenal gland, prostate, ovary and duodenum [116]. CYP2C9 metabolizes 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 [118], the antihypertensive losartan [119], the antidiabetic glipizide and the diuretic torasemide [120,121].

CYP2C19 has been detected in liver and duodenum [116,122]. Also CYP2C19 has been shown to metabolise several drugs such as S-mephenytoin, omeprazole and other important proton pump inhibitors [123], certain tricyclic antidepressant such as imipramine [124], the anxiolytic agent diazepam, some barbiturates [125] and the antimalarial drug proguanil [18]. CYP2C19 is highly polymorphic. Poor metabolizers (PMs) of CYP2C19 represent approximately 3-5% of Caucasians and of African-Americans and 12-100% of Asians groups [18]. Toxic effects can occur in PMs exposed to diazepam, and the efficacy of some proton pump inhibitors may be greater in PMs than in EMs at low doses of these drugs.

In human no differences in CYP2C isoforms between male and females have been reported [126].

§ 8.2. CYP2C in mouse

The mouse CYP2C family is larger and more complex than its human counterpart, with more than 10 members published to date, including CYP2C29, CYP2C37, CYP2C38, CYP2C39, CYP2C40, CYP2C44, CYP2C50, CYP2C54 and CYP2C55 [127,128,129], plus several unpublished new members [129]. Similar to 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 [130].

CYP2C29 is expressed in liver as well as in extrahepatic tissues including brain, kidney, heart, intestine, lung, adrenals, aorta, tests and ovary [129,131]. Among the CYP2Cs expressed in murine lung, CYP2C29 is the most abundant [130]. CYP2C37 is most abundant in liver, white blood cells and female adrenals [129], while CYP2C38 and CYP2C40 were found in liver, brain, kidney, lung, heart and intestine. In particular, CYP2C40 is the major CYP2C found in both kidney and intestine and it is the only enzyme found to produce the anti-inflammatory mediator, 16-HETE [132], whereas in human 16-HETE was mainly produced by polymorphonuclear leukocytes [133]. CYP2C44, a new member of CYP2C family, has been mainly detected in liver, kidney and adrenals [128]. CYP2C44 has the lowest homology with other known mouse CYP2Cs (50-60% identical at the aminoacid level). CYP2C44 does not metabolise the common CYP2C substrate tolbutamide and thus differs from CYP2C29, CYP2C38, and CYP2C39 isoforms.

Midazolam has been reported to be metabolized by CYP2C in addition to CYP3A [134], resulting in the formation of α-OH triazolam.
§ 8.3. CYP2C in rat

In rats, the CYP2C family includes several isoforms, such as CYP2C6, CYP2C7, CYP2C11, CYP2C12, CYP2C13, CYP2C22 and CYP2C23. The CYP2C family is the most abundant CYP2C isoform in the liver of the rat and is involved in the oxidation of dihydropyridines and aflatoxin B1 and in the hydroxylation of steroids [135]. There are sex-dependent differences in the expression of CYP2C family in rats, which are developmentally regulated and manifest in adult animals. Immunological data have shown that CYP2C12 is higher expressed in female than in male livers of adult rats, but those differences are not present in immature and old rats [23]. Also the CYP2C7 isoform, which catalyzes retinoic acid 4-hydroxylation and steroid 5α-reduction is female predominant [136]. 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 but not in female rats [137]. CYP2C11 is the predominant isoform in male rat liver comprising up to 50% of the total CYP content [137] and is also expressed in extrahepatic tissues such as kidney and intestine at lower levels [138,36]. Therefore the suppression of this isoform in the liver helps to explain the decline in drug-metabolizing capacity [136]. Also CYP2C13 is male specific, and is expressed not only in liver but also in extrahepatic tissue, such as in the rat brain [139]. In contrast, CYP2C6 is gender independently expressed [136] and it is detected in liver and at lower level also in intestine [36]. 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 CYP2C family in rat has been demonstrated to be regulated at the level of hypothalamic-pituitary axis [23] by the secretion of 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 [140], cyclophosphamide [141,142] and isosphamide 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 this cytotoxic agents induce. Also drugs such as phenobarbital [143], dexamethasone and also other foreign chemicals, such as ethanol, have been shown to suppress CYP2C11 expression in liver, probably due to their influence on testosterone serum level.

§ 8.4. CYP2C in dog

Despite the common use of the dog in safety evaluation as the most used non-rodent species, obliged for any new drug prior to use in man, knowledge concerning the canine cytochrome P450 system and in particular the CYP2C family is limited. Two canine CYP2C isoenzymes have been isolated so far, CYP2C21 and CYP2C41. 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 1 of the 9 dogs tested. In addition, the CYP2C41 gene was found only
in 4 dogs out of 28 dogs investigated [144]. Therefore this strong polymorphism in the CYP2C41 subfamily may be an important source of variability in the metabolic clearance of xenobiotics that are metabolized by CYP2C41 in dogs [144].

In addition, the metabolism of specific human CYP2C substrates, such as tolbutamide, warfarin and S-mephenytoin, is impaired in dog compared to human liver, illustrating an important species difference between dog and human drug metabolism [106].

§ 8.5. CYP2C in monkey

In monkey, the CYP2C family accounts for two isoforms, CYP2C20 and CYP2C43. These isoforms are both expressed in the liver and show an identity of 83% and 77% for the nucleotide and for the aminoacid sequences, respectively. Among the CYP2C isoenzymes in human, CYP2C43 shows the highest identity with CYP2C9 (95% and 92% in nucleotide and aminoacid sequences, respectively), followed by CYP2C19 (93% and 89%), CYP2C18 (86% and 80%) and CYP2C8 (84% and 78%). CYP2C43, but not CYP2C20, was able to metabolize S-mephenytoin, a probe substrate of CYP2C19 in human. In contrast, CYP2C43 was not able to metabolize tolbutamide, a probe substrate of CYP2C9 in human. Therefore monkey CYP2C43 appears to be functionally related to human CYP2C19 but not to human CYP2C9, although the N-terminal sequence (first 18 residues) was identical for CYP2C43 and CYP2C9 [145].

§ 8.6. Induction of CYP2C

Human CYP2C8, CYP2C19 and CYP2C19 are inducible isoforms. Compounds known to activate the pregnane X receptor (PXR) such as rifampicin and dexamethasone or the constitutively activated receptor (CAR) such as phenobarbital, induce CYP2C8, CYP2C9, and to a less extent CYP2C19 [146], although the precise mechanism of induction by xenobiotics has not been elucidated yet [147]. 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 [148,149].

§ 8.7. Inhibition of CYP2C

Sulfaphenazole is perhaps the most potent and selective inhibitor of CYP2C9 [50]. The mode of inhibition is via ligation to the heme iron of CYP2C9. In dog and in monkey, sulfaphenazole shows a similar inhibition profile, even though to less extent in comparison to human [67]. In contrast, in rat liver microsomes no inhibition of diclofenac metabolism by sulfaphenazole was found, thus indicating a difference between the active sites of human CYP2C9 and rat CYP2C9 related protein [67]. In addition, also theazole antifungal fluconazole [150], the HMG-CoA reductase inhibitors [151] and fluvastatin [151] are inhibitors of CYP2C9. The most relevant inhibitors of CYP2C19 are the SSRIs (selective serotonin re-uptake inhibitors), such as fluoxetine and fluvoxamine [152], whereas some antifungal drugs, such as miconazole, voriconazole and fluconazole are inhibitors of both CYP2C9 and CYP2C19 isoforms [153]. Inhibitors of CYP2C8 have been identified from a wide variety of therapeutic classes, such as
montelukast, salmeterol, ritonavir, ketoconazole, tamoxifen, quercetin, simvastatin and lovastatin [154].

§ 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 in rat only, the expression of CYP2C is sex dependent in adult animals. Substrate specificities are largely different between human and animal isoforms, and particularly CYP2C mediated metabolism in the dog is poorly representing human metabolism. In addition CYP2C is not expressed in all dogs, making prediction hazardous. CYP2C is inducible in man but to date, 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 mono-oxygenation of various chemicals including antidepressants (e.g. desipramine), β-blockers (e.g. propanolol), anti-arrhythmics (e.g. sparteine) and others such as dextromethorphan and methadone [155]. CYP2D was the first isoform shown to be polymorphic. Induction of CYP2D has not been reported to date.

§ 9.1. CYP2D in human

Although CYP2D6 is expressed at a low level in human liver accounting for about 4% of total P450 (12.8 pmol/mg microsomal protein) [45,156], this enzyme is involved in the biotransformation of 30% of drugs on the market [157,158]. In human, only one isoform, CYP2D6, is expressed in various tissues including the liver, kidney, placenta, brain, breast, lung and intestine [159,160]. CYP2D7 and CYP2D8 are inactive pseudogenes. Since it was discovered, CYP2D6 has been the most studied human genetic polymorphism in drug metabolism with more than 80 identified alleles within most human populations and racial groups. Problems related to polymorphism in drug metabolism became evident when sparteine [161] and debrisoquine [162] were found to be metabolized at different rates among individuals. Approximately 7 to 10% of the Caucasian population inherits mutant CYP2D6 alleles as an autosomal recessive trait [162], 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 poor metabolizers (PM, zero copies), intermediate metabolizers (one copy), extensive metabolizers (EM, two copies), and ultrarapid metabolizers (multiple copies). In addition, this genetic variation in CYP2D6 is associated with risk for diseases and cancer, for example Parkinson's disease, lung cancer, liver cancer and melanoma [163].

In the intestine, CYP2D6 is expressed in the duodenum and jejunum [102] and not in ileum and colon. Like CYP3A4, it is localized within the mucosal enterocytes. The mean specific enzyme content of jejunal microsomes was reported to be less than 8% of hepatic
CYP2D6 microsomal content (0.85 vs 12.8 pmol/mg) and there is extensive interindividual variability in protein content of both tissues [156].

§ 9.2. CYP2D in mouse

Only a few studies have been performed to characterize the CYP2D family in mouse. There are at least nine mouse CYP2D genes (CYP2D9, CYP2D10, CYP2D11, CYP2D12, CYP2D13, CYP2D22, CYP2D26, CYP2D34 and CYP2D40), but some of the isoenzymes have not been characterized for expression and function [164]. One isoenzyme CYP2D22 has been suggested to be the orthologue of human CYP2D6 [165] and it has been detected abundantly in liver, whereas intermediate levels of expression are seen in adrenal, ovary and mammary gland.

§ 9.3. CYP2D in rat

In rats, six isoforms (CYP2D1, CYP2D2, CYP2D3, CYP2D4, CYP2D5 and CYP2D18) have been identified by genomic analysis [166]. The rat and human CYP2D isoforms share a high sequence identity (>70%) [167]. Among these six, CYP2D5 and CYP2D18 have over 95% similarity in amino acid sequence to CYP2D1 and CYP2D4, respectively [168]. Similar to human CYP2D6, the six isoforms are expressed in various tissues such as liver, kidney, and brain [169]. The mRNA of each isoform shows a specific tissue distribution [170]. CYP2D2 and CYP2D3 mRNAs are mainly expressed in the liver, kidney, and small intestinal mucosa. In contrast CYP2D1/5 mRNAs is expressed in various tissues. CYP2D4/18 mRNAs are expressed in brain, adrenal glands, ovary, testis, in addition to liver, kidney and small intestinal mucosa [155]. CYP2D4 has also been identified in rat breast [171]. Therefore, the specificity tissue distribution of rat CYP2D isoforms suggest that each isoform has specific catalytic properties and plays specific roles in various tissues [155]. For example, R-mianserin was N-oxidated by only CYP2D1 while 8-hydroxylation was performed by all isoforms [172]. 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 [45]. CYP2D15 is 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 [173,174]. Two clones corresponded to variant full-length CYP2D15 cDNA (termed CYP2D15 WT2 and CYP2D15 V1), while the third was identified as a slicing variant missing exon 3 (termed CYP2D15 V2). Bogaards et al. [67] 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 [67]. Therefore, because of the similar enzyme kinetics and quinidine inhibition profile, dogs seem to be the most similar species to man with respect to CYP2D inhibition [67,45].
§ 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-aminoacid protein that is 93% identical to human CYP2D6 [175]. The recombinant CYP2D17 catalyzed 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 ortholog of human CYP2D6. In marmoset monkeys two isoforms, CYP2D30 and CYP2D19 has been isolated in two different female marmoset bred in different laboratories. Even though both isoforms have shown strong homologies in their nucleotide and amino acid sequences respectively [176], 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. Also the two marmoset recombinant enzymes are involved in the bufuralol metabolism. In particular 1’S-OH bufuralol metabolite was more produced by CYP2D30, whereas the 1'R-OH bufuralol metabolite was more produced by CYP2C19 [176]. Similar to human, quinidine exhibited inhibitory effect towards bufuralol 1’hydroxylation activities. In Japanese monkey (Macaca fuscata) a full length cDNA encoded a 497-aminoacid protein (designated CYP2D29) that is 96, 91 and 88% homologous to human CYP2D6, cynomolgus monkey CYP2D17 and marmoset monkey CYP2D19, respectively. Similar to human CYP2D6, this isoform catalyses the metabolism of debrisoquine and bufuralol [177].

§ 9.6. Inhibition of CYP2D

CYP2D6 is inhibited by a very low concentration of quinidine. Although not metabolized by CYP2D6 but by CYP3A4, quinidine conforms closely to the structural requirements for a substrate for the enzyme [178]. In addition to quinidine which is one of the most potent CYP2D6 inhibitors, some other compounds, such as the SSRIs and the HIV-I protease inhibitor ritonavir have a strong inhibitory interaction with CYP2D6 [179,180]. With regard to the other species, Bogaards et al. [67] 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 [67]. In rat, monkey and mouse the inhibition profile was different in comparison to human. Bogaards et al. [67] reported negligible (in rat and mouse) or low (in monkey) inhibitory effect by quinidine towards bufuralol 1’hydroxylase catalytic activity, whereas the quinine isomer weakly inhibited CYP2D in monkey and rat [67,135,181], but not in mouse [67].

§ 9.7. Conclusion

The CYP2D family shows genetic polymorphism resulting in variation in functional activity in drug metabolism in man. Similarly in other species, such as rat [182] and dog [173] 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 not to be inducible.

§ 10. CYP2E1

§ 10.1. CYP2E1 in animal species and man

CYP2E1 shows a quite 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 approximately 6% of total P450 in the liver and is involved in the metabolism of 2% of the drugs on the market [45]. The CYP2E1 appears to have a dual physiological role, namely a role in detoxification and in nutritional support. CYP2E1 is expressed in many tissues, such as the nose, the oropharynx (exposed to airborne xenobiotics), the lung and the liver. Also 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 to reach excessive levels. Its inducibility by ethanol was shown not only in experimental animals [183] but also in man [184]. In terms of its nutritional role, the upregulation of CYP2E1 plays a useful physiologic 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 [185]. 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 pathologic consequences. This is true not only when excess alcohol has to be metabolized, but also when CYP2E1 is confronted with an excess of ketones and fatty acids associated with diabetes and/or obesity [186].

A few drugs are metabolized by CYP2E1, such as acetaminophen, caffeine and chlorzoxazone, the latter being considered a marker of CYP2E1 activity [187]. Although relatively few drugs are oxidized by CYP2E1, the list of carcinogens that can be activated by CYP2E1 is quite extensive and includes benzene, styrene, acrylonitrile, and nitrosamines. CYP2E1 may generate reactive oxygen intermediates, such as superoxide radicals [188], which play a key role in liver injury because of the interaction with cellular proteins or DNA [189]. 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 [190] and vinylidene chloride [191] as well as anesthetics such as enflurane [192]. Enhanced metabolism and toxicity pertains also to a variety of prescribed drugs, including isoniazid, phenylbutazone and acetaminophen.

CYP2E1 activity is inducible by ethanol and by acetone in both rodents and non-rods. The regulation of CYP2E1 expression is complex, and involves transcriptional, post-transcriptional, translational and post-translational mechanisms [193]. CYP2E1 is transcriptionally activated in the first hour after birth. Xenobiotic inducers elevate CYP2E1 protein levels through both increased translational efficiency and stabilization of the protein from degradation, which appears to occur primarily through ubiquitination and proteasomal degradation [193].
Similar to human, many substrates such as organic solvents, nitrosamines and drugs such as paracetamol are metabolized by rodent CYP2E1. Therefore rodents, such as rats may be an appropriate model to study CYP2E1 dependent metabolism in man [45]. However, in dogs and monkeys some discrepancies with human 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 [67]. In monkeys, CYP2E1 activities in liver microsomes seem to be similar to human CYP2E1 [48], however inducibility of this enzyme by 3-methylcholanthrene (another typical inducer of the CYP1A) indicates significant differences in the mechanism of induction of CYP2E1 [194].

Disulfiram and diethyldithiocarbamate are mechanism-based inhibitors of CYP2E1 in man. Moreover, 3,4- and 3,5-dichlorophenyl derivates have recently being demonstrated to be potent inhibitors of human CYP2E1 [195].

In addition, diethyldithiocarbamate is a potent mechanism based inhibitor of 6 OH-chlorzoxazone formation in microsomes of rodents and non-rodents species [196], 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 all animal species. CYP2E1 plays a physiological role and is involved in the metabolism of few drugs. Similar to human, CYP2E1 is inducible by ethanol and acetone in rodents and non-rodents. In spite of some discrepancies, CYP2E1 is quite 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. CYP3A

The P450 3A subfamily (Table 1) plays very important roles 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-metabolizing enzymes because this subfamily is involved in the biotransformation of approximately 50% of therapeutic drugs currently on the market, although its content in the liver is only 30% of total P450. Some examples of drugs metabolized by CYP3A are terfenadine, the benzodiazepines midazolam and triazolam, quinidine, lidocaine, carbamazepine, nifedipine, tacrolimus, dapsone, erythromycin, dextromethorphan etc. [45,197]. In addition to drugs, CYP3A is involved in the oxidation of a variety of endogenous substrates, such as steroids, bile acids and retinoic acid [198]. Human express four CYP3A enzymes, CYP3A4, 3A5, 3A7 and 3A43. CYP3A4 and its related CYP3A5 are the most abundant CYP isoforms in human liver and are involved in the biotransformation
of the majority of drugs [199]. CYP3A4 and CYP3A5 are expressed in liver, stomach, lung, intestine and renal tissue. The level of CYP3A4 content is highest in 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) [200]. It is located at the apex of the enterocytes [201] and plays a major role in the first-pass metabolism of xenobiotics. CYP3A protein and catalytic activity decrease longitudinally along the small intestine. Although the levels of CYP3A in the intestine expressed per mg microsomal protein are generally 10 to 50% lower than those found in the liver, in some individuals CYP3A concentration is equal to or even higher than those in the liver [200]. This together with its strategic localisation at the tip of the villus makes that the 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 [202]. 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 been recently 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 about 20% of samples [203] and when expressed it accounts for one-third of CYP3A4 [204]. However, recent evidence indicates that CYP3A5 may represent more than 50% of the total CYP3A in some individuals [205]. 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 [206,207]. Furthermore, CYP3A5 is expressed in one-third of Caucasian livers and over one-half of African-American livers examined [205]. CYP3A7 and CYP3A43 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 to 5% of CYP3A4) [208,209].

§ 11.2. CYP3A in mouse

In mouse, there are six CYP3A isoforms identified so far. CYP3A11 and CYP3A13 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 to CYP3A11 [210]. Of the mouse CYP3A isoforms, CYP3A11 is the most similar to human CYP3A4, having 76% amino acid homology [211]. In addition, CYP3A11 is also expressed in the small intestine [212] like CYP3A4 in man. CYP3A16 has been identified as a fetal form, which diminishes rapidly after birth [213]. CYP3A25 appears primarily in liver and small intestine of newborn and adult mice with no evidence of gender bias in expression [214]. CYP3A41 [215] and CYP3A44 [216] 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 metabolized by mouse CYP3A isoforms [217] similar to human [218,219]. No strain related differences between nude and CD-1 mouse have been revealed in CYP3A11 expression [27].
§ 11.3. CYP3A in rat

In rats, CYP3A1/3A23 [220,224], CYP3A2 [221], CYP3A9 [222], CYP3A18 [223], and CYP3A62 have been reported as CYP3A forms. These CYP3A forms appear to be expressed in a sex-specific manner in rats. For example, CYP3A2 [225] and CYP3A18 [226] are male specific forms, whereas CYP3A9 is a female dominant form [226]. Recent studies from Matsubara et al. [227] have identified the new rat CYP3A62 form, and its expression profile is similar to that of human CYP3A4 and to rat CYP3A9. CYP3A62 is the predominant form in the intestinal tract, whereas CYP3A1 and CYP3A2 were detected only in the liver. In addition, CYP3A9 and CYP3A18 were mainly detected in the liver, although a low expression was detected also in the intestinal tract [227].

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 a typical human CYP3A inducer rifampicin [56]. Moreover, some discrepancies in the metabolism have also been revealed between rats and human: for example, many prototypical substrates of human CYP3A enzymes as dydropyridine calcium channel blockers (e.g. nifedipine) are not metabolized by rat CYP3A1 [228,229].

§ 11.4. CYP3A in dog

In dog, the CYP3A family accounts for two isoforms, including the CYP3A12 and CYP3A26. 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 [230]. The human isoforms CYP3A4 and CYP3A5 have been shown to exhibit some parallels when compared with canine CYP3A12 and CYP3A26 [230].

§ 11.5. CYP3A in monkey

In cynomolgus monkey CYP3A8 represents ~20% of the P450 in monkey liver [231], and it is 93% similar to the human CYP3A4 protein [194]. Taking into account the higher total P450 levels/mg liver microsomal protein in monkeys in comparison to human, this represents 4-5 times higher levels of CYP3A8 to CYP3A4/unit of liver.

In addition, the human 3A marker midazolam 1'-hydroxylase and the erythromycin N-demethylation had 4-fold and 19-fold higher activity, respectively, in cynomolgus monkeys than human, suggesting that there are some differences in affinity and enzymatic rates for CYP3A substrates [48].

§ 11.6. Induction of CYP3A

The induction of CYP3A family is extensively studied because of its importance on drug metabolism in man. The CYP3A protein is highly inducible by drug exposure, mainly trough transcriptional activation. The major part of CYP3A4 transcriptional activation is mediated via PXR [232]. Hence, the activation of PXR by xenobiotics is a good indicator of induction of CYP3A4 gene. In human and dogs, rifampicin is a strong inducer of CYP3A [47,110], but not in rat and mouse whereas dexamethasone and pregnenolone
16α-carbonitrile (PCN) are strong PXR activators and/or inducers of CYP3A [52,110]. In addition, dexamethasone induces CYP3A in human, but not in dog [56]. 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 different dramatically between species. Therefore, extrapolation of animal data with respect to the inducibility of the CYP3A subfamily in human can be problematic [233]. In addition to PXR activation, compounds that are poor PXR ligands may still be potent CYP3A4 activators through alternative routes. Such routes may include activation of alternative ligand-activated nuclear receptors, such as glucocorticoid receptor (GRα), constitutive androstane receptor (CAR) and the vitamin D receptor (VDR), which in turn activate gene transcription. Activation by GRα is likely to occur via a mix of interactions with sequences within the xenobiotic-responsive enhancer module (XREM) and also cooperative induction by other nuclear receptors, including PXR [234]. Similar to PXR, VDR and CAR heterodimerize with RXR and appear to be able to activate CYP3A4 gene expression through direct interaction with the PXR-responsive elements (PXRE). In particular, the DNA binding domains of CAR and PXR are rather similar in amino acid composition suggesting that these receptors bind to similar DNA motifs and are likely to share many target genes, leading to complex cross-regulation of drug-metabolizing enzymes. For example, phenobarbital is generally considered as a classic CAR-mediated inducer but it also appears to act through PXR. The mechanism of CAR activation is complex, involving translocation from the cytoplasm to the nucleus in the presence of activators, followed by further activation steps in the nucleus. In addition, CAR and PXR seem to have a very flexible, overlapping and low-affinity binding specificity that is ideally suited for the recognition of numerous xenobiotic compounds entering our body constantly.

§ 11.7. Inhibition of CYP3A

Ketoconazole is a potent and well-studied inhibitor of CYP3A in human and animals species and is often used in vitro and in vivo as a diagnostic inhibitor. The drug is not only an inhibitor of CYP3A (Ki < 1 µM), but it is also a CYP3A substrate, being metabolized into the imidazole ring which is also the site of its ligation to the heme [235]. 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 [40] and in rat [68], thus indicating a possible cross-reactivity with the more abundant CYP3A4 isoenzymes. In particular in rat, ketoconazole inhibited the activities of CYP1A2 and CYP2C6 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 [67].

Also other azole antifungals, such as itraconazole, have CYP3A inhibitory effect in man [236]. In addition, there are several examples of inhibitors acting as mechanism based or suicides inhibitors. The antibiotic macrolide erythromycin [237] is one example of mechanism-based inhibitors. Due to the large number of drugs metabolized by CYP3A4, being a potent inhibitor can have a detrimental effect on a compound’s marketability.
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 [238].

§ 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, PCN 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 20-50 fold. In addition, prominent interspecies differences in inhibition have been reported.

§ 12. Expert opinion and conclusions

For the development of new drugs, the investigation of drug metabolism mediated by cytochrome P450 and the evaluation of potential drug-drug interaction is essential. The experimental approach is based on animal drug-metabolizing systems and is used to predict kinetics and toxicity in human. However, this interspecies comparison suffers from certain limitations, because in different species, specific isoforms are expressed and even when a high degree of sequence identity in the aminoacid sequences exists between the isoforms, this does not automatically mean similar catalytic specificity [26]. In fact, none of the animal species are completely similar to man with respect to all P450 enzymes activities. However, similarities can be found for some specific CYP isoforms. For example CYP2E1, of which only one isoform is found up to now, shows no appreciable differences with respect to expression and catalytic activity between species according to several authors [67,26], and extrapolation between species appears to hold quite well. Regarding CYP1A, all species seem to express the two isoforms CYP1A1 and CYP1A2, albeit to a different extent and also different catalytic activity has been observed, and therefore some caution is required in extrapolation. CYP2C, CYP2D and CYP3A show substantial differences in terms of isoforms, expression, organ-specificity and catalytic activity. According to Bogaards et al. [67] and Zuber et al. [45], dog seems to be more similar to man for CYP2D activity, monkey for CYP2C activity, mouse and rat 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 and 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 nowadays 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. Besides, the assessment of the absolute amount of CYP isoforms, in different animals and in different organs, such as liver and intestine, could 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 be focused on the role of transporters in drug clearance in order to investigate their influence on metabolism.
Table 1. CYP enzymes of the major drug metabolizing CYPs family in man, mouse, rat, dog and monkey.

<table>
<thead>
<tr>
<th>Family</th>
<th>Subfamily</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
<th>Dog</th>
<th>Monkey</th>
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<tbody>
<tr>
<td>CYP1</td>
<td>A</td>
<td>1A1, 1A2</td>
<td>1A1, 1A2</td>
<td>1A1, 1A2</td>
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<td>1B1</td>
<td>1B1</td>
<td>1B1</td>
<td>1B1</td>
<td>1B1</td>
</tr>
<tr>
<td>CYP2</td>
<td>A</td>
<td>2A6, 2A7, 2A13</td>
<td>2A4, 2A5, 2A12, 2A22</td>
<td>2A1, 2A2, 2A3</td>
<td>2A13, 2A25</td>
<td>2A23, 2A24</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2B6, 2B7</td>
<td>2B9, 2B10</td>
<td>2B1, 2B2, 2B3</td>
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<td>2C29, 2C37, 2C38, 2C39, 2C40, 2C44, 2C50, 2C54, 2C55</td>
<td>2C6, 2C7*, 2C11*, 2C12*, 2C13*, 2C22, 2C23</td>
<td>2C21, 2C41</td>
<td>2C20, 2C43</td>
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<tr>
<td></td>
<td>D</td>
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<td>2D1, 2D2, 2D3, 2D4, 2D5, 2D18</td>
<td>2D15</td>
<td>2D17**, 2D19**, 2D29**, 2D30**, 2D42**</td>
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<tr>
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<td>2E1</td>
<td>2E1</td>
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</table>

* gender difference
** strain specific
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