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Mini Review

Cytochromes P450 and experimental models of drug metabolism

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Abstract

For the development of new drugs, evaluation of drug-drug interactions with already known compounds, as well as for better understanding of metabolism pathways of various toxicants and pollutants, we studied the drug metabolism mediated by cytochromes P450. The experimental approach is based on animal drug-metabolising systems. From the ethical as well as rational reasons, the selection of an appropriate system is crucial. Here, it is necessary to decide on the basis of expected CYP system involved. For CYP1A-mediated pathways, all the commonly used experimental models are appropriate except probably the dog. On the contrary, the dog seems to be suitable for modelling of processes depending on the CYP2D. With CYP2C, which is possibly the most large and complicated subfamily, the systems based on monkey (*Maccacus rhesus*) may be a good representative. The CYP3A seems to be well modelled by pig or minipig CYP3A29. Detailed studies on activities with individual isolated CYP forms are needed to understand in full all aspects of inter-species differences and variations.

Keywords: cytochrome P450 - drug metabolism - inter-species differences

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Introduction

Cytochromes P450 (CYP) are enzymes known for their role in metabolism of compounds of a rather nonpolar character [1, 2]. Their origin is dated to archaebacteria – one of the oldest organisms known. Recently, a CYP enzyme from bacteria *Sulfolobus solfataricus* has been characterized possessing extreme stability of its structure which apparently helped the enzyme to work even at high temperatures and pressures expected at the very beginning of life on the Earth [3]. Many CYP enzymes were characterized in the last fifteen years documenting the necessity of this system during the evolution. For example, the plants have been shown to need the CYP enzymes for biosynthesis of natural dyes as well as for of the poisons helping them to fight the natural enemies [4]. All the CYP enzymes known bind two atoms of oxygen (mostly the dioxygen molecule, O_2 , but in two cases of thromboxane synthase and allene oxide synthase, it may be also the –O–O– moiety of peroxide structure). The oxygen atoms are bound to the heme iron, of the same heme, as in hemoglobin or myoglobin molecule. The difference lies in the mode of interaction of the heme iron with the surrounding protein: Contrary to the hemoglobin iron, the iron atom is bound rather strongly to the anionic, thiolate sulfur of cysteine. This mode of binding gives the heme the properties necessary for oxygen activation (in other words, for splitting of the dioxygen molecule to two atoms). One atom of oxygen forms water molecule (this is why CYP enzymes were also named mixed function oxidases), the second is activated for introduction into the substrate molecule (this is why CYP enzymes are now included in the class of enzymes called monooxygenases, with systematic number 1.14.14.1). Hence, in most cases, a hydroxylated product is formed [1, 2]. Thus, a general reaction catalyzed by a CYP enzyme can be expressed by a scheme

$$RH + NADPH + H^+ + \mathbf{O_2} \rightarrow R\mathbf{O}H + NADP^+ + H_2\mathbf{O}$$

where RH is a substrate, oxygen atoms are shown in bold. The complete reaction scheme may be more complicated. The main types of reactions found to be catalyzed by this system are: (i) Hydroxylation of an aliphatic or aromatic carbon, (ii) Epoxidation of a double bond, (iii) Heteroatom (S-, N-, and X-) oxygenation and N-hydroxylation, (iv) Heteroatom (O-, S-, N-) dealkylation, (v) Oxidative group transfer, (vi) Cleavage of esters, (vii) Dehydrogenation, (viii) Reductive dehalogenation, azo-, nitro- reduction and (ix) Isomerization. All cytochromes P450 possess – as typical hemoproteins – also a peroxidase activity.

Mammalian CYP enzymes. CYP enzymes and drug metabolism in man

In mammals, CYP enzymes can be roughly divided in two classes. The first class is involved in biosynthesis of low-molecular weight regulators of various biological functions. Formation of steroid hormones from cholesterol is a typical example involving several very specific CYP enzymes [5]. The first step, cleavage of cholesterol side chain, is catalyzed by a specific CYP enzyme, CYP11A1 (formerly named P450scc for side chain cleavage). During the next steps, three other forms of CYP enzymes are involved (Fig. 1).

The numbering of CYP forms is based either on the characteristic position where they act (see Fig.1 – CYP enzyme catalyzing the hydroxylation in the position 21 of cholesterol is named CYP21A1) or sequentially. The P450 superfamily is divided into families (*e.g.* CYP1, which is with one of the oldest and most conserved structures) where the primary structure should be identical by more than 40%, in subfamilies (labeled with letters A, B etc.) In each subfamily, the similarity of the primary structure should be at least 55%. New forms of CYP should differ in its primary structure by more than 3% [5].

The second class of mammalian CYP enzymes takes part in metabolism of various compounds including drugs, food preservatives, many toxicants and carcinogens. Their site of action is very different, beginning with the organs where the drugs (or, generally speaking, xenobiotics) enter the intracellular compartments as liver and the gastrointestinal tract as a whole, lung, nasal mucosa up to organs and tissues as the kidney, blood, skin or blood cells [6]. The importance of P450s has been widely recognized only recently as several

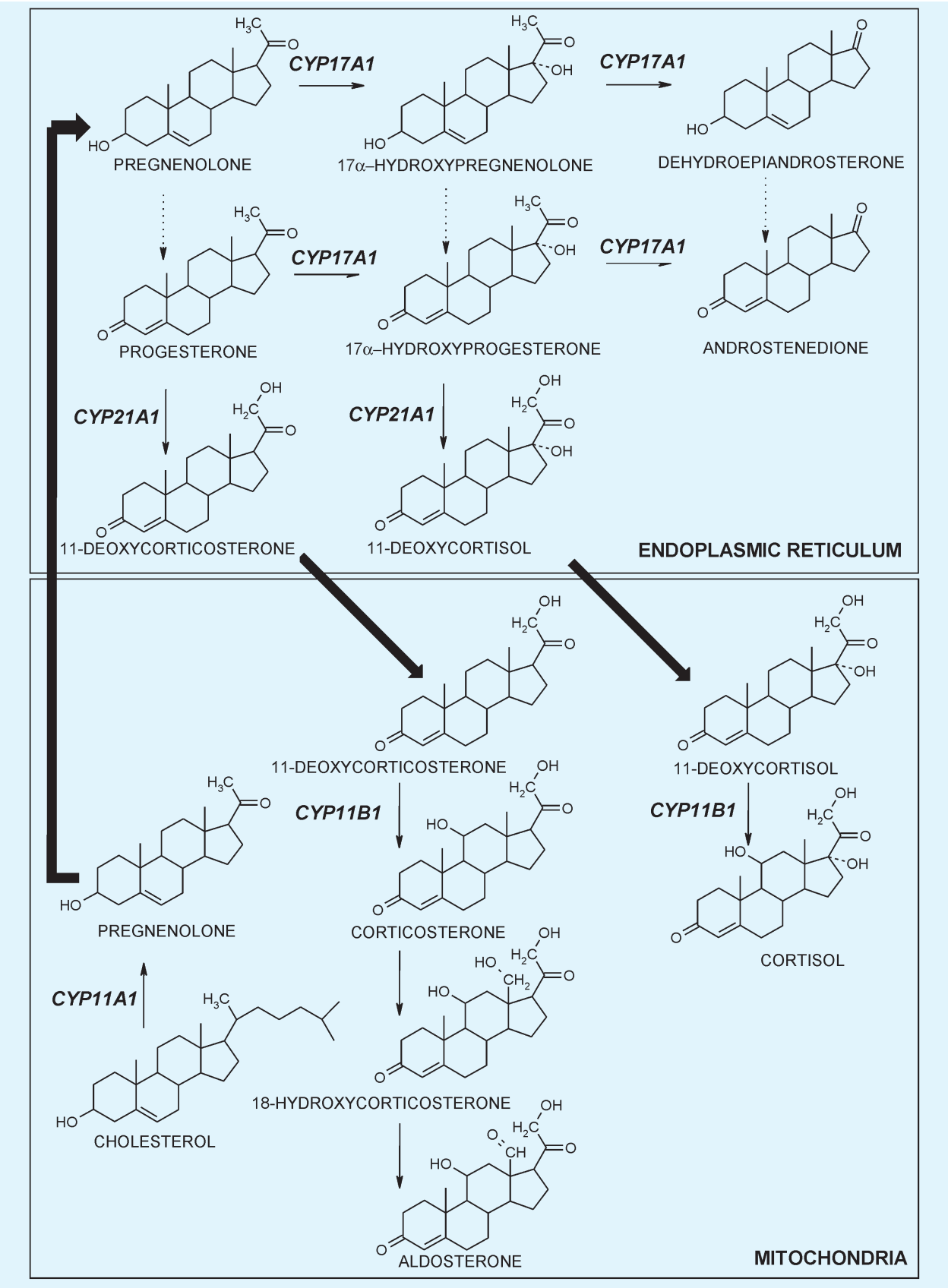

Fig. 1 Biosynthetic pathways of adrenal steroid hormones with individual CYP enzymes. Adapted from [46].

Table 1. Selected drugs known to be substrates or to interact with the most important CYP enzymes.

CYP3A4

Alfentanil	Diltiazem	Lovastatin	Salmeterol
Alprazolam	Docetaxel	Meloxicam	Saquinavir
Ambroxol	17 -estradiol	Methadone	Sertraline
Amiodaron	Ergot alcaloids	Mibefradil	Sildenafil
Amlodipin	Erythromycin	Midazolam	Simvastatin
Atrovastatin	Ethinylestradiol	Mifepristone	Sulfamethoxazol
Benzphetamine	Ethosuximide	Myconazole	Sulfentanil
Bupivacaine	Ethylmorphine	N-hydroxyarginine	Tacrolimus
Budesonide	Etoposide	Nefazodone	Tamoxifen
Buprenorphine	Felodipine	Nicardipine	Teniposide
Carbamazepine	Fenantyl	Nifedipine	Terfenadine
Citalopram	Finasteride	Niludipine	Terguride
Cisapride	Flutamide	Nimodipine	Testosterone
Clarithromycin	Gestodene	Nisoldipine	Tetrahydrocanabinol
Clozapine (also CYP1A2)	Granisetrone	Nitrendipine	Theophylline
Codeine	Hypericum extract	Paclitaxel (Taxol)	(mainly CYP1A2)
Colchicine	Ifosphamide	Paracetamol	Tolterodine
Cortisol	Indinavir	(mainly CYP2E1)	Triazolam
Cyclobenzaprine	Irinotecan	Prednisone	Trimethadone
Cyclophosphamide	Itraconazole	Progesterone	Troglitazone
Cyclosporin A, G	Ketoconazole	Proquanil	Troleandomycin
Dehydroepiandrosterone	Lansoprazole	Quinidine	Verapamil
Delaviridine	Levonorgestrel	Retinoic acid (Tretinoin)	Vinblastine
Dexamethasone	Lidocaine	Rifabutin	Vincristine
Dextromethorphan	Lisuride	Rifampicin	(R)-Warfarin
Diazepam	Loratadine	Ritonavir	Zolpidem
Digitoxin	Losartan	Ropivacain	

CYP2D6

Ajmaline	Dextromethorphan	Isoniazid (Inhibitor)	Quinidine (inhibitor)
Amitriptyline	Dexfenfluramine	Maprotilin	Risperidone
Bufuralol	Encainide	Mehoxyamphetamine	Sparteine
Bupranolol	Flecainide	(S)-Metoprolol	Tacrine (mainly CYP1A2)
Cinnarizine	Fluoxetine	Mexiletine	Tamoxifen
Citalopram	Flunarazine	Mianserin	Thioridazine
Clomipramine	Fluphenazine	Nortriptyline	Timolol
Chlorpromazine	Fluvoxamine	Paroxetine	Tramadol
Codeine	Galanthamine	Perhexiline	Trifluperidol
Debrisoquine	Haloperidol	Perphenazine	Trimepranol
Deprenyl	Hydrocodone	Propafenone	Tropisetron
Desipramine	Imipramine	Propranolol	Venlafaxine

CYP2C9

Antipyrine	Indomethacin
Diclofenac	Irbesartan
Dronabinol (THC)	Losartan
Carbamazepine	Meloxicam
Flurbiprofen	Phenytoin
Fluvastatin	Piroxicam
Glimpiride	Tolbutamide
Glipizide	Torsemide
Glibenclamide	(S)-Warfarin
Ibuprofen	

CYP2C19

Amitriptyline (also	Moclobemid
CYP1A2)	Omeprazole
Citalopram	Pantoprazole
Clomipramine	Phenytoin
Diazepam	Primidone
Imipramine	Proguanil
Indomethacin	Propranolol
Isoniazid (inhibitor)	Teniposide
Lansoprazole	(R)-Warfarin
(S)-Mephenytoin	

promising drugs have had to be withdrawn from the market because of life-threatening interactions with other drugs. The molecular principle of these interactions is either (i) an induction of a CYP enzyme by one drug, which may then influence the metabolism of another drug taken simultaneously or subsequently, or, (ii) an inhibition of the metabolism of one drug due to a competition with the second drug for the same CYP enzyme. An example of the first case is *e.g.* significant lowering of cyclosporine levels after taking of drugs containing hypericine (one of active components from plant *Hypericum perforatum*) which resulted in serious condition in transplanted patients because of strong induction of the CYP3A4 [7, 8]. The second mechanism (*i.e.* the inhibition) of drug interactions involves the interaction of non-sedative antihistamine drug terfenadine with azole antifungals as itraconazole or ketoconazole [9]. Here, the levels of terfenadine (struggling for the same CYP3A4) in patients exceeded the therapeutic level causing malignant arrythmia. The most striking example is the recent withdrawal of antihypertensive drug mibefradil from the market because of pharmacokinetic interactions with other drugs [10].

The sole fact that the CYP3A4 and its closely related form CYP3A5 are responsible for the majority of metabolic pathways of drugs (where the metabolism is known), being also the most abundant forms in human liver (from 30 to 60% of total CYP in the human liver, depending on many factors, as genetics, food etc., not fully understood yet) makes the CYP3A4 the most important CYP enzyme [11]. Hence, all its substrates may compete and the risk of drug interactions is possible. Among typical substrates which are strong inhibitors of this system, are already mentioned azole antifungals, macrolide antibiotics (not all of them – *e.g.* azithromycin is not an inhibitor), gestodene, ethynylestradiol, or substrates as statins, dihydropyridine calcium channel blockers. The other CYP enzymes taking part in drug metabolism are CYP2D6 (metabolizing *e.g.* beta blockers, many selective inhibitors of serotonin reuptake as fluoxetin or paroxetin, and tricyclic antidepressives), CYP2C9 (with substrates as S-warfarin, nonsteroid anti inflammatory agents as ibuprofen, peroral antiabetics), CYP2C19 (diazepam, tricyclic antidepresives as amitriptyline, imipramine, antiulcerotic drugs omeprazole and lansoprazole), CYP2E1 (with typical substrates paracetamol and inhalation anesthetics as halothane, and organic solvents as ethanol, acetone, acetonitrile, nitrosamines), CYP1A2 metabolizing theophylline, caffeine, clozapine, tacrine. Most drugs are metabolized by various ways in which more CYP enzymes take part. It is not the only aim of this minireview to list all the drug substrates of respective CYP enzymes, detailed information on the extent of the involvement of various CYPs in drug metabolism is given in paper of Bertz and Granneman [12], various information on the drug interactions is accessible through the web site [5]. Human CYP enzymes were reviewed in paper of Guengerich [13], a comprehensive review of drug metabolizing enzymes is given in [2].

Experimental animal models of drug metabolism

In the experimental pharmacology, there is an urgent need to understand the molecular mechanisms of drug metabolism. Not only the old drugs should be tested in more detail to avoid (and explain) unwanted reactions supposed to appear because of drug-drug interactions, but also the regulatory agencies demand this information when a new drug is entering the process of registration. Therefore, the experimental models are useful. These models are based mostly on animal liver microsomal CYP-containing systems or on recombinant human enzymes either expressed in viral or microbial host systems. Here, the comparisons of various animals will be briefly given to help the researchers to choose an appropriate animal-based model. Special reason for performing a detailed inter-species comparison holds for the minipig or pig: there is a growing chance that the pig organs or tissues, namely, from transgenic animals will be used in human therapy either for xenotransplantation as a source of tissues or cells for bioartificial organs [14]. The comparisons presented here are based on the available data and are grouped for each animal species. An alternative approach is to follow the individual CYP families, this approach has been used *e.g.* in systematic review of Guengerich [15] or in an older work of Smith [16].

Table 2. The main human liver microsomal P450 enzymes and their possible experimental models.

CYP	Rel. content in human liver (%)	Estim. fraction of drugs metabolized by indiv. CYP	Marker activity	Model system
1A2	12	4 %	caffeine	rat, rabbit, pig, minipig
2C9/10/19	20	11 %	diclofenac (2C9), (S)-mephenytoin (2C19)	monkey (*Maccacus mulatta*)
2D6	4	30 %	sparteine, debrisoquine, dextromethorphan	dog
2E1	6	2 %	chlorzoxazone	rat, rabbit, pig, minipig
3A4	30	52 %	nifedipine, erytromycin, alprazolam, dextrometorphan	pig, minipig

Rat

Cytochromes P450s from rats were one of the first isolated and characterized. In fact, one of the oldest known CYP enzymes is rat form $P450_d$ which has been shown to be similar to human counterparts. It is now named as the CYP1A2 enzyme known as conserved throughout the species with typical substrates as aromatic structures, preferably aromatic amines, but also polycyclic aromatic hydrocarbons and other planar structures. This form is inducible by polycyclic aromatic hydrocarbons as 3-methylcholanthrene [13] or by polychlorinated biphenyls [15]. Similar conclusion holds for the CYP2E1, which is in all species known inducible by ethanol, acetone and metabolizes organic solvents, nitrosamines and several drugs, as *e.g.* paracetamol (see above) [2,13,17]. To conclude, the rat may serve as a readily available model for liver microsomal metabolism dependent on these two CYP forms.

Unfortunately, rat is not a good model of metabolism dependent on the most important human CYP, namely, CYP3A4. The rat orthologous CYP3A1 (the main CYP3A form in the rat) is not induced by a typical CYP3A inducer rifampicine [18], and, which is much more important, many prototypical substrates of human CYP3A enzymes as dihydropyridine calcium channel blockers (*e.g.* nifedipine) are not metabolized by rat CYP3A1 or by other rat CYP3A forms [15,16]. The most abundant CYP subfamily of rat liver is the CYP2C having a role of human CYP3A enzymes – which is supported by the fact that not only the oxidation of dihydropyridines as well as of the aflatoxin B_1, but also hydroxylations of steroids are performed by rat 2C enzymes [19]. Another CYP enzyme important for drug metabolism, CYP2D6 (contributing to approx. 30%), is represented in rats by an orthologous CYP2D1 enzyme. Despite that the substrate specificities of the CYP2D6 and of the CYP2D1 are close, a specific inhibitor of human CYP2D6, quinidine, does not function well in the case of CYP2D1, but its stereoisomer quinine, is a potent inhibitor in this case [19, 20]. Also, one of the marker substrates of CYP2D6, dextro-methorphan, is metabolized specifically by another rat CYP2D enzyme, CYP2D2 [21]. There are also probably significant differences in mechanism of induction of the CYP enzymes as the rat CYP2D1 enzyme is inducible by 3-methylcholanthrene and phenobarbital [22], however, the human CYP2D6 is known not to be inducible and 3-methylcholanthrene induces rather human CYP1A2 [23] and phenobarbital is a classical inducer of human CYP3A, CYP2C and CYP2B forms [13].

Rabbit

Rabbit CYP enzymes were the first mammalian P450 isolated and characterized [24].
The rabbit CYP form LM4 is the CYP1A2 enzyme

and corresponds well to its human counterpart, and the same is true also for rabbit and human CYP2E1. Interestingly, a CYP2E2 enzyme is expressed in very young animals having almost identical structure and substrate specificity [25]. The second well-known rabbit liver microsomal form was the LM2, now CYP2B4. It has been widely used in many studies of CYP properties as the yields after induction by phenobarbital were very good. The human orthologue CYP2B6 is not widely expressed, however, its importance may increase as it has been found to activate cancerostatic agents as *e.g.* cyclophosphamide [26]. Contrary to human P450s, the rabbit 2C subfamily seems to be the most important, again (as it has been with the rat) taking a role of human CYP3A forms. It involves the main steroid hydroxylases (e.g. the testosterone 6β-hydroxylase is the rabbit CYP2C3) instead of the rabbit 3A6 [27].

Unfortunately, also the human CYP2D6 enzyme does not have a good partner between rabbit CYP forms. Genes of two rabbit CYP2D forms (CYP2D23 and 2D24) have been found [28], however, the proteins were not fully characterized yet. Hence, in the rabbit, the metabolism of one third of drugs used in human medicine involves other forms of CYP enzymes. Taking together, different properties of rabbit CYP3A, 2C and marginal importance of a CYP2D enzyme(s) exclude rabbits models from pharmacological studies.

Dog

Dogs (Beagle) are not often used as experimental animals, apparently from both ethical as well as rational reasons. The CYP1A enzymes seem to be somewhat different from the human ones. The antibodies against human CYP1A were shown to influence in dog microsomes the 6-hydroxylation of chlorzoxazone - a typical activity of CYP2E1 [29]. Moreover, beta naphthoflavone, a typical inducer of CYP1A forms in dog, has been shown to induce the same activity in dog microsomes [30]. Hence, the CYP1A and 2E1 forms in dog are not good models of the respective human CYP enzymes. Two CYP3A forms are expressed in dog, CYP3A12 and CYP3A26. However, dexamethasone, a typical inducer of 3A enzymes, does not induce these forms in dog [18, 30]. CYP2D15 is the major CYP form in dog with enzymatic activities similar to the human CYP2D6 [31]. This form seems to be a good model for human CYP2D6 enzyme justifying the use of dogs in studies based on metabolism mediated by this enzyme. The CYP2C21 and less expressed CYP2C41 were characterized form dog liver with activities not thoroughly examined, seemingly with very low tolbutamide hydroxylase activity [32]. Interestingly, the dog is the only mammalian species able to metabolize polycyclic aromatic hydrocarbons through its CYP2B11 enzyme [15].

Monkey

Macaca (*Maccacus rhesus*, Cynomolgus) and marmoset monkeys are the two most often used monkey species used as experimental animals. The CYP1A enzymes may differ in their activities between colonies of the same species [33], the CYP1A2 being less expressed in cynomolgus monkey than in the marmoset one [34]. CYP2E1 activities in liver microsomes seem to be similar to human CYP2E1 ones [33], however, inducibility of this enzyme by 3-methylcholanthrene (a typical inducer of the CYP1A forms) indicate significant differences in the mechanism of induction [35]. Unfortunately, also the 3A8 enzymes of cynomolgus monkey were shown in this work to have the same inducer. The monkey 2C enzymes (CYP2C20 has been sequenced) seem to be a good model for their human counterparts at least according to the microsomal activities, especially, in *Maccacus rhesus* [33]. If no significant differences are found in the future, the monkeys will become the only good animal models for metabolism mediated by CYP2C.

Minipig, pig

All the main activities typical for human CYP enzymes were found in liver microsomes of pigs and minipigs [36-38]. Many CYP forms were sequenced, however, only some of them were investigated in detail. The CYP1A forms of minipig or pig (named here collectively as (mini)pig) were not sequenced yet, however, their presence is now confirmed and the classical properties as *e.g.*

induction by beta naphthoflavone or polychlorinated biphenyls were confirmed as well [18, 39]. The pig CYP2E1 sequence is accessible in the GenBank, and the typical chlorzoxazone 6-hydroxylase activity was found, as well as the respective protein, by Western blotting in liver microsomes [38]. The CYP2D25 shares 75% sequence homology with human CYP2D6, however, the Western blotting gave controversial results [39] as well as the reports on low or absent debrisoquine activity indicate that the CYP2D form(s) of (mini)pigs may be different from the human counterpart [37, 38, 41]. (Mini)pigs apparently express many CYP2C forms whose sequences may be found in data banks (CYP2C32, 33, 34, 35, 36, 42, cf. *e.g.* [5]), however, detailed studies are still lacking [42]. Two CYP3A enzymes were found (3A29 and 3A39), the former seems to be the main form present both in (mini)pigs [43]. Their CYP enzymes do not differ significantly in their primary structures justifying the collective labeling (mini)pig used in the literature [43]. The isolated CYP3A29 form possesses a typical nifedipine oxidase activity (characteristic for human CYP3A4 and 3A5), it is inducible by phenobarbital, rifampicine and possibly also by dexamethasone [38,39]. Similarity of the primary structure of CYP3A29 and human CYP3A4 is reflected in the fact that pig liver microsomes and hepatocytes were reported to be good experimental model for drug metabolism mediated by human CYP3A enzymes [36-38, 43-45].

Conclusion

For the development of new drugs, evaluation of drug-drug interactions with already known compounds, as well as for better understanding of metabolism pathways of various toxicants and pollutants, we studied the drug metabolism mediated by cytochromes P450. The experimental approach is based on animal drug-metabolising systems. From the ethical as well as rational reasons, the selection of an appropriate system is crucial. Here, it is necessary to decide on the basis of expected CYP system involved. For CYP1A-mediated pathways, all the commonly used experimental models are appropriate except probably the dog. On the contrary, the dog seems to be suitable for modeling of processes dependent on the CYP2D. For CYP2C, which is possibly the most large and complicated subfamily, the systems based on monkey (*Maccacus rhesus*) may be a good alternative. The CYP3A seems to be well modeled by pig or minipig CYP3A29. Detailed studies on activities of individual isolated CYP forms are needed to understand in detail the inter-species differences and variations.

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