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Review

Cytochromes P450 and metabolism of xenobiotics

P. Anzenbacher* and E. Anzenbacherová

Institute of Pharmacology and Institute of Medical Chemistry and Biochemistry, Faculty of Medicine, Palacký University, Hněvotínská 3, 775 15 Olomouc (Czech Republic), e-mail: anzen@tunw.upol.cz

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Abstract. Cytochromes P450 (henceforth P450s) are involved in a variety of metabolic and biosynthetic processes. The number of known P450 enzymes exceeds 1000, while the endogenous substrates of most of them remain unknown. All P450 enzymes exhibit similarity in their structure and general mechanism of action; however, there are significant differences in the detailed function of individual enzymes as well as in the structures and properties of their active sites. This review discusses the properties of the most important P450 enzymes taking part in drug metabolism in humans. P450 3A4 is of paramount importance, because it is the most abundant P450 in the human liver and is known to metabolize the majority of drugs whose biotransformation is known. Genetically dependent variabilities of individual P450 activities and levels are described, documenting the importance of pharmacogenetics aimed at explaining differences in the response of the organism to various drugs.

Key words. Cytochromes P450; CYP; drug metabolism; xenobiotic.

Introduction

Cytochromes P450 (henceforth P450s) constitute a superfamily of heme enzymes found from bacteria to humans [1]. It is reasonable to suppose that a P450 enzyme is present in every living species on Earth, as this enzyme has been found in archaebacteria, in plants, and in various animal species. The number of P450s with known sequences, either as nucleotide sequences in GenBank or amino acid sequences from the SwissProt databases, approaches 1200. A specialized P450 database has been constructed recently and is available online [2]. Since 1991 [3], P450s (and their respective genes) are named as CYPs, the abbreviation CYP being followed by an Arabic numeral which expresses the family number, e.g. CYP21. This number may be associated with the function of the enzyme (here, the enzyme acts as a steroid 21-hydroxylase), or it may have been chosen rather arbitrarily. The P450s constituting a family should have similar sequences in their overlapping portions – the limit is 40%. If a family needs to be divided further, a subfamily is created based on higher degree of sequence similarity (here 55%); the subfamilies within one family are labeled sequentially as, e.g., CYP3A, CYP3B and so on. Individual members of a family or subfamily are labeled again by Arabic numerals (e.g., CYP3A4, CYP3A7). To represent an individual member, a new P450 sequence should differ by more than 3%.

In this review, individual P450s will be named as enzymes with CYP abbreviations (e.g., CYP3A4) and not as isoenzymes or isoforms; the labeling used will follow the most recent recommendations [1]. Only in cases where an old enzyme name is more familiar than the systematic one, will both names be used [e.g., for the P450 BM3 (CYP102)].

The reason for a different number of P450 enzymes in various species apparently reflects the need for the respective function: Whereas in bacteria, the number of P450s is up to 20, in humans about 60 are expected. In plants, the total number of P450s in one species has been esti-

* Corresponding author.

mated to be over 300, as recent results with the genome of *Arabidopsis thaliana* indicate [4]. Plants are believed to need their P450s for mainly two reasons – for the synthesis of pigments and growth regulators, and for the synthesis of plant toxins. Plants appear to fight a 'chemical war' as they cannot, in contrast to animals, save their lives by escape or by the use of specialized shielding structures.

Most attention has been focused on human P450s. Various P450 enzymes are involved in the biosynthesis of low-molecular-weight compounds acting as regulators at various levels and in different processes in the human organism, such as steroids, prostaglandins, thromboxanes, fatty acid derivatives and derivatives of retinoic acid [5].

A classical example is the involvement of CYP enzymes in the biosynthesis of various steroid hormones. The first step in the biotransformation of cholesterol, i.e., the cleavage of its side chain, is catalyzed by a P450 enzyme (CYP11A1, formerly called as P450scc for side chain cleavage). Other P450s which act in various steps of steroidogenesis belong to families or subfamilies with numbers expressing the site of their action, e.g., CYP11B, CYP17, and CYP21. Another example may be a recently discovered endogenous epithelial relaxation factor in coronary arteries, a derivative of arachidonic acid, which is formed by the P450 enzyme CYP2C10 [6]. However, in many cases, the endogenous substrate of a given P450 enzyme is not known.

This review focuses mainly on human P450s and the metabolism of xenobiotics, mostly drugs. We begin by discussing the similarities and differences between P450 enzymes, and then go on to describe the properties of individual human P450 enzymes most important for drug metabolism.

P450 enzymes: similarities and differences

Let us first try to identify the common properties of all P450 enzymes.

The most important property of all known P450s is their ability to bind and activate two atoms of oxygen. In most cases, it is the dioxygen molecule; examples of exceptions, described in detail in the literature, are thromboxane synthase (CYP5) and allene oxide synthase [7]. In both cases, the heme iron atom binds two atoms of oxygen from the substrate molecule – which is a peroxide. In the case of thromboxane synthase, the substrate is the prostaglandin H_2 which undergoes a rearrangement to thromboxane A_2; in the latter case, it is the hydroperoxylinoleic acid rearranged further to the allene oxide derivative. On the other hand, all P450s can bind hydrogen peroxide or peroxides and utilize one of their oxygen atoms for monooxygenations. Hence, most P450-catalyzed reactions may be expressed in a simple scheme (fig. 1).

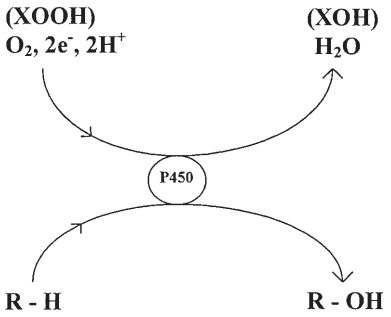

Figure 1. Scheme of P450 reactions. R–H, substrate; R–OH, hydroxylated product; XOOH, peroxide (X = H or organic residue); XOH, hydroxylated by-product.

The ability of all P450s to activate the Fe–O–O moiety (universal for all P450s) is determined by the mode by which the heme (here, heme *b*) is bound to apoprotein. The central atom of the heme macrocycle, i.e., the heme iron, is bound to the protein through the anionic, thiolate sulfur of a cysteine residue. This type of bond gives the heme moiety the ability to mediate the transfer of electron density to the dioxygen bound as the trans (sixth) ligand of the heme iron [8]. Hence, this bond also explains why the heme *b* (the same as in the well-known hemoproteins, hemoglobin and myoglobin) possesses here this unique property characteristic of all P450s, namely, the ability to activate the dioxygen for chemical reactions. The same type of bond (i.e., the heme-thiolate) has been found in chloroperoxidase and nitric oxide synthase (NOS) [7]. The NOSs are in fact very closely related to P450s [9]; however, more for historical than structural reasons they are not accepted in the P450 superfamily.

In the earlier literature, the P450 enzymes were according to recommendation of the International Union of Biochemistry, named as 'heme-thiolate proteins' to stress this fact and to avoid the unhappy label with '450' (expressing the position of the Soret maximum in the absorption spectrum of the complex of reduced P450 with carbon monoxide at 450 nm, which is characteristic for these heme enzymes as it is also determined by the thiolate bond). However, as this name was found to be misleading, because chloroperoxidases and NOS were found to be heme-thiolate proteins as well, the name 'heme-thiolate protein' for P450 enzymes has been completely abandoned.

The third common property (with the ability to activate the O–O bond as the first, and with the thiolate bond to the heme iron as the second characteristic property) of all P450 enzymes is their similar overall structure and shape. In other words, most of the secondary-structure elements observed in P450s as well as the overall folding pattern are conserved. To data, five structures of soluble microbial P450s are known [10] and one structure of the mam-

malian microsomal enzyme, rabbit CYP2C5 [11]. One of the reasons why the structure of the membrane-bound P450s is similar to that of the soluble ones is apparently the fact that there are no more defined membrane anchor domains in the microsomal P450 than just one at the very end of the N terminus; the association of P450s with the endoplasmatic reticulum also involves a hydrophobic protein surface formed by noncontiguous portions of the polypeptide chain [11].

Differences between P450s may be divided – an observer's viewpoint – into expected and unexpected ones. Among the unexpected ones, differences in the mechanism of enzyme reaction may be listed together with the observed effect of other substrates or effectors. The classical scheme (fig. 2) involves sequentially binding of substrate (RH) followed by conformational change leading to easier reduction of the heme iron; binding of an oxygen molecule should then take place followed by further reduction. However, recent studies have shown that the reduction may well happen even before the substrate is bound, depending on many factors, e.g., the state of aggregation, the interaction of P450s and their respective electron-donating reductases, and the particular P450 enzyme involved [12]. Simultaneous binding of more substrate molecules and their influence on the enzymatic reaction of P450s leading to sigmoidal kinetics has also been intensively studied, indicating that this property may not be limited to enzymes of the CYP3A and CYP2C family [13].

The expected differences are ultimately connected with the function of the individual P450s. Organisms apparently need to cope with many substrates and have been forced to utilize new substances to synthetize the correct molecules to regulate their metabolism, hence the appearance of new P450 species. Thus, the variability in substrate specificity, reflected in the variability of properties of the P450 active sites, is enormous. As the main focus of this review are the human drug-metabolizing P450s, the enzymes taking part in biotransformation and biosynthesis of endogenous compounds will not be included.

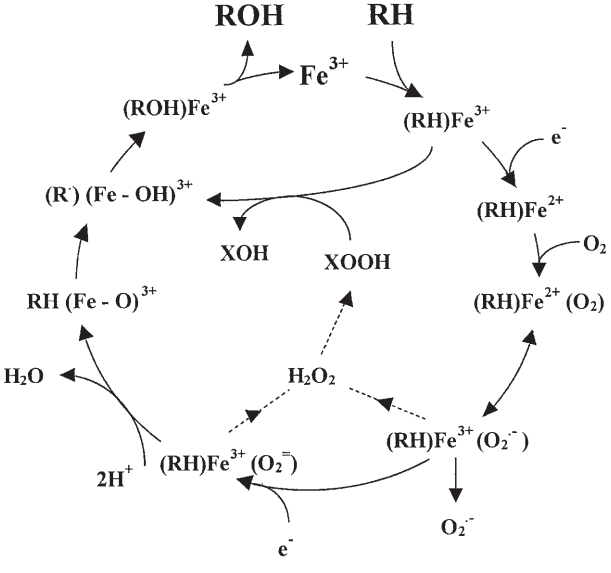

Figure 2. Catalytic cycle of P450 enzymes (symbols as in fig. 1).

In principle there are two approches to studying the active site of an enzyme whose crystal structure has not yet been resolved: (i) theoretical ones, taking advantage of the computer-aided comparison of structures, and (ii) experimental approaches, using mainly spectroscopic and molecular biology (site-directed mutagenesis) methods. Examples of the latter approach will be discussed for individual CYP enzymes below.

The first approach was recently reviewed in this journal [14]. The substrate recognition properties of several P450s are consistent with recently developed working models of P450 active sites. Most recently, Lewis [15] has pursued an inverse approach utilizing the structures of characteristic substrates of several P450s. Molecular templates of superimposed substrates have been shown to be complementary with the putative active site of the relevant enzymes, thus enabling possible prediction of P450 specificity from structure.

The active sites of P450s also differ in their stability to denaturation and their flexibility. On the basis of spectroscopic experiments realized under hydrostatic pressure or at different temperatures, the flexibility and stability of several P450 enzymes have been evaluated [16]. CYP1A2 possesses a stable conformation [17] and its active site seems to be rather inflexible (incompressible), while CYP3A4 denatures easily and its active site is more flexible. The bacterial, soluble CYP102 (BM3), whose crystal structure is known and which has served as a model for structural comparisons, is very stable against denaturation; however, its active site is flexible and compressible [16]. Hence, the flexibility of the active site is apparently not directly related to the ease with which the enzyme may denature, which seems to be property of the molecule as a whole.

Human drug-metabolizing P450 enzmyes

Human drug-metabolizing P450 enzymes are present, throughout the body. An overview of P450s which may take part in drug metabolism is presented in table 1. Formerly, the P450 enzymes were thought to be localized only in the liver and, as a minor fraction, in several other tissues where their role was confirmed [e.g. CYP11A1 (also P450scc) in the adrenals, with a known role in splitting the side chain of cholesterol]. As can be seen from the table 1, some P450s have are nearly ubiquitous distribution (3A4, 2D6, 1A1, 1B1, 2E1), among which, CYP3A4 seems to be the most important. On the other hand, this

Table 1. Human P450 enzymes metabolizing xenobiotics and drugs [2, 19, 20].

CYP	Localization	Typical substrate	Typical inductor
1A1	lung, liver,brain, GIT, lymphocytes, heart	PAH (polycycl. arom.hydrocarbons)	PAH, dioxins
1A2	liver	aromatic amines, PAH, caffeine	PAH, β-naphthoflavone, smoking
1B1	skin, brain, heart, lung, placenta, liver, kidney, GIT, spleen	PAH	dioxin
2A6	liver	coumarin, steroids	barbiturates, dexamethasone
2B1/2	brain	morphine	nicotine
2B6	liver, heart	nicotine	barbiturates
2C8	liver, kidney	retinoids, taxol	?
2C9/10	liver	tolbutamide, diclofenac	barbiturates, rifampicin
2C19	liver, heart	(S)-mephenytoin, omeprazole, diazepam	barbiturates, rifampicin
2D6	liver, brain, heart	antidepressives, β-blockers	–
2E1	liver, lung, brain, endothelium, heart, bone marrow	ethanol, nitrosamines, acetaminophen	ethanol, starvation
2F	lung	coumarins	?
3A4/5	liver, GIT, kidney, lung, brain, endothelium, placenta, lymphocytes	different – Ca channel blockers, cyclosporin, acetaminophen, taxol, steroids	steroids, barbiturates
3A7	fetus, placenta, (liver)	various, similar to 3A4	steroids, barbiturates
4A9/11	kidney	fatty acids	clofibrate
4B1	lung, placenta,	?	?
4F2/3	kidney	arachidonic acid derivatives	?

GIT, gastrointestinal tract; PAH, polycyclic aromatic hydrocarbons.

particular enzyme is not present in the heart, whereas CYP2D6, known to metabolize β-blocking agents, is present in the right ventricle and CYP2E1 is present in the regions of the human heart and in the major vessels [18]. This fact may be of paramount importance for targeting drugs to desired areas of the heart. The following sections will discuss individual P450 enzymes taking part in drug metabolism in the order of their importance.

A survey of liver P450 enzymes which are responsible for metabolic transformations of drugs is given in table 2. For each enzyme, its relative content in the (uninduced) liver is given together with the variability of its activity in liver microsomal samples. Genetic polymorphism is one of the most important factors contributing to the variability of individual levels of CYP enzymes. In general, alleles causing either defective, qualitatively altered, lowered, or enhanced activities of P450 enzymes have been identified for many CYPs. In fact, all the liver microsomal CYP enzymes may reasonably be expected to be polymorphic; the only case where polymorphism has not yet been confirmed is CYP2B6 whose levels in human liver are very low. The other major factors contributing to the variability in activities are enzyme induction [25, 26] and inhibition [25] (including diet [27]), age (showing a decrease in drug metabolism with age in both sexes) [28], and health status [29, 30]. The third column gives an estimate of the fraction of drugs of known metabolism which are biotransformed by a given P450 enzyme. The last co-

Table 2. Human liver microsomal P450 enzymes [19–24].

CYP	Relative content (%)	Variability	Estimate fraction of drugs metabolized by the P450	Marker activity
1A1	1	100	< 1%	none
1A2	12	40	4%	caffeine
2A6	4	30	< 1%	none
2B6	1	50	< 1%	none
2C9/10/19	20	20	11%	diclofenac (2C9) (S)-mephenytoin (2C19)
2D6	4	1000	30%	sparteine, debrisoquine, dextromethorphan
2E1	6	20	2%	chlorzoxazone
3A4	30	60	52%	nifedipine, erythromycin, alprazolam, dextrometorphan

lumn lists compounds used as probe drugs to estimate the in vivo activity of a particular isoform.

CYP3A4

This is apparently the most important P450 enzyme for drug metabolism in humans. This is not only because of its amount in the liver (which may be increased by induction to more than 60%) but mainly because it participates in the metabolism of the majority of drugs with known metabolic pathways. Unfortunately, it is not only a rather unstable enzyme with a complicated mechanism of action, which makes in vitro studies difficult, but it is also a P450 enzyme for which a suitable probe drug (in vivo 'marker' activity) has not yet been found [19, 24, 31]. Among the most recent candidates, the antianxiolytic drug alprazolam seems to be the most suitable [24]. CYP3A4 activity, as estimated from the levels of a known endogenous metabolic product, 6β-hydroxycortisol, relative to levels of cortisol as the substrate, changes during the day, with a maximum in the evening between 17 and 21 hours [32].

The spectrum of drugs known to be metabolized at least partly by CYP3A4 is extremely broad ranging from taxol to sildenafil (Viagra) [33]. This fact leads to the undesired possibility of drug interactions which may lead to extremely elevated or diminished levels of one of the interacting drugs. For example, simultaneous administration of a drug which blocks CYP3A4 (e.g., an azole antifungal) together with another drug with a relatively narrow therapeutic window (i.e., a drug which should be given in a relatively narrow range of dosages to avoid health risks) can lead to levels of the second drug approaching or exceeding the limits of safety. This was apparently the case for azole antifungals with terfenadine or cisapride. In both cases, increased levels of terfenadine or cisapride were documented to cause life-threatening heart arrythmias [31, 34]. Another possibility is that the first drug causes an induction of CYP3A4 activity which then metabolizes the second drug so efficiently that it is not available in the amount or level desired. Interaction of some anticonvulsants (a CYP3A4 inductors) with contraceptives containing etinylestradiol (CYP3A4 substrate) has been shown to cause 'pill failure' apparently because of this effect [35]. Among drugs which are taken as 'classical' CYP3A4 substrates with great interacting potential are calcium channel blockers of dihydropyridine structure (e.g., nifedipine), most of the macrolide antibiotics (e.g., erythromycin), and azole antifungals (e.g., clotrimazole, ketoconazole). The latter group, azole antifungals, are the most potent inhibitors of CYP3A activity with apparent inhibition constants in the micromolar range [31, 38]. Table 3 summarizes known drug substrates of this P450 enzyme and compounds (drugs) which are known to bind to it and to cause drug interactions.

Table 3. Drugs which are known to be substrates of (or to interact with) the CYP3A4 enzyme.

Acetaminophen (paracetamol)	Losartan
Alfentanil	Lovastatin
Alpidem	Meloxicam
Alprazolam	Methadone
Ambroxol	Mibefradil
Amitriptyline	Mifepristone
Astemizole	N-hydroxyarginine
Atorvastatin	Nevaripine
Benzphetamine	Nicardipine
Bupivacaine	Nifedipine
Brotizolam	Niludipine
Budesonide	Nimodipine
Buprenorphine	Nisoldipine
Carbamazepine	Nitrendipine
Citalopram	Omeprazole
Cisapride	Oxodipine
Clarithromycin	Paclitaxel (Taxol)
Clozapine	Pantoprazole
Codeine	Progesterone
Colchicine	Propafenone
Cortisol	Proquanil
Cyclobenzaprine	Quinidine
Cyclophosphamide	Rapamycin
Cyclosporin A , G	Retinoic acid (Tretinoin)
Dapsone	Rifabutin
Dehydroepiandrosterone	Ritonavir
Delaviridine	Ropivacain
Dextromethorphan	Sameterol
Diazepam	Sequenavir
Digitoxin	Sertindole
Diltiazem	Simvastatin
Docetaxel	Sulfamethoxazole
17β-estradiol	Sulfentanil
Erythromycin	Tacrolimus
Ethinylestradiol	Tamoxifen
Ethylmorphine	Teniposide
Etoposide	Terfenadine
Felodipine	Terguride
Fentanyl	Terbinafine
Finasteride	Testosterone
Flutamide	Tetrahydrocannabinol
Gallopamil	Theophylline
Gestodene	Tolterodine
Granisetrone	Triazolam
Haloperidol	Trimethadone
Hypericum extract	Troglitazone
Ifosphamide	Troleandomycin
Imipramine	Verapamil
Indinavir	Vinblastine
Irinotecan	Warfarin (R-)
Ivermectin	Zatosetron
Lansoprazole	Zonisamide
Lidocaine	Zopiclone
Lisuride	
Loratidine	

Data taken from refs. 36 and 37 and original literature.

Interactions are not only observed with drugs: constituents of the diet may also take part in drug interactions. An example of this effect is the well-known inhibitory action of grapefruit juice, which was discovered rather serendipitously [39]. In this case, the in vivo effect can be

quite dramatic with a single glass of grapefruit juice resulting in fivefold increases in the values of the main pharmacokinetic parameters such as the C_{max} (maximal level) and AUC (area under pharmacokinetic curve) for dihydropyridine beta-blocking agents (e.g., nifedipine). The molecular basis is apparently the inhibition of CYP3A4 (possibly the intestinal one) by flavonoids present in grapefruit juice [40].

The enormous variability of substrates for CYP3A4 together with the paramount importance of this enzyme has been reflected in attempts to understand the structure of its active site. CYP3A4 has been subjected to site-directed mutagenesis together with homology modeling which has led to the identification of residues 210–211 and 360–375 as constituents of the active site [41, 42]. One of the keys to elucidation of the broad substrate specificity of this enzyme may be the recent finding of more, possibly three, substrate binding sites [43]. Hence, the active site seems to be a rather spatious part of the molecule which confirms earlier suggestions [19] as well as results of studies on its flexibility [16].

The variability of CYP3A4 activities in liver samples has been studied extensively to assess the genetic contribution to this effect [44]. Levels of CYP3A4 activity and expression vary up to 60-fold [19, 22, 44], and understanding the basis of this marked variability is critical for individualized treatment with CYP3A4 drug substrates and may help to forecast drug-drug interactions mediated by this enzyme. Although several CYP3A4 alleles have been recently reported [45], the responsibility of a particular allele (or alleles) for this variability has not yet been established. Statistical analyses of data on the administration of CYP3A4 substrates were reviewed to evaluate the genetic component of variability in CYP3A4 activity, and showed that at least 60% or more of the variability is under genetic control [44]. Hence, further molecular genetic investigations are warranted to identify the genetic loci at the CYP3A4 gene or elsewhere in the genome which contribute to regulation of CYP3A4 activity.

CYP1A2

This enzyme, for a long time known as 'P-448' was first characterized in rat liver microsomes after induction with methylcholanthrene [46]. In earlier models, it was believed to be the 'bad' P450 responsible for unwanted effects such as activation of polycyclic aromatic hydrocarbons and other carcinogens and toxicants. However, there are two members of the CYP1A subfamily in humans: CYP1A1 and CYP1A2 [19]. The former is the true benzo(a)pyrene hydroxylase, which is, however, localized mostly in extrahepatic tissues being present in the liver in significant amounts only after induction, e.g., by smoking. CYP1A2 is mainly a hepatic enzyme with substrates as aromatic amines, polycyclic aromatic hydrocarbons; among drugs metabolized preferentially by this enzyme are acetaminophen, caffeine, clozapine, phenacetin, tacrine, and theophylline. Most of the drugs are also substrates of other CYP enzymes, as the metabolic pathways are in most cases complicated, involving the formation of more products or alternative routes. This is why CYP1A2 substrates include drugs such as fluvoxamine, imipramine, clomipramine, naproxen, ondansetron, propafenone, terbinafine, verapamil, and (R)-warfarin.

Levels of CYP1A2 are known to be variable depending on induction by known factors (see table 2) or diet. For example, brassica vegetables increase and apiaceous vegetables decrease CYP1A2 activity [47]. In another study, charcoal-grilled hamburgers (150 g beef, 15–20% fat, 10 min grilled on each side, consumed once daily for 5 days) caused an increase of CYP1A2 activity by 14.1% ($P<0.05$) [48]. A role of CYP1A2 in chemical carcinogenesis is recognized – heterocyclic and aromatic amines, certain nitroaromatic compounds or aflatoxin B_1 (which may, however, also be present in contaminated food) belong to substances activated by this enzyme [49].

The CYP1A2 enzyme is known to be genetically polymorphic [45]; one allele has been shown to be connected with decreased in vivo activity. However, detailed study of CYP1A2 phenotyping is needed, because a functionally significant polymorphism has not yet been confirmed [50]. An interesting question is the physiological role of this enzyme [51], as it tends to be expressed constitutively in mammals.

This CYP protein is not only conserved among species, but is also rather stable against denaturation [16, 17]. Its active site is able to accommodate planar molecules of moderate volume and basicity [15].

CYP2D6

CYP2D6 is possibly the most popular cytochrome P450 among physicians and other health professionals, because of its genetic polymorphism (causing the presence of three main phenotypes of oxidative metabolism of drug substrates of this enzyme) [53]. These three phenotypes are classified as the slow metabolizers (with defective CYP2D6 alleles), the extensive (or rapid) metabolizers (with the wild-type allele or with alleles having nucleotide changes not causing altered activity of CYP2D6), and the ultrarapid ones with multiple genes for the functional CYP2D6 enzyme [54]. Figure 3 documents the differences in the levels of a drug in human plasma caused by the poor-metabolizer phenotype; the volunteers were given propafenone as a probe drug [55]. The most frequently used probe drugs for determining CYP2D6 phenotype are sparteine, debrisoquine, and dextromethorphan (see also table 2). Extensive studies have shown that in the Caucasian population, there are about 7% de-

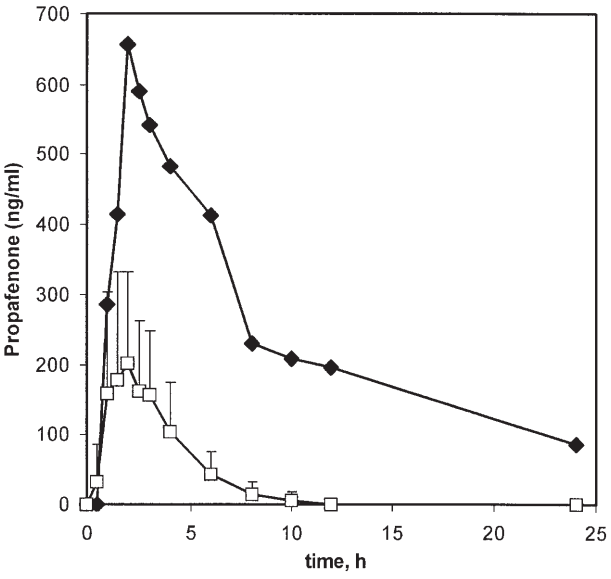

Figure 3. Time dependence of propafenone in human blood plasma. Data for a poor metabolizer (◆) and for extensive metabolizers (□, errorr bars, SEM, N = 13).

Table 4. Substrates (drugs) of the CYP2D6 enzyme.

Ajmaline	Hydrocodone
Amitriptyline	Imipramine
Bufuralol	Methoxyamphetamine
Bupranolol	Metoprolol
Captopril	Mexilitine
Cinnarizine	Mianserin
Citalopram	Nortriptyline
Clomipramine	Ondansetron
Chlorpromazine	Paroxetine
Codeine	Perhexiline
Debrisoquine	Perphenazine
Deprenyl	Propafenone
Desipramine	Propranolol
Dextromethorphan	Risperidone
Dexfenfluramine	Sparteine
Encainide	Thioridazine
Flecainide	Timolol
Fluoxetin	Tramadol
Fluvoxamine	Trifluperidol
Flunarizine	Trimepranol
Fluphenazin	Tropisetron
Galanthamine	Tomoxetine
Haloperidol	Venflaxine

Data from refs. 19, 37, and 54 and original literature.

fective CYP2D6 genes, whereas in the Asian population the frequence of defective alleles is almost one-half [54]. The total number of CYP2D6 alleles known to date approaches 80 [45]. The CYP2D6 poor-metabolizer phenotype is associated with Parkinson's disease [56]; on the other hand, this phenotype should protect against urinary bladder cancer [57] and possibly also against development of lung carcinoma [58].

For CYP2D6, variability of activities in human liver samples may well be ascribed to genetic polymorphism as CYP2D6 is not inducible. The impact of decreased activity of CYP2D6 on drug treatment may be extremely important, because elevated levels of tricyclic antidepressants may lead to cardiotoxic effects, in the case of inhibitors of selective reuptake of serotonin it may lead to nausea, and with antiarrythmics, undesired and life-threatening arrythmias may develop [54]. The list of CYP2D6 substrates (table 4) is rather representative; principal substrates are β-adrenoreceptor blockers and tricyclic antidepressants.

The substrates of the CYP2D6 enzyme are known to possess a basic atom (nitrogen), which has led to speculations on the presence of a carboxyl residue in the active site. The substrates are mostly rather hydrophilic [15, 52]. Modeling of the active site and site-directed mutagenesis studies favor the residues 301 (Asp) and 374 (Val or Met) as parts of the substrate-binding site [59].

CYP2C

This subfamily of CYP enzymes comprises four main members: CYP2C8, 2C9, 2C10, and 2C19. The first enzyme does not seem to be very important for drug metabolism, as only retinoic acid (Tretinoin), warfarin, and taxol (paclitaxel) have been shown to be its substrates. On the other hand, CYP2C9 and 2C19 metabolize important drugs and their polymorphism is known. CYP2C10 differs from CYP2C9 very little in its structure and substrate specificity [19], and these two enzymes are often considered together. The marker activities of CYP2C9 are tolbutamide 4′-hydroxylation, (S)-warfarin 7-hydroxylation, or diclofenac 4′-hydroxylation. However, CYP2C19 was very recently shown to also contribute to the metabolism of tolbutamide; hence, possibly, both the latter activities are more specific for this enzyme [60]. As can be seen from table 5, typical substrates of CYP2C9 are nonsteroidal antiinflammatory drugs and hypoglycemics. Three defective alleles of the CYP2C9 gene are known, two of which cause decreased CYP2C9 activity [45]. It is particularly important for metabolism of hypoglycemics, where decreased clearance of these drugs may cause severe hypoglycemia, or of (S)-warfarin, where bleeding is a known adverse effect. CYP2C9 substrates should be weakly anionic (to establish an ionic bond with a putative cationic site of the protein) and fairly lipophilic [52, 61].

The CYP2C19 enzyme is highly polymorphic with 3% of slow metabolizers present in Caucasians but almost 20% in Asian populations [54]. An interesting fact is that the presence of defective alleles (nine [45]) in the genome abolishes CYP2C19 activity, which is not the case with other known polymorphisms. The function of an enzyme may often be taken by another one, although with much lower efficiency. In addition, most of drugs are meta-

Table 5. Drug substrates of CYP2C9 and 2C19.

2C9	*2C19*
Amitriptyline	Amitriptyline
Antipyrine	Carisoprodol
Diclofenac	Clomipramine
Dronabinol (THC)	Diazepam
Carbamazepine	Imipramine
Flurbiprofen	Lansoprazole
Glimepiride	Omeprazole
Glipizide	Proguanil
Glibenclamide	Propranolol
Ibuprofen	(S)-mephenytoin
Indomethacin	(R)-warfarin
Losartan	
Phenytoin	
Piroxicam	
Tolbutamide	
Torsemide	

Data from refs. 2 and 19 and from the original literature.

bolized by several CYP enzymes, and hence alternative routes may help in this situation. However, with omeprazole (a typical 2C19 substrate, only partly metabolized also by CYP3A4), in slow metabolizers, 12-fold higher AUCs were observed than those found for extensive metabolizers [54].

The substrates of CYP2C19 (table 5) should be neutral or weakly basic and moderately lipophilic, and able to take part in hydrogen bonds with corresponding partners in the protein [15, 52]. Site-directed mutagenesis has been used to find sites important for the activity of this enzyme in comparison to the CYP2C9 [62].

CYP2E1

The 2E subfamily is present in mammals in one form labeled 2E1. This enzyme is known for its involvement in the metabolism of ethanol. For a long time, it was thought to be responsible for at least a part of ethanol metabolism as a 'microsomal ethanol oxidizing system', [63, 64]. CYP2E1 is now known to take part in the biotransformation not only of ethanol and acetone (both are also CYP2E1 inducers) but also of many small-molecule substrates such as halogenated hydrocarbons (1,1,1-trichloroethane, 1,2-dichloropropane, carbon tetrachloride, chloroform, ethylene dibromide, ethylene dichloride, halothane, methylchloride, methylene dichloride, vinylchloride and trichloroethylene, most of which are hepatotoxic), acetaldehyde, benzene, and styrene [19, 37, 65]. Among drugs, it is known for its ability to metabolize volatile anasthetics such as halothane, enflurane, isoflurane, and sevoflurane, acetaminophen (paracetamol), phenacetin and chlorzoxazone, which is used as a selective in vivo probe for its activity [66]. Another group of CYP2E1 substrates are the nitrosamines [65]. With acetaminophen (paracetamol) as a substrate, it has been shown to take part (together with CYP3A4) in the formation of reactive quinoneimine, which can bind to hepatic and renal proteins (fig. 4) [65, 67]. Hence, CYP2E1 is involved in chemical activation of many carcinogens, procarcinogens, and toxicants.

Levels of CYP2E1 are induced not only by ethanol, acetone, pyrazole, or isoniazide, but also by pathological states such as diabetes or starvation. Oxygen exposure is also a known inductor of this enzyme. Induction or, more precisely, an increase in levels of this enzyme, seems to be achieved by post-translational events such as protein stabilization or inhibition of degradation [64, 68]. Although more than ten alleles of the CYP2E1 gene are known and some are associated with the formation of proteins with altered activity [45], no confirmation of markedly differing CYP2E1 phenotypes has been presented to-date. CYP2E1 activities may be decreased by an effect of some food components, such as the diallylsulfide present in garlic or onion and after consumption of cruciferous vegetables [27].

As the substrates of CYP2E1 are relatively small, neutral, and relatively hydrophilic molecules, its active site is not expected to be spacious, and probably forms one or two hydrogen bonds with the substrate close to the site of metabolism [15, 52].

Conclusion

The time has arrived when cytochromes P-450 are emerging from the academic laboratories and are becoming the focus not only of clinical pharmacologists, but also of health practicioners and pharmaceutical companies [69]. In the next few years, one can expect wide genetic screening of the CYP system aimed at identifying those patients likely to experience toxicity or unwanted drug-drug interactions. At the same time, this screening will enable us to optimize therapeutic efficiency [70]. We can easily foresee a future when information on an individuals CYP genotype will be, together with blood group identification, a normal part of the medical record of every citizen. Knowledge about the human genome [71] will help us to resolve the question of the number of CYP genes and alleles; however, detailed study of substrates and further characterization of the respective CYP enzymes must necessarily follow.

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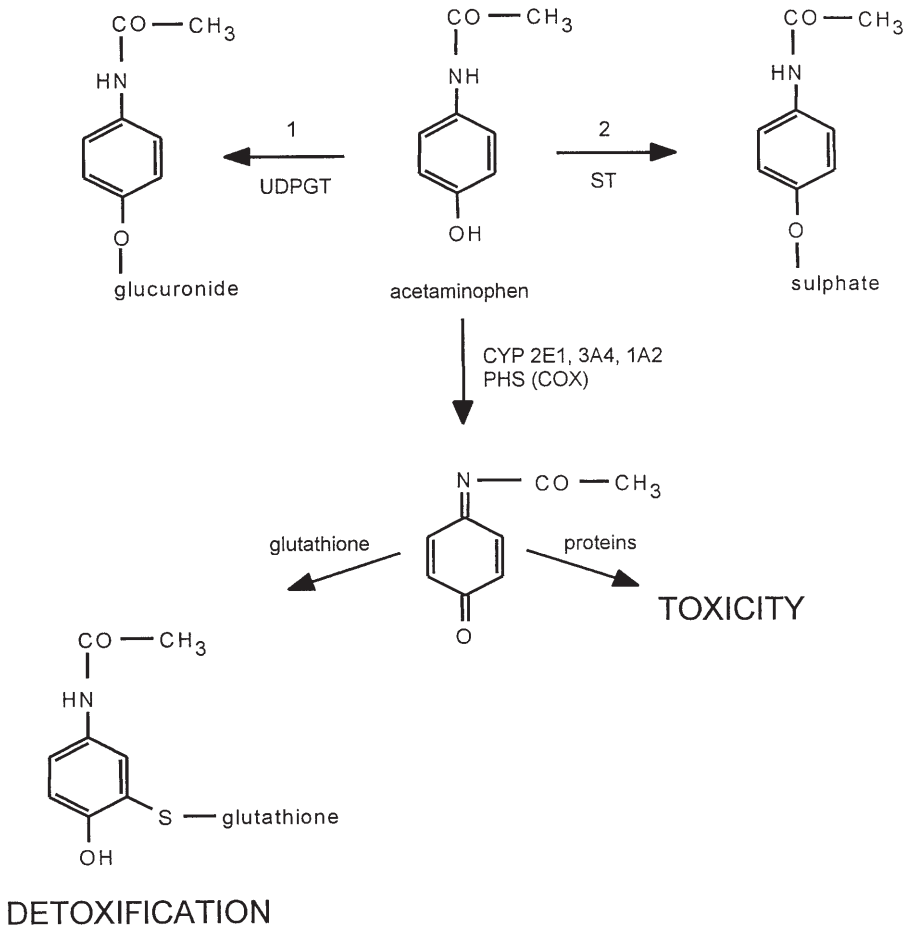

Figure 4. Scheme of the main metabolic transformations of acetaminophen (paracetamol). 1, glucuronidation with UDP-glucuronosyltransferase (UDPGT); 2, sulfation with phenolsulfotransferase (ST); 3, oxidation to N-acetylbenzoquinone imine with CYP2E1, 3A4, 1A2 and prostaglandin H synthase (PHS, cyclooxygenase, COX).

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