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Research Paper

Molecular mechanisms of the microsomal mixed function oxidases and biological and pathological implications

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ABSTRACT

The cytochrome P450 mixed function oxidase enzymes play a major role in the metabolism of important endogenous substrates as well as in the biotransformation of xenobiotics. The liver P450 system is the most active in metabolism of exogenous substrates. This review briefly describes the liver P450 (CYP) mixed function oxidase system with respect to its enzymatic components and functions. Electron transfer by the NADPH-P450 oxidoreductase is required for reduction of the heme of P450, necessary for binding of molecular oxygen. Binding of substrates to P450 produce substrate binding spectra. The P450 catalytic cycle is complex and rate-limiting steps are not clear. Many types of chemical reactions can be catalyzed by P450 enzymes, making this family among the most diverse catalysts known. There are multiple forms of P450s arranged into families based on structural homology. The major drug metabolizing CYPs are discussed with respect to typical substrates, inducers and inhibitors and their polymorphic forms. The composition of CYPs in humans varies considerably among individuals because of sex and age differences, the influence of diet, liver disease, presence of potential inducers and/or inhibitors. Because of such factors and CYP polymorphisms, and overlapping drug specificity, there is a large variability in the content and composition of P450 enzymes among individuals. This can result in large variations in drug metabolism by humans and often can contribute to drug–drug interactions and adverse drug reactions. Because of many of the above factors, especially CYP polymorphisms, there has been much interest in personalized medicine especially with respect to which CYPs and which of their polymorphic forms are present in order to attempt to determine what drug therapy and what dosage would reflect the best therapeutic strategy in treating individual patients.

Introduction

The cytochrome P450 mixed function oxidase system plays a major role in the metabolism of important endogenous substrates such as in cholesterol biosynthesis and cholesterol conversion to bile acids, formation of steroid hormones, androgens and estrogens, metabolism of vitamin D_3 to the active 1,25-dihydroxyvitamin D_3, omega hydroxylation of fatty acids, as well as biotransformation of exogenous xenobiotics. The biological effectiveness and the potential toxicity of many drugs are strongly influenced by their metabolism, much of which is accomplished by P450-dependent monoxygenase systems. The wide array of chemical reactions performed by P450 makes this enzyme one of the most versatile catalysts known. The liver, lung and skin microsomal P450s in particular are important in converting lipophilic xenobiotics including drugs, insecticides, carcinogens, food additives, and environmental pollutants to more polar compounds which are easier to excrete. Intestinal CYPs, especially CYP3A4, may be very important in promoting first pass metabolism of many drugs. Since many of these compounds, lose their activity or potency after being metabolized to polar and excretable metabolites, P450 was considered to be important as a cellular detoxification system. However, with certain compounds although the parent xenobiotic is not toxic, metabolism by the P450 system can generate reactive intermediates which are highly toxic e.g. CCL_4, nitrosamines and acetaminophen.

The term P450 designates a broad family of heme-containing proteins found in bacteria, yeast, plants, invertebrates and vertebrates. About 150 forms of P450 have been identified and a nomenclature based on structural homology, largely deduced from the corresponding cDNAs is used to classify these multiple forms of P450 [1]. The nomenclature is based on evolutionary relationships between CYP450 enzymes and not on similarity in substrate profiles because of the overlapping substrate profiles of many CYP

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enzymes and the ability of multiple CYPs to modify a single substrate at the same or even at different sites [2]. Many in vitro studies using isolated microsomal fractions or intact hepatocytes or cell lines have provided critical and basic information on drug metabolism by CYPs despite the relative short term limitations of such systems including lack of conjugation and other cytosolic enzymes, loss of CYPs in culture, limited number of liver functions expressed, lower levels of CYPs compared to in vivo. Studies to improve such in vitro systems in order to be more reflective of the in vivo state are an important research front.

Several of the contributions to the common theme series in Redox Biology entitled "Role of CYP2E1 and Oxidative/Nitrosative stress in the Hepatotoxic Actions of Alcohol" discuss the P450 enzyme CYP2E1. The goal of this brief overview is to summarize the molecular mechanisms of the cytochrome P450 microsomal drug oxidation system and perhaps be helpful as an educational tool analogous to the Graphical Review by Dr. B. Kalyanaraman on "Oxidants, Antioxidants and Disease Mechanisms" published in Redox Biology [3].

Cytochrome P450 General characteristics

The presence of a carbon monoxide-binding pigment in rat liver microsomes was initially reported in 1958 [4,5]. The oxidized and reduced spectrum of one member of the cytochrome P450 family of enzymes, CYP2E1, is shown in Fig. 1. The pigment, when reduced, displayed a maximal absorbance at a wavelength of 450 nm when binding carbon monoxide and was called P (pigment)-450. Spectral evidence revealed that P450 was a heme-containing protein [6] and P450 plus cytochrome b_5 accounted for most of the hemoproteins found in liver microsomes. The content of cytochrome P450 (nmol/mg microsomal protein) can be calculated from the ferrous carbon monoxide P450 versus ferrous P450 difference spectrum as described in detail in [7]. Cytochrome P450 was subsequently shown to function as the oxygen-activating oxidase associated with microsomal oxygenation reactions such as steroid C-21 hydroxylation, xenobiotic hydroxylation and oxidative dealkylations [8,9]. Elevated activity of the microsomal mixed function oxidase system after in vivo administration of certain drugs was shown to be related to cytochrome P450 and its inducibility [10]. CYPs are b-type cytochromes, containing protoporphyrin IX as the prosthetic group.

Cytochrome P450 is present in various vertebrates, invertebrates and plants. In mammals, P450, while present at highest levels in microsomes from liver (where it plays a major role in detoxification reactions) is also present in microsomes from kidney, small intestine, lungs, adrenal cortex, skin, brain, testis, placenta and other tissues [9]. Mitochondria, especially from liver and endocrine tissue, contain P450. The nuclear envelope and plasma membranes contain low amounts of P450 [11,12]. In plants, P450s are involved in the synthesis of lignins and alkaloids.

Most P450s are made up of about 400–500 amino acids with molecular weights of about 50,000 Da. About half of the amino acids are non-polar. While earlier studies proposed P450 to be buried in the membrane, it is now recognized that much of the

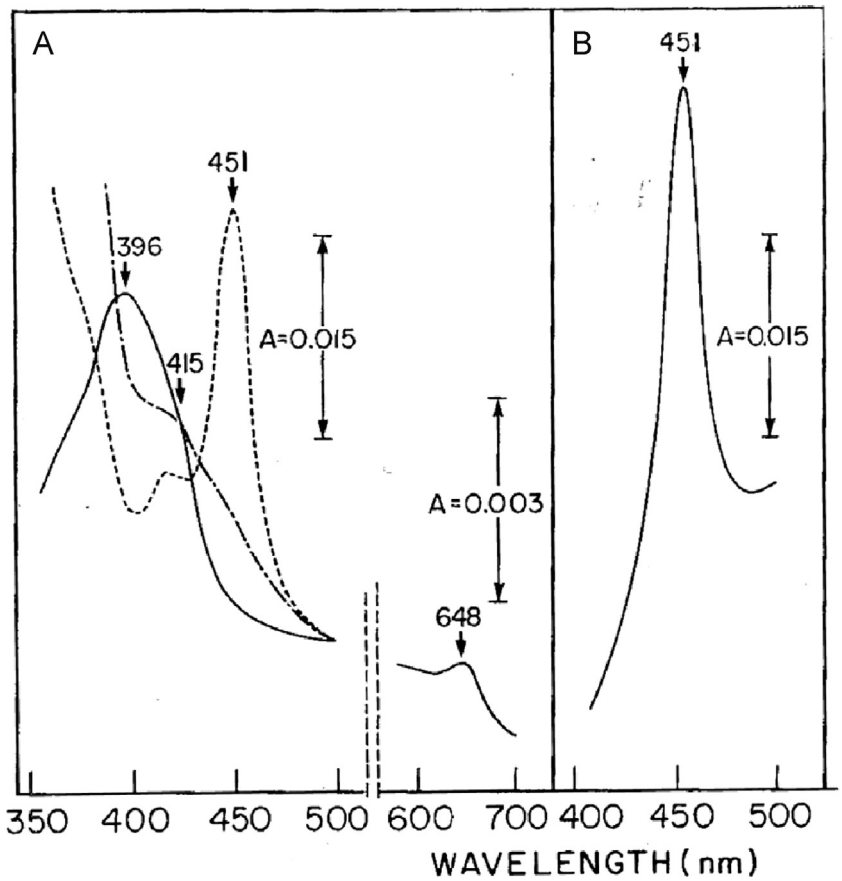


Fig. 1. Spectral characteristics of cytochrome P450. A: Absolute spectra of CYP2E1 purified from pyrazole-treated rats [101]. The sample cuvette contained 0.39 nmol of CYP2E1 in 0.1 M KPi, pH 7.4 buffer, 20% glycerol, 0.1 mM EDTA, 0.2% Emulgen 911 and 0.5% sodium cholate in a final volume of 1 ml. The reference cuvette was identical except for the omission of the CYP2E1. The scanned spectra were: oxidized CYP2E1 (solid line); dithionite reduced CYP2E1 (dot/dashed lines -.-.-.-.-.); carbon monoxide-bound reduced CYP2E1 (dashed line - - - -). B: The CO-CYP2E1 difference spectrum. Dithionite-reduced CYP2E1 (0.39 nmol/ml) was present in the sample and reference cuvettes. The sample cuvette was saturated with CO and spectra recorded over the indicated wavelengths.

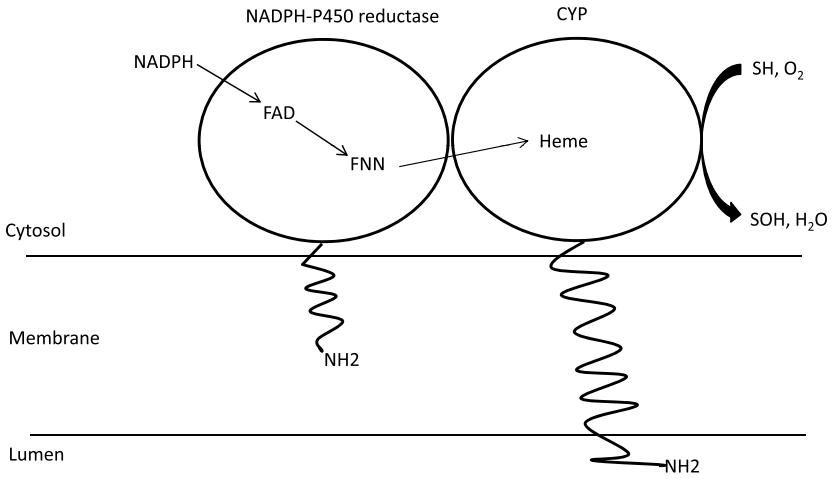


Fig. 2. Cartoon of the membrane topography of cytochrome P450 and of NADPH-cytochrome P450 reductase. Electrons for the reduction of molecular oxygen are provided by the reductant/cofactor NADPH which is generated by the pentose phosphate pathway or mitochondrial transhydrogenase, and transferred via NADPH-cytochrome P450 reductase to the heme of cytochrome P450. Both the NADPH-P450 reductase and P450 are microsomal membrane associated, large parts of these enzymes protrude into the cytosol and are attached to the membrane via their amino terminus.

P450 sticks out of the membrane and is attached at the amino terminus to the microsomal membrane (Fig. 2). Similarly, the NADPH-cytochrome P450 reductase (discussed below) also largely sticks out of the membrane (Fig. 2). The axial ligand for the heme iron is a cysteine residue which is well conserved through evolution near the carboxy terminus of the protein and attaches the heme to the protein. The sixth coordination position of the iron may be water or be empty in the oxidized state and oxygen or carbon monoxide in the reduced state. The addition of a substrate may displace the water with subsequent effects on the redox potential of the heme. The oxidation reduction potential as determined by titrations with redox dyes is very low, with a midpoint potential of about −0.34 to −0.40 V. Agents that react with sulfhydryl groups or disrupt hydrophobic interactions convert P450 to an inactive form called P420, based on the absorption maximum of the ferrous P420–carbon monoxide complex. Conversion of P450 to P420 usually results in loss of substrate-induced spectral changes discussed below.

Substrates may change the optical properties of P450, so called substrate binding spectra [13,14]. The substrate binding may promote the high spin state of the P450 with an absorption maximum at about 390 nm and a trough at about 420 nm (Type I substrate binding spectrum) or promote transition to the low spin state with an absorption maximum at about 417 nm and a trough at about 390 nm (Type II substrate binding spectrum) [13,14]. Fig. 3 shows the binding spectra when increasing concentrations of DMSO or the chemical pyrazole, known ligands for CYP2E1, are added to purify CYP2E1. Type II binding spectra were produced. The inset of Fig. 3 shows a Lineweaver–Burk plot of these data in order to calculate the spectral dissociation constants for DMSO (21 mM) or pyrazole (0.04 mM) binding to CYP2E1. The substrate binding spectra of ligands for cytochrome P450 can be intensified after induction of the specific CYP which interacts with the ligands. For example, CYP2E1 was induced by chronic ethanol feeding and microsomes from the ethanol-fed rats and the dextrose-pair fed controls were incubated with pyrazole or 4-methylpyrazole [15]. The magnitude of the Type II binding spectrum was intensified with the microsomes from the ethanol-fed rats and the affinity for pyrazole and 4-methylpyrazole, as determined from Hanes–Wolf plots was elevated by ethanol treatment in association with a 3–5 folds increase in CYP2E1 content [15].

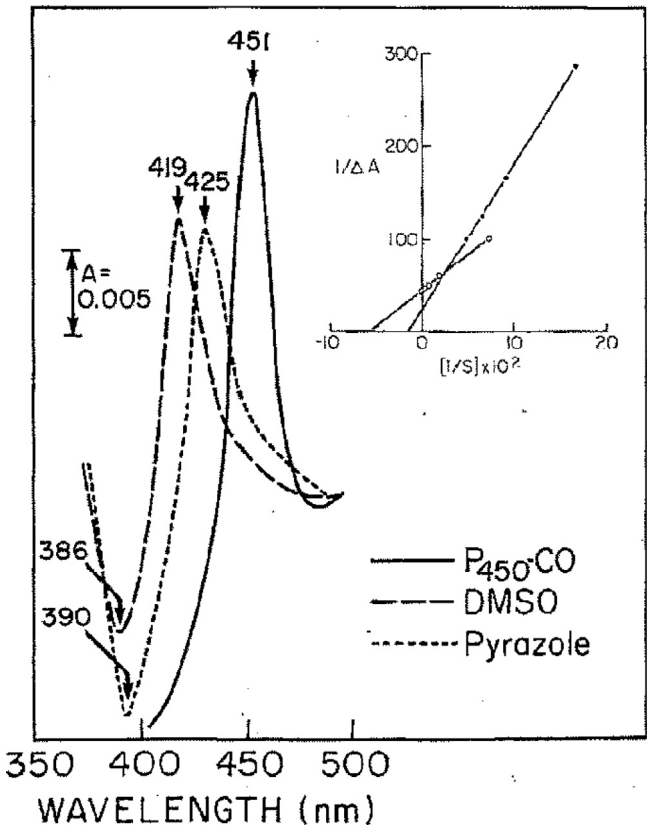


Fig. 3. Substrate binding spectra to CYP2E1. CYP2E1 and various concentrations of the CYP2E1 ligands DMSO (14–364 mM) or pyrazole (6–51 μM) were added and the spectrum was recorded over the indicated wavelengths until no further changes were observed. A CO-CYP2E1 binding spectrum is shown by the solid lines with a peak at 451 nm. DMSO produces a type II substrate binding spectrum with CYP2E1 with a peak at 419 nm and trough at 386 nm. Pyrazole also produces a type II binding spectrum with a peak at 425 nm and trough at 390 nm. A Lineweaver–Burk plot of the concentration dependence was linear (insert) and the spectral dissociation constants were calculated to be 21 mM for DMSO and 0.04 mM for pyrazole. Results are from Ref. [101].

Electron transfer to P450

Reducing equivalents from either NADH or NADPH are required for P450 to activate molecular oxygen for subsequent mixed

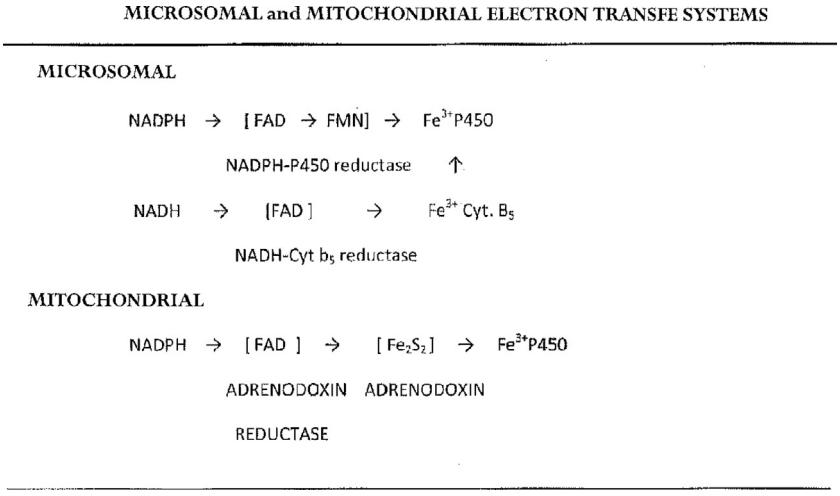


Fig. 4. Electron transfer to cytochrome P450. NADPH is the preferred reductant for microsomal cytochrome P450. Electrons are passed from NADPH to the FAD cofactor of the reductase which passes electrons to the FMN cofactor which than reduces the heme of P450. NADH is less effective than NADPH in reacting with the P450 reductase and reducing P450. NADH effectively reduces the FAD component of the NADH-cytochrome b_5 reductase which then reduces the second major heme enzyme in microsomes, cytochrome b_5. The system is important in fatty acid desaturation. However, it is not very efficient, relative to the NADPH cytochrome P450 reductase in reducing the heme of cytochrome P450. For reduction of mitochondrial cytochrome P450, iron sulfur proteins are required. The scheme depicts the roles of the FAD adrenodoxin reductase in reducing the adrenodoxin iron–sulfur protein and subsequently, mitochondrial cytochrome P450.

function oxidation. The first P450 system to be purified and reconstituted from its molecular components was the 11 β-hydroxylase system of adrenal cortex mitochondria [16]. This system was separated into a P450-containing membrane fraction and a soluble NADPH-P450 reductase activity; the latter was further resolved into an iron–sulfur protein and a flavoprotein reductase and the sequence

$$\text{NADPH} \rightarrow \text{flavoprotein} \rightarrow \text{iron sulfur protein} \rightarrow \text{P450}$$

was established [16]. A similar electron transfer pathway that used NADH in place of NADPH was found for the camphor hydroxylase system of the bacterium Pseudomonas putida [17]. Iron sulfur proteins, while required for mitochondrial P450 electron reduction are not involved in microsomal P450 electron reduction. The microsomal drug metabolism system contains two major components, the P450 and its reductant, the NADPH cytochrome P450 reductase [18] (Fig. 4). The mammalian microsomal cytochrome P450 reductase contains 2 mol of flavin, one FAD and one FMN, per mol of reductase enzyme. The activity of NADPH-cytochrome P450 reductase can be assayed via its ability to reduce other acceptors, usually ferric cytochrome c, following the increase in absorbance at a wavelength of 550 nm as described in [7]. The reductase is also active in providing reducing equivalents for microsomal heme oxygenase activity. A recent review of the roles of the reductase in physiology, pharmacology and toxicology can be found in [19].

Cytochrome P450s use molecular oxygen and reducing equivalents to catalyze the monooxygenation of a variety of substrates (RH) by the following general reaction:

$$\text{RH} + \text{NAD(P)H} + \text{O}_2 \rightarrow \text{ROH} + \text{NAD(P)} + \text{H}_2\text{O}$$

This reaction is referred to as a mixed function oxidase reaction since one atom of oxygen is incorporated into the substrate (hydroxylation) while the other is reduced to water. P450s cannot directly accept electrons from NADH or NADPH, therefore a cytochrome P450 reductase containing FMN and FAD is necessary to reduce the heme of microsomal P450. For mitochondrial P450 reduction, a FAD containing reductase and an iron–sulfur protein are necessary (Fig. 4). For microsomal P450 reduction, NADPH rather then NADH is the preferred reductant for transferring reducing equivalents to the NADPH-P450 reductase and then to cytochrome P450. NADH generally is about 10% as effective as NADPH in promoting microsomal P450 activity. NADH transfers electrons to the FAD containing flavoprotein NADH-cytochrome b_5 reductase which reduces cytochrome b_5 (Fig. 4). Experiments with inhibitors, antibodies and reconstitution experiments with purified enzymes have provided clear evidence for the major role of the NADPH-P450 reductase in reducing P450. The reduced b_5 may to some extent reduce P450. In some cases, NADH may further increase NADPH-dependent P450 catalytic activity by providing the second electron required for the P450 catalytic cycle (described below). The NADH/ NADH cytochrome b_5 system is normally involved in transferring electrons to the cyanide sensitive factor required for fatty acid desaturation. A summary of the electron transfer pathways involved in P450 catalyzed reactions [9,20,21] is shown in Fig. 4.

Rat liver microsomes contain about 0.05 nmol/mg protein of NADPH-cytochrome P450 reductase and 0.05 nmol/mg of NADH-cytochrome b_5 reductase, about 0.5 nmol/mg of cytochrome b_5 and about 0.5–1 nmol P450/mg protein. Therefore, the molar ratios of the two cytochromes are about an order of magnitude greater than that of the corresponding reductase. Molecular weights of cytochrome b_5, the NADH reductase and the NADPH reductase are about 17,000, 40,000 and 80,000 Da, respectively [9,22], while that for cytochrome P450s are about 46–60,000 Da depending on the molecular form. Fig. 5 shows the absorption spectrum of oxidized cytochrome b_5 and after its reduction with dithionite. The two reductases and b_5 are typical integral membrane proteins that are amphipathic, attaching to the microsomal membrane via a short hydrophobic region and containing a large hydrophilic catalytic domain which protrudes into the cytosol of the cell and thus are accessible to reducing equivalents. As mentioned above, most of the P450 also protrudes out of the microsomal membrane, being attached to the membrane at its amino terminus. The amino terminus is believed to anchor P450 to the endoplasmic reticulum and to act as a microsomal membrane insertional signal [23,24]. For example, removal of the amino terminus of CYP2E1 promotes its translocation to the mitochondria [25].

Phase 1 (I) and phase 2 (II) drug metabolism

The general reaction catalyzed by cytochrome P450s is to oxidize lipophilic substrates to more hydrophilic products e.g.

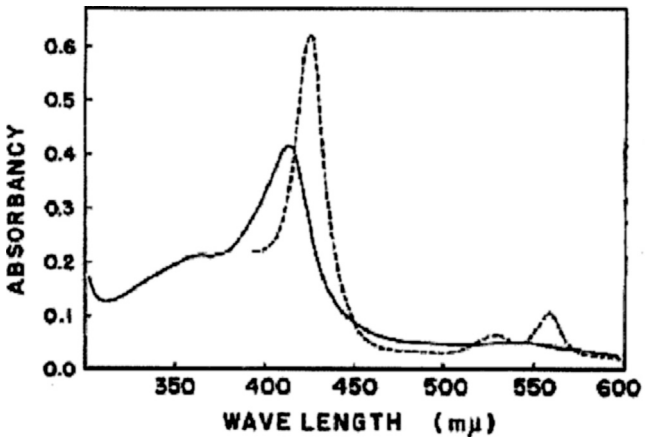


Fig. 5. Absorption spectrum of microsomal cytochrome b_5. Solid line is the spectrum for oxidized cytochrome b_5 with a peak at 412 nm. The dashed line is the absorption spectrum after reduction with dithionite, with a peak at 423 nm. Reduced cytochrome b_5 does not bind CO.

RH→ROH. This is referred to as phase 1 drug metabolism and generally involves oxidation, reduction and hydrolysis reactions and is catalyzed by a number of enzymes, the most important being CYPs [26,27]. The phase 2 metabolic reactions catalyze conjugation reactions of lipophilic compounds with endogenous cofactors such as glucuronic acid, sulfate, glutathione or acetate (acetyl CoA). Phase 2 conjugation of a drug can occur in the absence of phase 1 metabolism. The hydroxylated product produced from phase 1 reactions can also be conjugated by conjugation enzymes to more hydrophilic products, easier to excrete. Often, phases 1 and 2 pathways work together to help in the removal of the xenobiotic. An example is shown in Fig. 6 for the conversion of benzene to phenyl sulfate. Among the conjugation enzymes (and the conjugated product) are those promoting glucuronidation, the UDP-glucuronyl transferases (R-Glucuronide), sulfation by sulfotransferases (R-Sulfate), acetylation, by N-acetyl transferases (acetylated R), methylation by methyltransferases (R-CH_3) and glutathionylation by various glutathione transferases (R-SG) (Fig. 6). The conjugation reactions are not only important for excretion of the drug but also help to minimize drug toxicity. For example, the widely used drug acetaminophen (N-acetyl-p-aminophenol) is normally metabolized largely by glucuronidation and sulfation to form nontoxic acetaminophen-conjugated products. A small amount of acetaminophen can be oxidized by CYPs, especially CYP2E1 to a reactive N-acetyl-p-quinone imine (NAPQI), which alkylates proteins, especially mitochondrial proteins. This reactive, toxic metabolite can be removed by conjugation with GSH. Acetaminophen toxicity is magnified when conjugation with glucuronic acid or sulfate is compromised or after intake of very high levels of acetaminophen which saturate the conjugation pathways thereby increasing formation of the reactive NAPQI. NAPQI toxicity is magnified especially when glutathione conjugation is impaired because of depletion of hepatic levels of GSH e.g. by alcohol, thereby causing accumulation of NAPQI [28,29]. These reactions are summarized in Fig. 7.

Catalytic activity of cytochrome P450

There is tremendous diversity in the reactions catalyzed by cytochrome P450, which may be one of the most versatile catalysts known [30–33]. The reactions catalyzed by P450 may be broadly categorized as: metabolism of endogenous constituents including steroids, cholesterol, bile acids, vitamins, fatty acids, and eicosanoids; conversion of lipophilic exogenous xenobiotics into more polar products which can be conjugated to more soluble products for detoxification and removal from the body and metabolism of certain xenobiotics such as CCL_4, acetaminophen, benzene, halothane and nitrosamines into more reactive products that are toxic or carcinogenic. Conversion of drugs into toxic products as a consequence of metabolism by the microsomal mixed-function oxidase system is a major reason for failure of many drugs in pre and post clinical trials [34–36].

Oxidative reactions that are catalyzed by P450 enzymes have been characterized and discussed by Guengerich and colleagues [34,37,38] and representative ones are shown in Fig. 8 – aliphatic hydroxylations, aromatic hydroxylation, epoxidation, N-dealkylation, O-dealkylation, S-dealkylation, N-hydroxylation, oxidative deamination, oxidative denitrosation, oxidative dehalogenation, oxidative desulfuration, sulfoxidation. P450 can also catalyze reductive reactions under anaerobic or hypoxic conditions e.g. nitro reduction of nitropyrenes, azo reduction (aminoazobenzene),

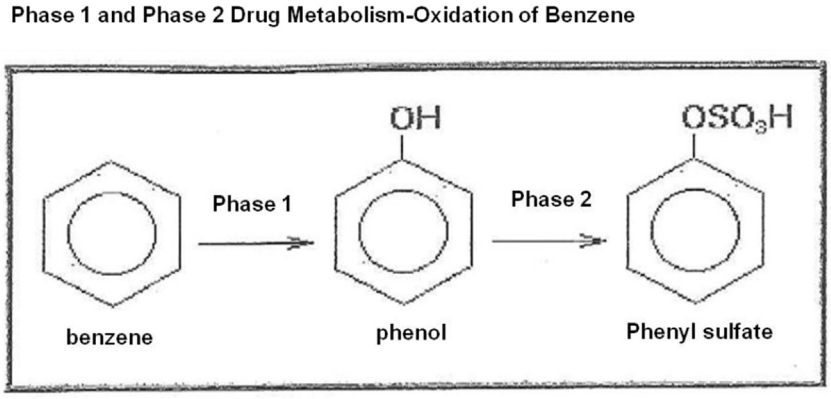


Fig. 6. Phase 2 conjugation enzymes.

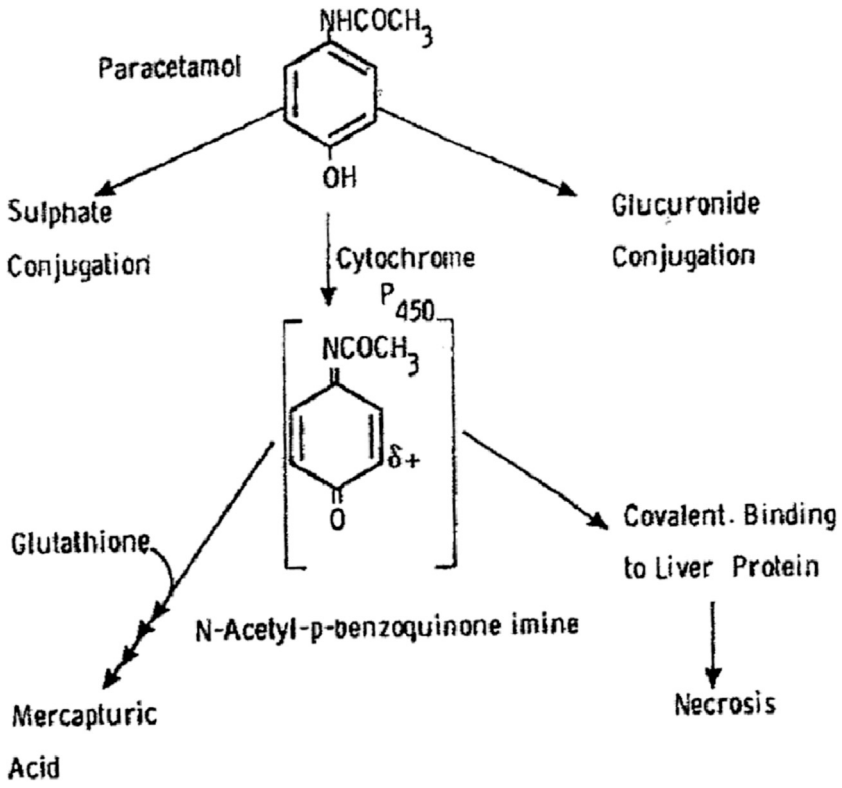


Fig. 7. Phases 1 and 2 acetaminophen metabolism. Acetaminophen consumed in "normal" amounts can be removed by direct conjugation by sulfotransferase or glucuronidation by glucuronyl transferases to produce acetaminophen sulfate or acetaminophen glucuronide, which are excreted. When present at high levels, the conjugation reactions become limiting and acetaminophen can be oxidized by several CYPS, especially CYP2E1 to form the reactive N-acetyl-p-benzoquinone imine. The quinone imine can be removed by conjugation with GSH. However, when formed in high amounts at high levels of acetaminophen or when GSH levels are low because of liver disease or alcohol intake, the reactive quinone imine forms covalent adducts with proteins, especially mitochondrial proteins. Impairment of mitochondrial bioenergetics leads to liver necrosis. Acetaminophen toxicity is one of the leading causes of liver damage and emergency room visits.

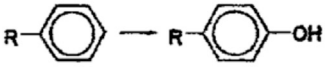
reductive dehalogenation of CCL_4 to the trichloromethyl radical. The latter plays a critical role in the use of CCL_4 in toxicological reactions to cause lipid peroxidation followed by liver damage, fibrosis and cirrhosis. In general, some of these reactions may be carried out by one form of P450 while others may be carried out by many forms. Some substrates may be oxidized by one form of P450 at a low concentration but another form at a high concentration e.g. low levels of the cancer-inducing N,N-nitrosodimetylamine are demethylated by CYP2E1 (low K_m demethylase) whereas higher concentrations are demethylated by several P450s including CYPs 2B1 and 2B2 [39]. The structural features necessary for a substrate to be oxidized by a particular P450 are still not clear despite much investigation.

The cytochrome P450 catalytic cycle

A general mechanism for microsomal cytochrome P450 catalysis as described in Refs. [20,32–35] is shown in Fig. 9. Substrates for P450s have K_m values ranging from nM to mM. Binding of the substrate to the oxidized ferric P450 (RH, step 1) may or may not be rapid (depending on the P450 and the substrate) and may or may not change the oxidation/reduction potential of the heme. Binding of substrate to the heme of P450 leads to displacement of water as the 6th ligand to the heme iron, changing the spin state of the iron from low spin to high spin [14]. A change to a more positive redox potential would accelerate acceptance of electrons from the NADPH reductase to reduce the heme from the ferric to the ferrous heme (step 2). Molecular oxygen binds rapidly to the substrate–ferrous P450 (step 3) to form the ternary P450-oxygen–substrate complex (oxy-P450). Carbon monoxide competes favorably with oxygen for binding to ferrous P450 thereby inhibiting P450 activity and providing the basis for detecting and quantifying P450 in biological systems at 450 nm as the ferrous P450– carbon monoxide complex [7]. The ferrous O_2 complex can display a resonance structure in which an electron from the ferrous is transferred to the O_2 to produce the ferric–O_2^- complex. Decay of this complex produces the ferric-P450–substrate complex with release of superoxide anion radical, the focus of production of normally small amounts of reactive oxygen intermediates during the P450 cycle [40–42]. The decay of the P450–substrate-superoxide complex is dependent on the nature of the substrate, the form of P450 and the efficacy of input of a second electron to reduce the oxy ferrous P450 to the peroxy P450 complex (substrate–ferrous P450-superoxide or substrate–ferric P450-peroxide) (step 4). Decay of the latter produces H_2O_2 plus substrate–ferric P450. The second electron may come from the NADPH P450 reductase (like the first electron) or it may come from reduced cytochrome b_5 [43,44]. The ability of cytochrome b_5 to provide the second electron to reduce oxy-P450 and continue the P450 cycle may explain the ability of b_5 to stimulate (or inhibit) certain P450-dependent reactions [45]. Subsequent steps of the cycle (steps 5–8) are not very clear but it is believed that heterolytic cleavage of the O–O bond of peroxy P450 yields H_2O and a ferryl type oxidant (step 5). The ferryl like oxidant abstracts a hydrogen from the substrate (RH) to produce a substrate radical ($R^{\bullet}$, step 6) and the bound equivalent of a hydroxyl radical (FeOH). Rapid oxygen rebound to the substrate radical (radical recombination) yields the hydroxylated substrate product (step 7) and regenerates the ferric P450 (step 8) [20,30,32–35].

With such a complex overall reaction pathway, it is difficult to ascertain what step(s) is rate limiting for P450-catalyzed reactions.

Example of the general type of oxidation reactions catalyzed by the cytochrome P450-containing monooxygenation

REACTION	EXAMPLE
Aliphatic hydroxylation	$R-CH_2-CH_2-CH_3 \rightarrow R-CH_2-CHOH-CH_3$
Aromatic hydroxylation	
Epoxidation	$R-CH=CH-R' \rightarrow R-\underset{O}{CH-CH}-R'$
N-, O-, or S-dealkylation	$R-\overset{H}{(N, O, S)}-CH_3 \rightarrow R-(NH_2, OH, SH) + CH_2O$
Deamination	$R-CH_2-NH_2 \rightarrow R-\overset{O}{\overset{\|}{C}}-H + NH_3$
N-hydroxylation	$R-NH-\overset{O}{\overset{\|}{C}}-CH_3 \rightarrow R-NOH-\overset{O}{\overset{\|}{C}}-CH_3$
Sulfoxidation	$R-S-R \rightarrow R-\underset{O}{\overset{\|}{S}}-R'$
Desulfuration	$R_1R_2\overset{S}{\overset{\|}{P}}-X \rightarrow R_1R_2\overset{O}{\overset{\|}{P}}-X + S$
Oxidative Dehalogenatic	$R-\underset{H}{\overset{X}{\overset{\|}{C}}}-H \rightarrow R-\underset{H}{\overset{X}{\overset{\|}{C}}}-OH \rightarrow R-\overset{O}{\overset{\|}{C}}-H + HX$

* X=halogen

Fig. 8. General type of reactions catalyzed by cytochrome P450 enzymes. Other reactions, not shown, include aryl migration, ring contraction, ring formation, ring coupling, dimer formation, group migration (shown in Ref. [33]).

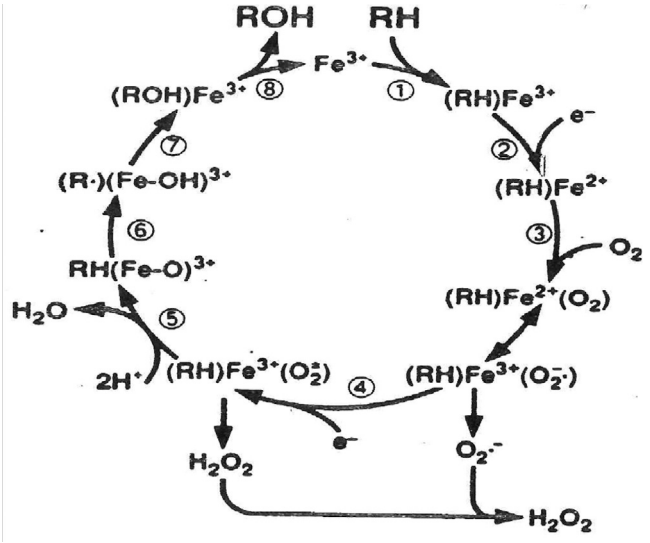


Fig. 9. The CYP catalytic cycle. Please see text for discussion. Oxygen surrogates such as hydroperoxides can replace NADPH, oxygen and the NADPH-cytochrome P450 reductase in promoting certain CYP-catalyzed reactions by forming active oxygenated–CYP complexes. For example:
$RH + Fe^{3+}$ (CYP) → RH-Fe^{3+} (CYP)
RH-Fe^{3+} (CYP) + XOOH → XOH + R• (Fe–OH)$^{3+}$ (CYP)
R• (Fe–OH)$^{3+}$ (CYP) → ROH-Fe^{3+} (CYP) → ROH + Fe^{3+} CYP.

It appears that depending on the P450 and the substrate, different steps may be rate-limiting. Input of the first and especially the second electron may at times be limiting. Substrate may facilitate entry of the first electron [46]. Carbon–hydrogen bond breakage of the substrate to produce the substrate radical may be limiting based upon kinetic isotope effects with deuterated substrates [47]. Cytochrome b_5 may stimulate, have no effect or inhibit. Decay of the oxygenated P450 complex may vary among different forms of P450 and thereby limit the monoxygenase reaction e.g. CYP2E1 appears to be a "loosely coupled" form of P450 associated with elevated rates of production of superoxide and H_2O_2 [48,49]. Depending on the form of P450, there may be a 1:1:1 stoichiometry between the amount of oxygen consumed, the amount of product formed and the amount of NADPH used. In some cases, the stoichiometry is poor unless other products of molecular oxygen reduction such as superoxide or peroxide or especially water are taken into account. For example, with CYP2E1, a considerable amount of the oxygen and NADPH consumed produces water [48]. Excellent reviews on the P450 catalytic cycle can be found in Refs. [42,50,51].

So-called oxygen surrogates may replace NADPH, oxygen and the reductase in promoting certain P450-catalyzed reactions. Agents such as hydroperoxides (e.g. cumene hydroperoxide) can react directly with P450 to produce an active oxygenated P450 complex that can oxygenate certain substrates [52]. Usually these reactions are much more rapid than the NADPH-dependent reactions indicating that electron transfer from the reductase to P450 is a limiting step in the overall P450 mechanism.

Multiple forms of cytochrome P450s

The very broad substrate specificity, the differential effect of inducers and inhibitors on P450 reactions, the multiple bands on

SDS-gels, and multiphasic Lineweaver–Burk plots, all pointed to the presence of many forms of P450 enzymes. This was confirmed by purifications of different P450s and by molecular biology. It is now recognized that more than 150 separate forms of P450 exist. The human genome project has identified 57 human genes coding for the various P450 enzymes (http://drnelson.utmem.edu/CytochromeP450.html) OR (http://drnelson.uthsc.edu/human.P450.table.html).

Numerous names were given to these different forms as they were purified or identified and the older literature can be confusing on nomenclature. A systematic nomenclature is now used which assigns proteins into families and subfamilies based on their amino acid similarities. Proteins within a family exhibit greater than 40% homology while those with at least 55% homology are in the same subfamily. The written notation is an Arabic number designating the gene family followed by a letter for the subfamily and an Arabic number for individual genes within a subfamily. Thus CYP1A1 and CYP1A2 are two individual P450s in family 1 subfamily A.

One way of categorizing the multiple forms of human CYPs is by the substrates they oxidized (Table 1, derived from Ref. [37]). About one half of the human CYPs metabolize endogenous substrates such as steroids, bile acids, fatty acids, eicosanoids and vitamins. The other 50% function to metabolize xenobiotics [37]. In general, members of the CYPs 1, 2 and 3 families are the most important in catalyzing oxidation of exogenous drugs and are generally present in highest amounts in human liver (Table 2, derived from Ref 26,31,34,37 53). In the P450 reactions with drugs, 90–95% of clinical drugs are metabolized by 5 of the 57 human CYPs: 1A2, 2C9, 2C19, 2D6 and 3A4 [54]. CYPs 3A4/5 oxidize about 40% of the drugs used clinically. CYPs 2C, 2D and 1A also metabolize many clinically-used drugs. Together, CYPs 2A6, 2B6 and 2E1 oxidize less than 10% of clinical drugs (Table 2). Recently, Rendic and Guengerich [55] reported on the contributions of human P450 enzymes in carcinogen metabolism. Their Fig. 2B of Ref 55 showed the fraction of P450 activation reactions for carcinogens attributed to individual human P450 enzymes as follows: CYP1A1, 20%; CYP1A2, 17%; CYP1B1, 11%; CYP2A6, 8%; CYP2E1, 11%; CYP3A4, 10%; others 23%. Members of the CYP4 family are active in metabolizing fatty acids, especially arachidonic acid while CYPs 7, 11, 17 and 19 are most important in steroid metabolism (Table 1). CYPs 24A1, 27B1 and 26B1 play a role in vitamin D and retinoic acid metabolism, respectively (Table 1). Approaches that are used to predict or estimate the contribution of an individual CYP to the metabolism of a drug include comparison of rates measured with recombinant P450s, correlation of the catalytic activity of the drug with marker activities of individual CYPs, use of specific selective inhibitors or antibodies against the CYP and evaluation of the drug metabolism remaining activity, and determining the effect of a specific CYP inducer on the drug oxidation activity [54].

Table 3 summarizes some representative substrates for the major human liver drug metabolizing CYPs. Table 4 summarizes representative inhibitors and inducers (discussed below) for the major human liver drug metabolizing CYPs.

Differences in expression of specific CYPs and active CYPs in humans can be due to differences in gene regulation and the presence of genetic polymorphisms. CYPs 2A6, 2C9, 2C19 and 2D6 in particular display polymorphisms [56,57]. As an example, a listing of many cytochrome P450 genes and their polymorphisms can be found in Table 1 of Ref. [53] and in http://www.imm.ki.se/CYPalleles/. The effect of the polymorphism may include formation of an inactive CYP enzyme, CYP gene deletion, formation of an unstable CYP which rapidly degrades, formation of a CYP with lower affinity for the cytochrome P450 reductase, formation of a CYP with altered substrate specificity or altered substrate affinity, or even increased CYP activity due to a gene duplication [58]. An example of such effects for polymorphisms of CYP2D6 is shown in Table 5 (derived from [58,59]).

Table 1
Classification of CYPS based on the substrates they oxidize.

Sterols	Xenobiotics	Fatty acids	Eicosanoids	Vitamins
1B1	1A1	2J2	4F2	2R1
7A1	1A2	4A11	4F3	24A1
7B1	2A6	4B1	4F8	26A1
8B1	2A13	4F12	5A1	26B1
11A1	2B6		8A1	26C1
11B1	2C8			27B1
11B2	2C9			
17A1	2C18			
19A1	2C19			
21A2	2D6			
27A1	2E1			
39A1	2F1			
46A1	3A4			
51A1	3A5			
	3A7			

Modified from Refs. [33,34,37]. Other CYPs such as CYP2A7, 2S1, 2U1, 2W1, 3A43, 4A22, 4F11, 4F22 4V2, 4X1, 4Z1, 20A1, 27C1 are referred to as orphan CYPs as specific substrates have not been identified [37].

Table 2
Human liver drug metabolizing CYPS-content and % drug metabolism.

CYP	Percent drug metabolized	Percent of total human liver P450
1A2	10–12	5
2A6	3–5	2
2B6	3–5	2–4
2C8/9	15	11
2C19	7–9	5
2D6	30	20–30
2E1	3–5	2–4
3A4/5	35–40	40–45

Induction of cytochrome P450s

The ability of certain xenobiotics to elevate their own metabolism has long been known and the mechanism proposed was that the agent increased the content of the P450 responsible for its metabolism. Table 4 lists classical inducers of liver microsomal P450s: for example, polycyclic aromatic hydrocarbons and barbiturates such as phenobarbital were among the first identified inducers of CYP1A1 and CYP2B1/2B2 respectively, while glucocorticoids such as dexamethasone increase CYP3A4 and ethanol elevates CYP2E1. Not all forms of P450 are inducible especially the steroid metabolizing P450s characteristic of the 11, 17, 19, 21 and

Table 3
Representative substrates for human liver drug metabolizing CYPS.

CYP1A2-	tacrine, theophylline, propranol, imipramine, amitryptyline, phenacetin, caffeine, nifedipine.
CYP2D6-	debrisoquine, sparteine, bufuralol, codeine, desipramine, nortriptyline, dextromethorphan.
CYP2E1-	ethanol, acetaminophen, chlorzoxazone, halothane, enflurane, nitrosamines, benzene, CCl_4.
CYP2C9-	tolbutamide, mephenytoin, S-warfarin, diclofenac. ibuprofen, phenytoin, sulfamethoxazole.
CYP2C19-	propranolol, clomipramine, diazepam, omeprazole, progesterone, cyclophosphamide.
CYP2A6-	coumarin, nitrosamines, aflatoxin, nicotine, cotinine, testosterone.
CYP3A4/5-	nifedipine, cyclosporine, simvastatin, quinidine, midazolam, lovastatin, dexamethasone.

Table 4
Representative CYP inhibitors and inducers.

Isozyme	Inhibitor	Inducer
CYP1A2	Cimetidine, ellipticine, benzo(a)pyrene, furafylline, α-naphthoflavone.	Phenobarbital, omeprazole, rifampin, ritonvair, cyclic aromatic hydrocarbon
CYP2D6	Quinidine, yohimbine, haloperidol, fluoxetine, thioridazine, bupropion.	Dexamethasone, carbamazepine, phenytoin, rifampin, ritonavir
CYP2C9	Amiodarone, chloramphenicol, ritonavir, ketoconazole, fluconazole	Rifampin, phenobarbital, phenytoin
CYP2C19	Cimetidine, fluoxetine, omeprazole, ritonavir, ketoconazole, sertraline	Carbamazepine, phenytoin, rifampin, phenobarbital
CYP2E1	Chlormethiazole, 4-methylpyrazole, disulfiram, diallyl disulfide	Ethanol, acetone, isoniazid pyrazole, diabetes
CYP3A4/5	Cimetidine, ketoconazole, ritonavir, azole anti-fungals, grapefruit juice	Dexamethazone,barbiturates, carbamazepine, phenytoin, rifampin

Table 5
Polymorphisms of CYP2D6.

Major variant	Mutation	Consequence
2D6*2Xn	Gene duplication	Increased activity
2D6*4	Defective splicing	Inactive enzyme
2D6*5	Gene deletion	No enzyme
2D6*10	P34S, S486T	Unstable enzyme
2D6*17	T107L, R296C, S486T	Reduced affinity for substrate

Several of the major variants of CYP2D6, the nature of the mutation and the consequences of the polymorphism on content and/or activity of CYP2D6 are shown. Results modified from Refs. [58,59].

26 families. Not all P450s in a particular family are inducible e.g. CYPs 2B and 2E are inducible (by phenobarbital or ethanol respectively) but CYPs 2A, 2C and 2D are not. Induction of most (but not all) P450s involves activation of the respective gene and increased de novo protein synthesis. In some cases, specific cell receptors which interact with the inducing agent have been identified e.g. induction of CYP1A1 by polycyclic aromatic hydrocarbons such as 3-methylcholanthrene involves initial binding of the inducer to the Ah receptor, translocation into the nucleus and eventual activation of the CYP1A1 gene. Induction of CYP2E1 by ethanol is complex and involves both transcriptional and post-transcriptional mechanisms [60]. The CYP2E1 gene is under transcriptional regulation during development and in rats is activated immediately after birth. Following fasting or induced diabetes, CYP2E1 mRNA is increased several fold due to post-transcriptional mRNA stability [61]. The elevation of CYP2E1 by many low molecular weight chemicals including ethanol. acetone, pyrazole is largely due to protein stabilization and increases in protein half-life [60,62,63]. Ethanol, at very high levels can also increase CYP2E1 by a transcriptional mechanism and increased mRNA synthesis [64]. Thus, multiple mechanisms can exist by which a cytochrome P450 such as CYP2E1 can be induced.

In many cases, induction of a specific P450 by a chemical inducer requires binding of the inducer to a nuclear receptor, followed by translocation of the receptor-inducer complex into the nucleus and subsequent interaction and activation of the gene for the P450 [65,66]. Nuclear receptors involved in activating P450 genes include the constitutive androstane receptor (CAR), the pregnane X receptor (PXR), the peroxisome proliferator-activated receptors (PPARs), the aromatic hydrocarbon receptor (AhR) and the farnesoid X receptor (FXR). The Ah receptor in cooperation with its binding partner ARNT is involved in induction of CYPs 1A1, 1A2 and 1B1, critical CYPs involved in carcinogenesis; CYPs 1A1 and 1B1 are mainly extrahepatic. PXR promotes the induction of CYP3A after forming a complex with its binding partner retinoid X receptor (RXR); CYP3A4 is responsible for metabolism of many drugs in humans. PPARs are activated by fatty acids and lipid lowering drugs and induce fatty acid oxidation enzymes such as peroxisomal acyl CoA oxidase to promote fat oxidation and removal. Genes induced by CAR include CYPs 2B6,2C9 and 3A4 as well as several phase 2 conjugation enzymes. Examples of drugs and substrates for these nuclear receptors include omeprazole for AhR, phenobarbital for CAR, rifampin for PXR, bile acids for FXR, fibrates for PPAR.

Table 4 lists representative inducers and inhibitors of the major human liver drug metabolizing CYPs [67].

Sex, age and species differences

Levels of the hepatic microsomal mixed function oxidase system are very low during fetal development but increase rapidly soon after birth. There are varying patterns of development depending on the form of P450. In general, drug metabolism activities stabilize by early adulthood. Studies in rats of varying ages have suggested that aging is often accompanied by a decline in metabolism of certain drugs (e.g. [68]). Sex differences, especially in rats in metabolism of certain drugs have been reported and certain forms of P450 are present in males e.g. constitutive CYPs 2C11 and 2C13 whereas other forms are present in females e.g. CYP2C12. Male rats can metabolize certain drugs faster than females whereas the opposite occurs with other drugs [69].

In mammals, there are wide differences in drug metabolism by different species. For example, the half life of the barbiturate hexobarbital has been reported to be 19, 60, 140, and 260 min and estimated to be 360 min for mice, rabbits, rats, dogs and humans, respectively [70], results in general agreement with the in vitro rates of P450-catalyzed metabolism. Imidazole can elevate CYP2E1 in rabbits but not in rats [60]. Strain differences in drug oxidation are frequently observed in mice. Qualitative and quantitative differences in the amount of and form of cytochrome P450s present account for many of these aging, sex and species differences found. Human genetic polymorphisms, as discussed above, not only in drug metabolism P450 enzymes but also in phase 2 conjugation enzymes such as individuals who are rapid acetylators versus slow acetylators are especially important in drug development, efficacy and toxicity. For example, the drugs sparteine and debrisoquine are metabolized by CYP2D1 [71]. Poor metabolizers of these drugs are deficient in CYP2D1 and represent a high risk group with respect to adverse drug effects [71].

Diet can have major effects on drug metabolism by modulating CYP activity [72,73]. Poor nutrition can depress P450-catalyzed drug oxidations by lowering synthesis and induction of CYPs, by effects on heme biosynthesis thus altering holoCYP formation and by modulation of cofactor availability. Various components in the diet can affect hepatic drug oxidation for example, St. John's wort can increase hepatic levels of CYP3A4 [74]. CYP inhibitors and inducers are present in foods [75]. This is especially notable for components in grapefruit juice such as naringin which is a potent inhibitor of CYP3A4. Consumption of grapefruit juice is known to alter the half life and bioavailability of many important clinical drugs e.g. statins. Isothiocyanates present in cruciferous vegetables and diallyl disulfide present in garlic inhibit CYP2E1 catalytic activity. Alcohol increases levels of CYP2E1. Charring of food or smoke can induce CYP1A2 which can activate many carcinogens. Levels of CYPs can vary in the fasted versus the fed nutritional state; for example, levels of CYP2E1 are increased after 24 h of starvation [60].

Liver disease can alter the elimination and half life of xenobiotics by altering blood flow to the liver due to hepatic vascular resistance or intrahepatic shunting or extrahepatic shunting of drugs given orally. Impaired synthesis of plasma proteins such as albumin may interfere with drug transport of highly protein-bound drugs in the blood. A decrease in the number of viable hepatocytes or lower activity of residual hepatocytes will lower P450-catalyzed drug oxidation. Cofactor availability, not always considered, may also be compromised by glycogen depletion or pentose cycle impairment and under certain conditions, NADPH may be a limiting factor in overall drug oxidation by P450 [76]. There is a zonal distribution of P450 in the liver acinus as the centrilobular region of the liver generally contains a higher amount of P450 than the periportal zone. For example, CYP2E1 is present, especially after induction by ethanol in the centrilobular zone of the liver [77]. Interestingly, this may, along with other factors (lower oxygen tension, lower levels of GSH), contribute to the initiation and increased toxicity of agents oxidized by CYP2E1 to reactive products including CCl_4, acetaminophen, benzene and ethanol itself in the centrilobular zone. A review of the effects of diseases and environmental factors on human CYPs can be found in [78].

Reactive oxygen formation (ROS)

As mentioned above, small amounts of ROS such as superoxide radical anion and H_2O_2 are produced during the P450 catalytic cycle and cytochrome P450 enzymes are a significant source of ROS in biological systems, especially tissues like the liver where P450 is present in high amounts. Several factors determine the generation of ROS by P450s including the specific form of P450, entry of the second electron into the P450 cycle, the presence of substrate and nature of the substrate [40–43]. The toxicity of many reagents is due, in part, to increased production of ROS when they are metabolized by cytochrome P450s e.g. CCL_4, halogenated hydrocarbons, benzene, acetaminophen, anesthetics, nitrosamines etc. CYP2E1 appears to be significant generator of ROS and this may play a role in alcohol-induced liver toxicity [79]. Using CYP2E1 as the representative P450 enzyme, some examples of ROS production by microsomes are briefly presented below. Fig. 10 shows the generation of superoxide from liver microsomes isolated from chronic ethanol-fed rats (in which CYP2E1 is elevated) and their pair-fed dextrose controls [80,81]. Superoxide was assayed by electron resonance spectroscopy from the interaction of superoxide with a chemical probe which produces a stable nitroxyl radical with defined splitting constants. Superoxide was produced when either NADPH or NADH was the microsomal reductant, however, rates of superoxide production were about 3–4 times greater with NADPH (Fig. 10 spectra b and c) than with NADH (Fig. 10 spectra d and e) with either microsomes from the ethanol-fed or the control rats, in agreement with NADPH being the preferred reductant for the liver microsomal mixed function oxidase system. The rates of superoxide production by microsomes from the ethanol-fed rats were greater with NADH or especially NADPH as the reductants as compared to the microsomes from the control rats. All ESR signals were decreased more than 80% by superoxide dismutase validating the role of superoxide in producing the ESR signal. The increase in the ESR signal by the microsomes from the ethanol-fed rats was blunted by an antibody against CYP2E1, suggesting that induction of CYP2E1 by ethanol played a role in the increase in microsomal superoxide generation.

Fig. 11 shows that chronic ethanol feeding for 4 weeks increased liver lipid peroxidation as compared to the pair-fed

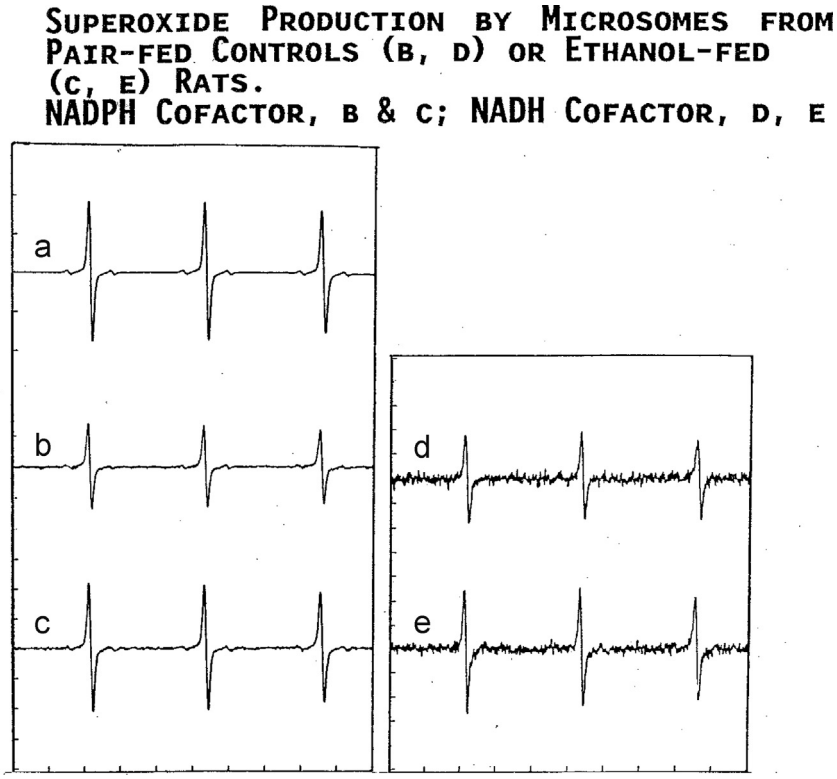


Fig. 10. Microsomal production of superoxide anion radical. Microsomes were isolated from chronic ethanol-fed rats and dextrose pair-fed controls. Generation of superoxide was assayed by determining the superoxide dismutase-sensitive production of the nitroxyl radical formed from the interaction of superoxide with 1-hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidine. Either NADPH (spectra b and c) or NADH (spectra d and e) were used as cofactors. ESR measurements were carried out at room temperature in a flat quartz cuvette using a Bruker E-300 spectrometer. Spectrum a is that of 0.04 mM of a standard nitroxyl radical. Splitting constants for the resulting triplet were $A_N = 16.0$ G and $g = 2.005$. Results are from Refs. [80,81].

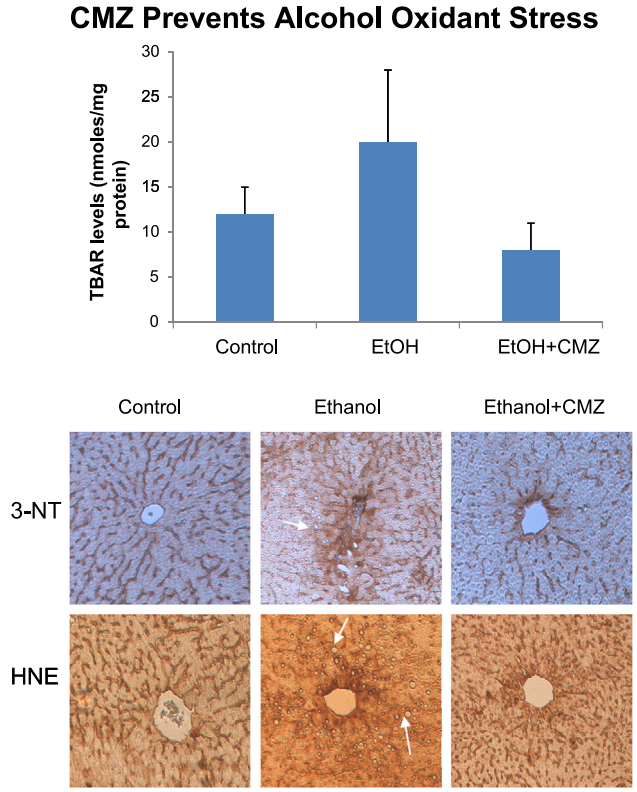


Fig. 11. Chlormethiazole (CMZ) lowers alcohol-induced oxidant stress. SV129 female mice were fed the Lieber-DeCarli ethanol or dextrose liquid diets for 4 weeks. At 2 weeks, some of the ethanol-fed mice were also treated with the CYP2E1 inhibitor, CMZ, at a dose of 50 mg/kg body wt, IP, every other day and fed ethanol for the remaining 2 weeks. Lipid peroxidation in liver homogenates was determined by a thiobarbituric acid (TBARs) assay. Immunohistochemical staining for 3-nitrotyrosine (3-NT) protein adducts or 4-hydroxynonenal (4-HNE) protein adducts was carried out using anti-3-NT or 4-HNE antibodies, followed by a rabbit ABC staining system (Santa Cruz). Results are from Ref. [82].

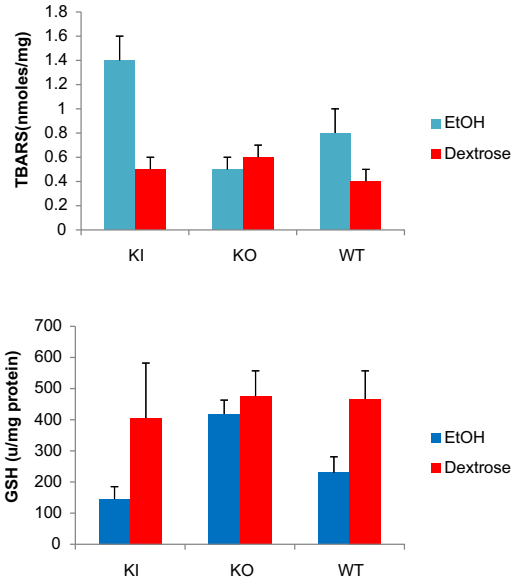


Fig. 12. Alcohol-induced oxidant stress is lower in CYP2E1 knockout (KO) mice. CYP2E1 KO mice and CYP2E1 knockin (KI) mice in which the human 2E1 transgene was introduced into the corresponding mouse null mice to produce a humanized CYP2E1 in the mouse background in the absence of the mouse CYP2E1, were kindly provided by Dr. Frank Gonzalez, NCI, NIH [83,84]. SV129 female wild type (WT) and the KO and the KI mice were fed the ethanol or dextrose diets for 4 weeks. Liver homogenates (1:10) were prepared in 150 mM KCl and assayed for TBARs and for total GSH levels. Results are from Ref. [85].

controls as determined by a TBARs assay. Immunohistochemistry showed that the ethanol feeding increased levels of 3-nitrotyrosine protein adducts and 4-hydroxynonenal protein adducts. Increases in these adducts occurred mostly in the pericentral zone of the liver acinus, the area where alcohol injury in the liver is initiated. Chlormethiazole, a potent inhibitor of CYP2E1, blocked these increases in TBARs and 3-NT and 4-HNE adducts (Fig. 11) [82]. Another approach to evaluate the role of a CYP in ROS generation is to use knockout (KO) mice [83,84]. Fig. 12 shows that chronic ethanol treatment increased levels of TBARs in wild type (WT) mice about 2-fold. No such increases were observed in CYP2E1 KO mice, whereas even greater increases in TBARs were seen in CYP2E1 KI mice in which high levels of CYP2E1 were restored in the KO mice [85]. Similar results were found with respect to hepatic GSH levels; GSH was lowered by ethanol feeding in WT and KI mice but not in KO mice (Fig. 12). Thus, CYPs such as CYP2E1 can produce ROS and increases in ROS production can occur due to induction of CYP2E1. Use of specific inhibitors or appropriate knockout mice are tools to allow evaluation of the role of a specific CYP in ROS production or the elevation in ROS production by a specific treatment.

Personalized medicine

Personalized medicine refers to the customization of healthcare using molecular analysis and tailoring an individual's medical approach and treatment according to his/her genetic information [86,87]. Selection of an individual's appropriate and optimal therapy is based, in part, on the patient's genetic content. This has been successfully applied to pharmacogenomics of the applied therapy which is largely based on evaluation of the presence or absence of specific CYPs and of CYP polymorphisms which are present. Personalized medicine can be used to predict a person's risk for a particular disease based on the presence or absence of a specific gene. With respect to drug therapy, the drug chosen and the dosage used may be due, in part, to the content of CYPs and the presence of certain CYP polymorphisms. Knowledge of the CYPs present and their polymorphic forms can be useful for inclusion or exclusion of certain individual's in clinical trials of new drugs and may increase safety and lower adverse outcomes caused by the drug being tested [88,89]. This information may also lower trial and error approaches to find the most appropriate therapy effective for the patient [88,89]. According to the Personalized Medical Coalition, 137 FDA-approved drugs have pharmacogenomic information in their labeling and 155 pharmacogenomic biomarkers are included in FDA-approved drug labeling. Many of these labels include information on the role of CYPs and effect of polymorphic CYPs on metabolism of the drug [88,89].

The major factor for interindividual differences in drug response is the variable pharmacokinetics, which are due mainly to differences in the content and activity of the CYPs which metabolize the drug. About 50% of human drug metabolism is mainly carried out by the highly polymorphic CYP enzymes, CYP2C9, 2C19 and 2D6 (Table 2). Resulting phenotypes in response to a drug may depend on the specific polymorphism e.g single nucleotide polymorphism, insertion, deletion, copy number variation (Table 5 for CYP2D6) which can give rise to ultrarapid metabolizers (UMs), extensive metabolizers (EMs), intermediate metabolizers (IMs) and poor metabolizers (PMs) [53,56,59]. Problems associated with UMs include lack of a response to the drug when administered at

the normal drug maintenance concentration and perhaps formation of toxic metabolites. PMs have a risk of no therapeutic response and increased risk of adverse drug reactions. For example, for the CYP2D6*1/*2XN which displays gene duplication, there is poor or no response to antiemetics or antidepressants; the latter was associated with increased suicide risk [88]. For the CYP2D6*4 or *5 polymorphisms, which are PMs, there is reduced response to antianalgesics [88]. Besides detoxifying and eliminating drugs, the liver microsomal CYPs are often required to activate certain prodrugs to the active form. Many opiod analgesics are activated by CYP2D6 and in view of the many polymorphisms of CYP2D6, many individuals are relatively insensitive to the analgesic actions of e.g. codeine and there is much interindividual variation in the efficacy of pain relief when "normal" doses of codeine or ethylmorphine are administered. A good example on how CYP2D6 polymorphisms affect the necessary maintenance dosage of a drug is the study by Zanger et al. [90]. The doses of nortriptyline required to achieve therapeutics were about 50 mg in PMs, about 50–100 mg in IMs, 100–150 mg in EMs and 500 mg in UMs. Such differences in required maintenance dosages were in accordance with rates of bufuralol hydroxylation, a marker substrate for CYP2D6 activity.

Warfarin is a widely used oral anticoagulant but has a narrow therapeutic window and there is difficulty in management of effective maintenance doses. Warfarin is metabolized by CYP2C9 and ethnic differences in warfarin maintenance doses requirements are related to CYP2C9 polymorphisms [91]. African-Americans require a higher warfarin dose (about 6 mg/day) while Asians require a lower warfarin maintenance dose (about 3.5 mg/day) compared to Caucasians (about 5 mg/day) [92]. There are many polymorphic forms of CYP2C9; Caucasians possess mainly the CYP2C9*2 and *3 forms and Asians contain mainly the CYP2C9*3 form. These polymorphisms reduce CYP2C9 metabolism of warfarin leading to requirement of lower maintenance doses than in African-Americans in which these two polymorphic forms of CYP2C9 are lower and the authors concluded that additional studies on polymorphic forms of CYP2C9 are needed [92]. Allele frequencies also can differ between Caucasians and Orientals e.g. the CYP2D6*4 allele shows a 12–21% frequency is Caucasians but only a 1% frequency is Orientals while the CYP2D6*10 allele has a frequency of 1–2% in Caucasians but a 50% frequency in Orientals [58], likely contributing to differences in drug responses between these two ethnic groups. Table 3 of Ref. [59] shows the ethnic distribution of the most common variant alleles of CYP2D6 among Caucasians, Asians, Black Africans and Ethiopians/ Saudi Arabians e.g. the latter have the highest frequency of the CYP2D6*2Xn allele which displays gene duplication and increased enzyme activity; such considerations play critical roles in personalized medicine.

Besides the importance of phase I CYPs in personalized medicine, there are also polymorphisms in phase 2 conjugation enzymes which play significant roles in individual differences to drugs e.g. the N-acetyl transferases which can cause the "slow acetylator" phenotype with respect to certain drugs such as isoniazid or hydralazine or sulfamethoxazole [93].

In view of the importance of the liver microsomal CYPs in personalized medicine, identifying which CYPs and which polymorphic forms are present is of major value in choosing the most appropriate drug and drug dosage. The concept of CYP on a chip has been developed as a major tool for this. For example, the AmpliChip CYP450 (Roche Molecular Diagnostic) test evaluates the CYP2D6 genotype and makes phenotype predictions [94]. This chip allows simultaneous analysis of 33 CYP2D6 alleles [94]. Rabsamen et al. [95] verified the genotyping accuracy of the chip for the 5 major CYP2D6 alleles and confirmed these results with real time PCR. An updated version of the AmpliChip contains 3 CYP19 alleles in addition to the 33 CYP2D6 alleles with the goal "to help clinicians determine therapeutic strategy and treatment doses for therapeutic metabolism by the CYP2D6 and CYP2C19" [96].

Conclusions

Because of many of the above described factors modulating levels of CYPs as well as polymorphisms of many CYPs, there is large variability in content of the xenobiotic metabolizing CYPs in families 1, 2 and 3 in human livers, which likely accounts for the large variability in drug oxidation by humans. Guengerich evaluated the levels of CYPs 1A2, 2E1 and 3A4 in 18 human liver samples [37]. There were 5–10 folds differences in levels of each of the 3 CYPs among the 18 samples [37]. Because of this variability in levels of a specific P450, the presence of multiple CYPs with overlapping substrate specificity and the ability of a CYP to metabolize many structurally distinct substrates, there is extensive overlapping substrate specificities which likely contributes to drug–drug interactions and adverse drug reactions [97]. Attempts to minimize such adverse effects will require further studies on structure–function relationships of CYP enzymes, further information on links between CYP polymorphisms and disease/toxicity, identification of what are the substrates for orphan CYPs [98], and developing bioinformatic models to predict whether certain drugs and chemicals may be toxic or carcinogenic or act to induce a CYP. Development of designer CYPs which may be useful in removal of toxins, pollutants, oil *etc.* and engineering CYPs to extend their catalytic capabilities as discussed in Refs. [99,100] are likely to further extend the activities of this versatile family of enzymes.

Acknowledgments

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