Bioactivation of chemicals by cytochromes P450

Costas Ioannides*

Molecular Toxicology Group, School of Biomedical and Molecular Sciences,
University of Surrey, Guildford, Surrey, GU2 7XH, UK
Tel.: +44 1483 689709, Fax: +44 1483 686401
E-mail: c.ioannides@surrey.ac.uk

ABSTRACT

Most chemical carcinogens require metabolism to generate highly reactive intermediates that manifest their carcinogenicity following covalent interaction with DNA. This bioactivation process is catalysed chiefly by cytochromes P450, a superfamily of enzymes localised primarily in the liver. The carcinogenic activity of a chemical is largely dependent on the profile and concentration of cytochromes P450 at the time of exposure.

INTRODUCTION

In order to survive and thrive in an increasing chemical environment, the living organism had to adapt and develop processes that would allow it to readily eliminate chemicals (xenobiotics), thus preventing them from accumulating with detrimental consequences. Chemicals that reach the systemic circulation are by nature lipophilic so as to traverse readily lipoid barriers such as the intestinal lining. Humans are exposed involuntarily, on a daily basis, to thousands of such chemicals, largely through the diet, that can not be exploited advantageously. However, humans are not adequately equipped to remove lipophilic compounds through excretion. For example, renal excretion of lipophilic chemicals by glomerular filtration and active tubular secretion may be effectively antagonised by passive reabsorption, so that overall renal excretion is minimal. As a result, in order to facilitate excretion, lipophilic chemicals must be metabolically converted to hydrophilic products, and this is achieved by a process referred to as 'biotransformation'. Consequently, the human body has developed a number of broad-specificity enzyme systems that can convert lipophilic chemicals to hydrophilic, readily excretable metabolites. Biotransformation of chemicals occurs in two distinct phases; during phase I a functional group is generated, e.g. –OH, either by insertion or unmasking, that allows the chemical to proceed to phase II metabolism where it conjugates with endogenous substrates, such as sulphate, glucuronic acid and glutathione, to produce highly hydrophilic metabolites that can be removed efficiently by renal and/or biliary excretion. For example, 7-hydroxycoumarin can be generated either by the hydroxylation of coumarin (insertion of an oxygen atom) or the *O*-deethylation of 7-ethoxycoumarin (unmasking). The hydroxylated metabolite is further conjugated with either sulphate or glucuronic acid and excreted (Figure 1).

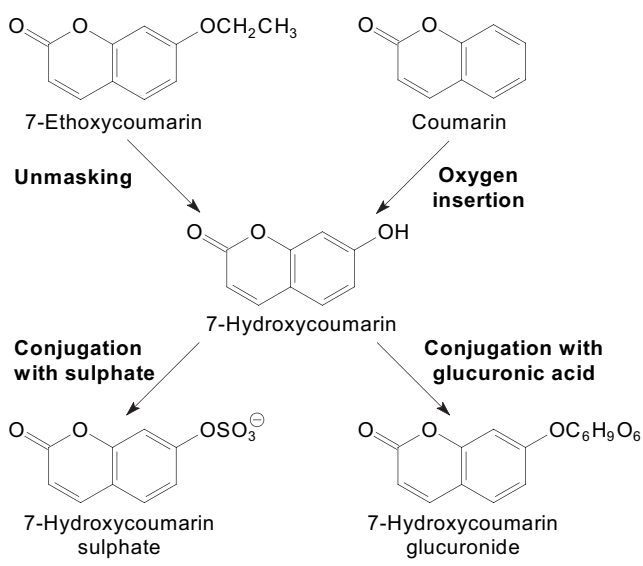

Figure 1. Phase I and Phase II metabolism.

* Professor Costas IOANNIDES was an invited speaker at The First International Environmental Best Practices Conference, 07-10 August 2006, Olsztyn, Poland

THE CYTOCHROME P450 ENZYME SYSTEM

Although a number of enzyme systems contribute to the biotransformation of chemicals (Ioannides 2002a), undoubtedly the most important is cytochrome P450, a ubiquitous haemoprotein, localised on the endoplasmic reticulum (Danielson 2002); it acts primarily as a Phase I oxygenase, although it can also operate as a reductase under conditions of low oxygen tension (Guengerich 2002). The function of the cytochrome P450 system is not confined to exogenous chemicals but it also plays a key role in the metabolism, both biosynthesis and catabolism, of endogenous substrates including hormones such as steroids and melatonin, eicosanoids such as prostaglandins, vitamins such as vitamin D, sterols such as cholesterol and fatty acids such as lauric acid. Indeed, it is believed that all cytochrome P450 proteins originate from the same ancestor that was initially characterised by narrow specificity metabolising endogenous chemicals, but evolved to a broad-substrate enzyme system as a response to the diversity of exogenous chemicals it is now exposed to.

The cytochrome P450 achieves its broad specificity by existing as a superfamily of enzymes that is divided into a number of families; each family in turn is further subdivided to subfamilies that may comprise one or more enzymes. For example, family one is composed of two subfamilies, A and B (Figure 2). The former contains two enzymes – CYP1A1 and CYP1A2 – whereas the latter consists of a single enzyme, CYP1B1. Classification of enzymes within the same family or subfamily is based strictly on structural similarity; enzymes sharing a structural similarity of 40% belong to the same family, whereas if similarity exceeds 55% they are classified within the same subfamily.

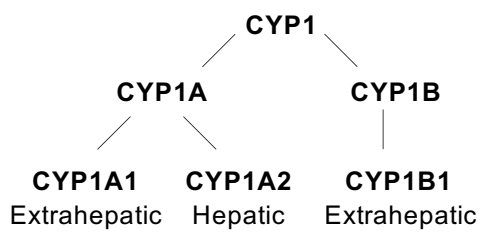

Figure 2. The CYP1 family.

It has long been recognised that individuals can display markedly different response to the biological activity of the same dose of a chemical, and it was suspected that differences in metabolic handling, at least in part, may be the underlying mechanism. Indeed, subsequent work established that cytochrome P450 activity in humans can be modulated by a number of factors including exposure to phytochemicals through the diet or intake of herbal products (Ioannides 1999, 2002b), and the presence of disease (Ioannides et al. 1996), but the most important appear to be genetic makeup (Ingelman-Sundberg 2005), and concurrent or prior exposure to chemical resulting in induction (Xu et al. 2005) or inhibition (Zhou et al. 2005). Xenobiotic-metabolising cytochromes P450 may be polymorphically expressed and, if this is not appreciated and the necessary steps taken to adjust drug dosage accordingly, it may have a dramatic impact in clinical therapeutics (Gardiner and Begg 2006; Ingelman-Sundberg 2005).

PIVOTAL ROLE OF CYTOCHROMES P450 IN THE TOXICITY OF CHEMICALS

It was realised in the 1970s that, in most cases, the toxicity of chemicals was mediated by metabolites, i.e. metabolism led to the generation of toxic entities; this observation challenged the long-held view that cytochromes P450, as well as other enzyme systems participating in xenobiotic metabolism, were exclusively involved in the deactivation and elimination of chemicals, but pointed out that metabolism could in fact assume the reverse role i.e. convert innocuous chemicals to reactive, toxic metabolites with deleterious consequences to the body, this process being frequently referred to as 'metabolic activation' or 'bioactivation'. Thus inert chemicals can acquire chemical reactivity through metabolism. The reactive intermediates are electophiles that readily engage into covalent binding with nucleophiles; thus, they interact covalently with vital cellular macromolecules such as DNA and protein, giving rise to different forms of toxicity. Alternatively, reactive intermediates may interact with molecular oxygen generating reactive oxygen species and, in this way, cause toxicity indirectly (Figure 3). Simultaneously, a chemical may be subjected to alternative metabolic pathways that may lead to inactive metabolites. Thus, the balance of activation/deactivation is crucial in determining the metabolic and toxicological fate of a chemical, and any factor that perturbs this balance will also influence the biological outcome.

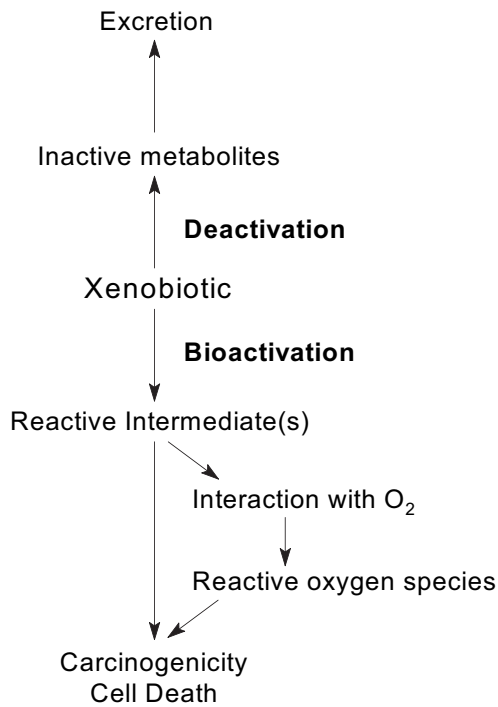

Figure 3. Generation of reactive intermediates.

Cytochromes P450 and drug toxicity

Paracetamol (acetaminophen), a widely used drug, helps illustrate the importance of bioactivation in drug toxicity; it is primarily metabolised by phase II conjugation, since it already possesses a functional group, the principal metabolites being the sulphate and glucuronide conjugate. To a minor extent it undergoes a cytochrome P450-mediated oxidation to generate a reactive quinoneimine, which the body effectively neutralises through conjugation with glutathione (Figure 4). As a result, no adverse interaction takes place with hepatic proteins, and paracetamol enjoys a remarkable record of safety at therapeutic level. However, the oxidation pathway which is normally a minor metabolic route can become more dominant when the activation pathway is selectively stimulated. One of the cytochrome P450 enzymes catalysing the bioactivation of paracetamol is CYP2E1, whose expression is enhanced by chronic intake of alcohol, a selective inducer of this enzyme. As a result chronic alcoholics are vulnerable to the hepatotoxicity of this drug (Seeff et al. 1986; Zimmerman and Maddrey 1995).

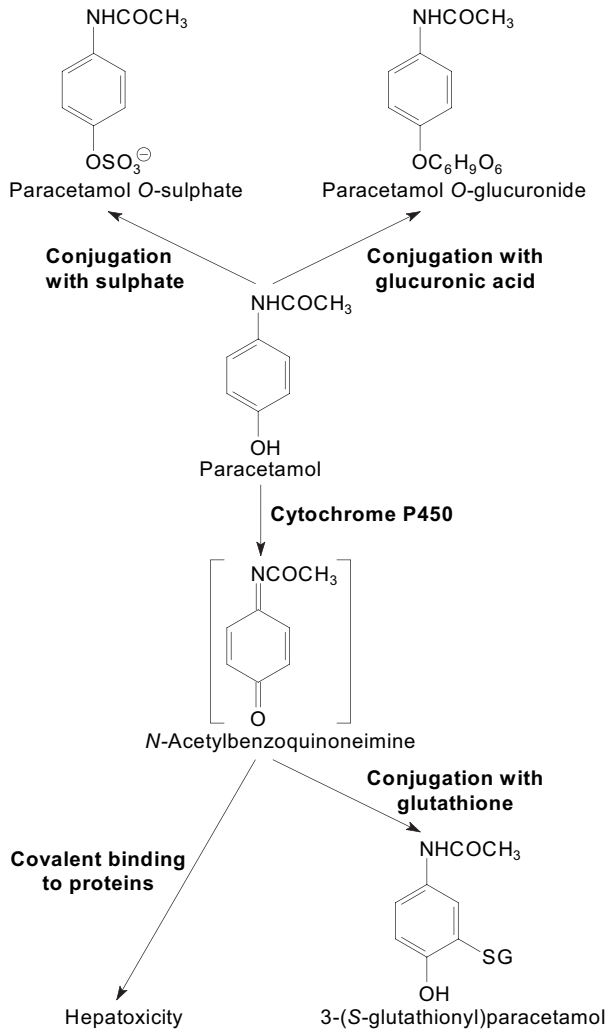

Figure 4. Metabolism of paracetamol.

Cytochromes P450 and chemical carcinogenesis

The vast majority of chemical carcinogens require metabolic activation to form genotoxic intermediates that elicit their carcinogenicity. Although every enzyme system, both phase I and phase II, has the potential to contribute to the bioactivation process, cytochromes P450 are the most closely associated with chemical carcinogenesis. They are involved in the bioactivation of most chemical carcinogens, either alone or in conjunction with other enzyme systems. For example, cytochrome P450 proteins, such as CYP1A2 and CYP3A4, are responsible for the metabolic activation of the mycotoxin aflatoxin B_1 (Figure 5). On the other hand, the bioactivation of 4-aminobiphenyl requires the interplay of cytochrome P450 enzymes and conjugation systems (Figure 6). The CYP1 family is responsible for the first oxidative step to produce the hydroxylamine, which is subsequently converted to the unstable acetoxy- and sulphatoxy-esters that break down spontaneously to release the nitrenium ion, the putative entity that interacts with DNA.

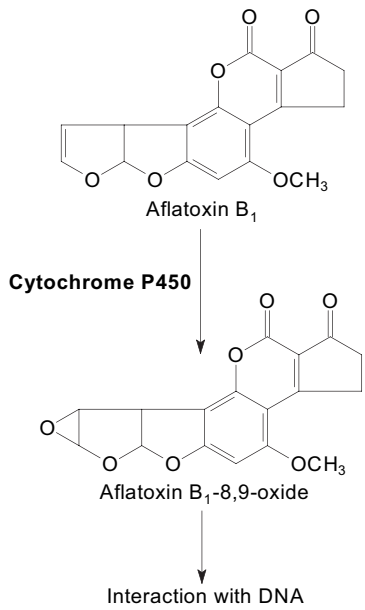

Figure 5. Bioactivation of aflatoxin B_1.

A number of cytochrome P450 enzymes may participate in the metabolism of a chemical carcinogen, but may display regio--selectivity, i.e. metabolise the molecule at different sites. As a result, a particular cytochrome P450 enzyme may be responsible for the activation process whereas others are limited to catalysing deactivation pathways. The bladder carcinogen 2-naphthylamine is activated selectively by CYP1A2 through N-hydroxylation, whereas many isoforms catalyse its ring-oxidation at positions 1- and/or 6-, both being deactivation reactions, as these phenols are conjugated and excreted (Hammons et al. 1985) (Figure 7). So clearly, for chemical to display carcinogenicity it is not sufficient to have the propensity of produce a reactive intermediate but, in addition, at the time of exposure, the living organism must

possess the necessary enzymic complement to favour the activation pathways. Thus the cytochrome P450 profile at the time of exposure regulates the fate of a chemical as it determines whether the activation or deactivation pathways predominate. For example, the cynomolgous monkey lacks the enzyme, namely CYP1A2, that is responsible for the activation through *N*-hydroxylation of the heterocyclic amine 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and is consequently refractive to its carcinogenicity, whereas rodents catalyse the activation of this carcinogen and are susceptible (Ogawa et al. 1999).

Since the liver is the tissue endowed with the highest cytochrome P450 activity, it would be logical to expect it to serve as the major site of carcinogen activation and, as a consequence, should be the principal target of chemically-induced tumours. In reality, however, the breast, colon, prostate, bladder and lungs, tissues which in comparison with the liver have low cytochrome P450 levels and limited metabolic capability, are more frequent sites of tumour formation. What apparently appears to happen is that the reactive intermediates of carcinogenic compounds can somehow be transported to other tissues where they can initiate tumours. For example, the dihydrodiol-epoxides, the principal DNA-binding metabolites of polycyclic aromatic hydrocarbons, produced largely in the liver may be transferred into other tissues where they can interact with DNA (Wall et al. 1991). Similarly, *N*-hydroxylamines, formed as a result of cytochrome P450 oxidation, undergo *N*-glucuronidation and in this form can reach the urinary bladder where, under the prevailing acidic conditions, they release the hydroxylamine that is converted to the nitrenium ion, the DNA-binding entity (Zenser et al. 1999).

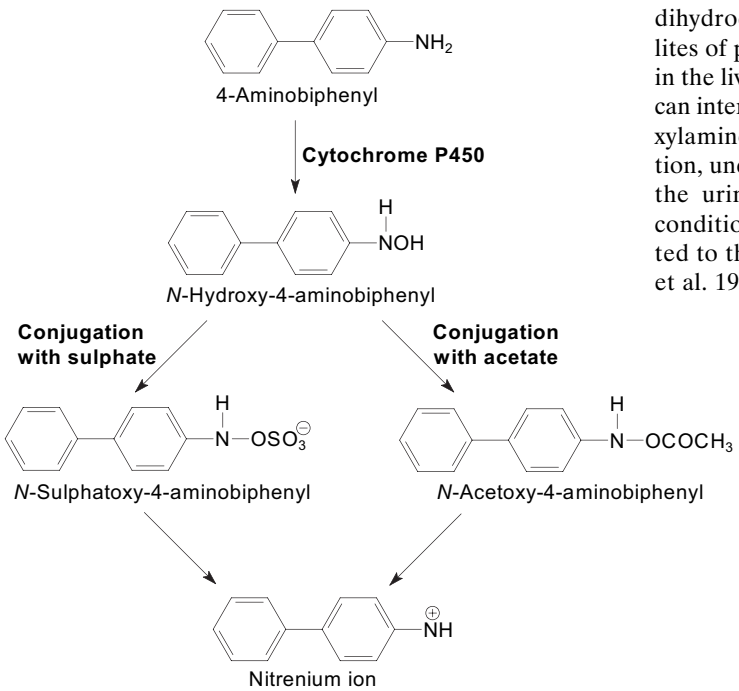

Figure 6. Bioactivation of 4-aminobiphenyl.

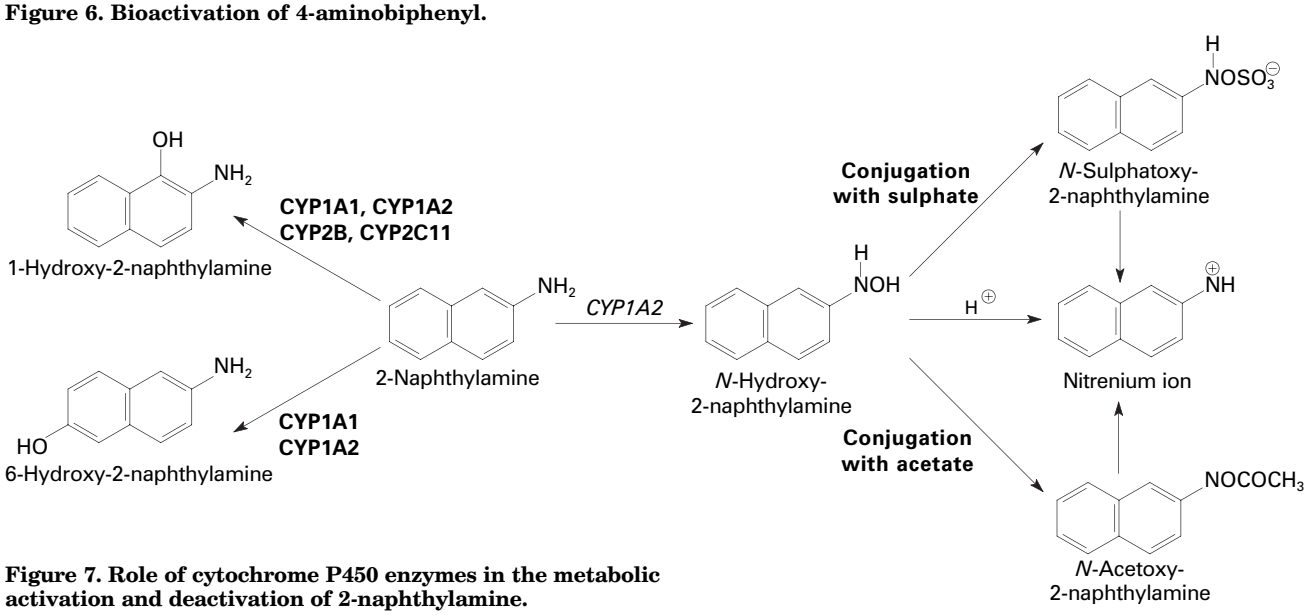

Figure 7. Role of cytochrome P450 enzymes in the metabolic activation and deactivation of 2-naphthylamine.

Induction of cytochromes P450 and chemical carcinogenesis

A characteristic of many chemical carcinogens is that, on repeated administration, they can selectively and markedly induce the cytochrome P450 enzymes that are active in their metabolism, thus influencing the rate of metabolism as well as the metabolic profile. Thus, the first dose of a carcinogen is metabolised by a different, constitutive cytochrome P450 complement, whereas subsequent doses are metabolised by a different complement of cytochromes P450, arising as a result of induction. CYP1 is probably the most inducible family, being induced by planar compounds in the liver and extrahepatic tissues of animals and humans (Christou et al. 1995; Ioannides and Parke 1990). Treatment with the polycyclic aromatic hydrocarbon, 3-methylcholanthrene, can elevate the expression of this family in rats so that it comprises as much as 80% of the total hepatic cytochrome P450 content (Ryan and Levin 1990). Indeed, in many cases carcinogenic activity within a class of compounds, may be related to the propensity to induce the bioactivating cytochrome P450 protein, particularly in the case of CYP1-mediated bioactivation. For example, DBA/2 mice, a strain refractive to CYP1 induction, were less susceptible to the carcinogenicity of benzo[*a*]pyrene compared with the C57/BL strain, where CYP1 activity is readily inducible (Nebert 1989). In another animal study, of ten generations of rats exposed to 3´-methyl-4-dimethylaminoazobenzene, a CYP1-activated carcinogen, the 4th to 8th generations were resistant to the carcinogenicity of this chemical compared to the other generations; the resistant generations also displayed poor CYP1 inducibility and, consequently, their ability to bioactivate this carcinogen was diminished (Yano et al. 1989; Yoshimoto et al. 1985). These studies support the premise that the ability of a chemical to stimulate its own activation pathways may be a critical factor in determining its carcinogenic activity, and help explain the markedly different carcinogenic activity between structurally-related chemicals, even isomers (Ioannides 1990). For example, between isomers, carcinogenic potency could be related to the ability to upregulate CYP1A. Benzo[*a*]pyrene, 4-aminobiphenyl and 2-naphthylamine, all established carcinogens, were far more potent inducers of hepatic CYP1A than their non-carcinogenic isomers benzo[*e*]pyrene, 2-aminobiphenyl and 1-naphthylamine (Ayrton et al. 1990a, 1990b). Similarly, it was reported that the carcinogenic activity of polycyclic aromatic hydrocarbons was related to their ability to induce CYP1A1 and CYP1B1 activity (Shimada et al. 2002). In order to further define this relationship, extensive studies were undertaken to correlate CYP1A induction, binding to the Ah receptor that regulates the CYP1 induction process, and carcinogenic activity among isomers or structurally related compounds. Such studies revealed that the treatment of animals with the carcinogenic 2-acetylaminofluorene resulted in the induction of both CYP1A isoforms, whereas the 4-isomer increased the levels of only CYP1A2; in concordance with these findings, the 2-isomer binds to the Ah receptor markedly more avidly than the 4-isomer (Ioannides et al. 1993). In studies involving six azobenzenes, a correlation was established between binding to the Ah receptor and CYP1A induction on the one hand, and carcinogenic activity on the other (Cheung et al. 1994). A similar picture emerged when isomeric diaminotoluenes or diaminonaphthalenes and related compounds were used as models (Cheung et al. 1996, 1997). In addition to CYP1 induction, the Ah receptor mediates many other activities including protein kinase C, whose activation culminates in accelerated DNA replication, dedifferentiation and cellular proliferation, critical stages in the carcinogenesis process (Sogawa and Fujii-Kuriyama 1997). It may be, therefore, that an increase in the promotion stages of carcinogenesis, mediated by the Ah receptor, is also an important aspect, in addition to CYP1 induction. It is pertinent to point out that Ah receptor knockout mice were resistant to the carcinogenicity of benzo[*a*]pyrene (Shimizu et al. 2000).

CONTRIBUTION OF INDIVIDUAL CYTOCHROME P450 FAMILIES/SUBFAMILIES TO THE BIOACTIVATION OF CARCINOGENS

Although all xenobiotic-metabolising cytochrome P450 enzymes can contribute to the bioactivation of chemical carcinogens, there are pronounced differences in the role of individual families/ subfamilies, the most active being the CYP1 family and the CYP2E subfamily, whereas the CYP2D subfamily makes virtually no contribution (Ioannides and Lewis 2004).

CYP1 in the bioactivation of chemical carcinogens

CYP1 is a small family comprising two subfamilies, 1A and 1B. The former contains two proteins whereas the latter contains a single protein (Figure 2). CYP1A2 is primarily expressed in the liver, but the hepatic levels of CYP1A1 and CYP1B1 are very low. In contrast, the latter two proteins are present in appreciable amounts in extrahepatic tissues (Bhattacharyya et al. 1995; Guengerich 1990). It is probably the most conserved family within the phylogenetic tree so that the human CYP1 proteins share extensive structural similarity and display similar substrate specificity to the orthologous rodent proteins.

The substrates of the CYP1 family are essentially lipophilic planar molecules, composed of fused aromatic rings and characterised by a small depth and a large area/depth^2 ratio, and their planar nature presumably facilitates their interaction with DNA (Lewis et al. 1986, 1987). The CYP1 family is believed to be responsible for the activation of more than 90% of known carcinogenic chemicals (Rendic and Di Carlo 1997). Most ubiquitous environmental and dietary carcinogens to which humans are frequently exposed, such as polycyclic aromatic hydrocarbons, heterocyclic amines and mycotoxins, are molecularly planar in nature, and therefore favoured substrates of the CYP1 family. The CYP1 family, through arene oxidation and *N*-oxidation activates polycyclic aromatic hydrocarbons, aromatic amines, aflatoxins, aminoazobenzenes and heterocyclic amines (Ioannides and Parke 1990; Kim et al. 1998;

Shimada et al. 1996). Generally, CYP1A1 is more effective in catalysing arene oxidation and CYP1A2 *N*-oxidation (Ioannides and Parke 1990). However, CYP1B1 is also an excellent catalyst of the arene oxidation of polycyclic aromatic hydrocarbons, and can catalyse both of the cytochrome P450-mediated oxidations that are necessary to produce the ultimate carcinogenic species, the dihydrodiol epoxides (Kim et al. 1998). Its importance in the bioactivation of polycyclic aromatic hydrocarbons is highlighted by the observation that lymphoma incidence following exposure to 7,12-dimethylbenz[*a*]anthracene was much lower in CYP1B1-null mice compared with the wild-type mice (Buters et al. 1999). All three members of the CYP1 family have also been shown to be effective in the activation of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a metabolite of nicotine (Smith et al. 1992). Both CYP1A proteins, especially CYP1A1, are involved in the hydroperoxide-dependent oxidation of catechol oestrogens including the carcinogenic synthetic stilbene, diethylstilboestrol, to the genotoxic quinones (Roy et al. 1992). CYP1B1 hydroxylates 17β-oestradiol, primarily at the 4-position and appears to be the principal catalyst of this hydroxylation pathway, whereas CYP1A1 metabolises the steroid less efficiently, at different positions (Hayes et al. 1996). In subsequent studies, CYP1A2 was shown to be an important catalyst of the 2- and 4-hydroxylations of oestradiol and oestrone (Yamazaki et al. 1998). It has been suggested that the 4-hydroxyoestradiol can directly interact covalently with DNA, or be a precursor for such a genotoxic metabolite, probably a quinone or a semiquinone, which may also act as a generator of genotoxic free radicals and may be involved in the aetiology of breast cancer. Interestingly, CYP1B1 appears to be over-expressed in many human tumours (Murray et al. 1997) which is in contrast to other cytochrome P450 proteins whose levels are usually depressed in tumours (Ioannides et al. 1996). Because of its prominent role in the activation of chemical carcinogens, high levels of CYP1 are considered undesirable (Ioannides and Parke 1993). A number of studies have linked CYP1 activity to human lung cancer (Anttila et al. 1991; Bartsch et al. 1992).

CYP2E in the bioactivation of chemical carcinogens

In both humans and animals the CYP2E subfamily consists of a single enzyme (CYP2E1), the only exception being the rabbit where two proteins appear to be expressed. CYP2E1 is expressed in both hepatic and extrahepatic tissues and, in common with the CYP1 family, is one of the most conserved subfamilies in animal species, and the orthologues share similar substrate specificity.

CYP2E1 metabolises small molecular weight compounds, and its substrates are characterised by a small molecular diameter of $<$ 6.5 Å (Lewis et al. 1993). It plays a dominant role in the metabolism of small molecular weight carcinogenic compounds such as azoxymethane, benzene, 1,3-butadiene, nitrosamines like dimethylnitrosamine and nitrosopyrrolidine, and halogenated hydrocarbons such as carbon tetrachloride and vinyl chloride (Guengerich and Shimada 1991; Sohn et al. 2001; Yamazaki et al. 2002). Indeed, benzene and carbon tetrachloride failed to induce toxicity in *CYP2E1*-knockout mice whereas severe toxicity was seen in the wild-type animals (Valentine et al. 1996; Wong et al. 1998). A major and toxicologically important characteristic of the CYP2E subfamily is its high propensity to generate reactive oxygen species (Ronis et al. 1996). CYP2E1 may therefore facilitate carcinogenesis by two distinct mechanisms, namely the oxidative activation of chemicals and by the generation of reactive oxygen species.

Bioactivation of chemical carcinogens by other cytochrome P450 proteins

The role of CYP2A enzymes in the bioactivation of environmental chemical carcinogens is not extensive. They appear to make a significant contribution to the human hepatic metabolic activation of *N*-nitrosamines, including a number of tobacco nitrosamines, and of 1,3-butadiene (Duescher and Elfarra 1994; Kamataki et al. 2002; Yamazaki et al. 1992). A role has also been ascribed for the CYP2A subfamily in the activation of the fungal contaminant aflatoxin B_1 (Aoyama et al. 1990). Rat CYP2A3 and human CYP2A6, which are present in olfactory mucosa, can bioactivate the nasal toxin 2,6-dibenzonitrile and the carcinogen hexamethylphosphoramide (Liu et al. 1996). Human CYP2A13 displays high activity in the bioactivation of the nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Su et al. 2000).

The CYP2B subfamily, although involved in the metabolism of many chemical carcinogens, it appears to direct the metabolism primarily towards the formation of inactive metabolites, so that a deactivating role has been ascribed to this subfamily (Ioannides and Parke 1990). For example, CYP2B enzymes can metabolise effectively aromatic amines and amides, but they are unable to carry out *N*-hydroxylation, the activation pathway, and only catalyse ring-oxidations that lead to biologically inactive metabolites (Figure 7) (Astrom and De Pierre 1985; Hammons et al. 1985). However, they participate in the bioactivation of a number of long-chain nitrosamines to genotoxic metabolites (Shu and Hollenberg 1997). The human CYP2B6 protein has also been shown to contribute to the activation of aflatoxin B_1 and NNK (Code et al. 1997).

The CYP2C subfamily appears to play a very minor role in the bioactivation of chemical carcinogens (Shimada et al. 1989). In rats, however, members of the CYP2C family are efficient in converting aflatoxin B_1 to the reactive 8,9-epoxide (Figure 5) (Shimada et al. 1987) whereas human CYP2C enzymes can bioactivate polycyclic aromatic hydrocarbons, but much less effectively compared with CYP1A1, and thus may contribute to their hepatic bioactivation (Shou et al. 1996). No major role in the bioactivation of chemicals is associated with the CYP2D subfamily.

CYP3 is the most abundant family in human liver, comprising a single subfamily, the principal protein being

CYP3A4, and among cytochrome P450 enzymes it is the most active contributor to drug metabolism (Rendic and Di Carlo 1997). It is also present at high concentrations in the intestine (McKinnon et al. 1995) where it is responsible for the first-pass (presystemic) metabolism of many drugs. A number of carcinogenic chemicals have been shown to be bioactivated by the CYP3A subfamily, the most important being aflatoxin B_1 and dihydrodiols of polycyclic aromatic hydrocarbons in whose metabolism other cytochrome P450 proteins also participate (*vide supra*) (Aoyama et al. 1990; Kitada et al. 1990; Shimada et al. 1989). Members of the CYP3A family have also been shown to contribute to the bioactivation of 6-aminochrysene (Yamazaki et al. 1995) and of the heterocyclic amine 2-amino-3-methylimidazo-[4,5-*f*]quinoline (IQ) (Kitada et al. 1990).

The role of the various cytochrome P450 enzymes in the bioactivation of chemical carcinogens, and their inducibility are summarised in Table 1.

CONCLUSIONS

Most toxic compounds are chemically inert, precluding any interactions with cellular macromolecules. In the liver primarily, but also in other tissues, however, these chemicals are metabolically transformed to highly reactive intermediates capable of irreversible interactions with cellular components with adverse consequences. Clearly, toxicity is not simply a consequence of the intrinsic molecular structure of the chemical, but is also determined by the complement of enzymes involved in the bioactivation/deactivation of the chemical, especially cytochromes P450, at the time of exposure. Thus, for a chemical to provoke toxicity at least two prerequisites must be fulfilled: (a) the chemical must be, or must have the propensity to be metabolically converted to a reactive intermediate(s), and (b) the living organism, at the time of exposure, must possess the necessary enzyme(s) required for the activation of the chemical. The overall contribution of an individual cytochrome P450 enzyme in the bioactivation of chemicals will depend not only on its catalytic activity, but also on its tissue levels as well as the tissue concentration of the chemical. At high tissue concentrations of the chemical, more than one cytochrome P450 enzyme may be involved, as low affinity enzymes may also contribute significantly to metabolism. Obviously, the amount of reactive intermediate produced, and hence incidence and degree of toxicity, will be largely dependent on the competing pathways of activation and deactivation, and whatever factor influences this delicate balance of activation/deactivation of a chemical will also impact on its biological activity and safety.

Undoubtedly the initial view that the cytochrome P450 enzyme system functions simply in the metabolism of xenobiotics to readily excretable metabolites, to enable the elimination of these from the body is anachronistic on the face of mounting evidence that this system can also convert innocuous chemicals to toxic products, detrimental to the welfare of the living organism.

Table 1. Xenobiotic-metabolising cytochromes P450: inducibility and their role in the bioactivation of chemical carcinogens.

Cytochrome P450 subfamily	Role in chemical carcinogenesis	Major classes of activated carcinogens	Inducibility
CYP1A	Very extensive	PAH, AA, HA, MC, NNK, AAB	Very high
CYP1B	Very extensive	PAH, AA, HA, MC, NNK, AAB, Oestrogens	Very high
CYP2A	Moderate	NA, OP	Moderate
CYP2B	Moderate	NA, OP	High
CYP2C	Minor	PAH	Moderate
CYP2D	Poor	NNK	Not inducible
CYP2E	Extensive	NA, HH	High
CYP2F	Not evaluated	–	Not evaluated
CYP2G	Not evaluated	–	Not evaluated
CYP2J	Not evaluated	–	Not evaluated
CYP3A	Moderate	PAH, MC, PA	High

PAH, Polycyclic aromatic hydrocarbons; AA, Aromatic amines; HA, Heterocyclic amines; MC, Mycotoxins; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; AAB, Aminoazobenzenes; OP, Oxazophosphorines; NA, Nitrosoamines; HH, Halogenated hydrocarbons; PA, Pyrrolizidine alkaloids.

REFERENCES

Anttila, S., E. Hietanen, H. Vainio, A-M. Camus, H.V. Gelboin, S.S Park, L. Heikkila, A. Karjalainen, H. Bartsch. 1991. Smoking and peripheral type of cancer are related to high levels of pulmonary cytochrome P450IA in lung cancer patients. International Journal of Cancer 47: 681-685.

Aoyama, T., S. Yamano, P.S. Guzelian, H.V. Gelboin, F.J. Gonzalez. 1990. Five of 12 forms of vaccinia virus-expressed human hepatic cytochrome P450 metabolically activate aflatoxin B_1. Proceedings of the National Academy of Sciences USA 87: 4790-4793.

Astrom, A., J.W. De Pierre. 1985. Metabolism of 2-acetylaminofluorene by eight different forms of cytochrome P-450 isolated from rat liver. Carcinogenesis 6: 113-120.

Ayrton, A.D., M. McFarlane, R. Walker, S. Neville, C. Ioannides. 1990a. The induction of P450 I proteins by aromatic amines may be related to their carcinogenic potential. Carcinogenesis 11: 803-809.

Ayrton, A.D., M. McFarlane, R. Walker, S. Neville, M.M. Coombs, C. Ioannides. 1990b. Induction of the P450 I family of proteins by polycyclic aromatic hydrocarbons. Possible relationship to their carcinogenicity. Toxicology 60: 173-186.

Bartsch, H., M. Castegnaro, M. Rojas, A-M. Camus, K. Alexandrov, M. Lang. 1992. Expression of pulmonary cytochrome P4501A1 and carcinogen adduct formation in high risk subjects for tobacco--related lung cancer. Toxicology Letters 64/65: 477-483.

Bhattacharyya, K.K., P.B. Brake, S.E. Eltom, S.A. Otto, C.R. Jefcoate. 1995. Identification of a rat adrenal cytochrome P450 active in polycyclic-hydrocarbon metabolism as rat CYP1B1- demonstration of a unique tissue-specific pattern on hormonal and aryl-hydrocarbon receptor-linked regulation. Journal of Biological Chemistry 270: 11595-11602.

Buters, J.T.M., S. Sakai, T. Richter, T. Pineau, D.L. Alexander, U. Savas, J. Doehmer, J.M. Ward, C.R. Jefcoate, F.J. Gonzalez. 1999. Cytochrome P450 CYP1B1 determines susceptibility to 7,12-dimethylbenz[*a*]anthracene-induced lymphomas. Proceedings of the National Academy of Sciences USA 96: 1977-1982.

Cheung, Y-L., S.M. Puddicombe, T.J.B. Gray, C. Ioannides. 1994. Mutagenicity and CYP1A induction by azobenzenes correlates with their carcinogenicity. Carcinogenesis 15: 1257-1263.

Cheung, Y-L., J. Snelling, N.N.D. Mohammed, T.J.B. Gray, C. Ioannides. 1996. Interaction with the aromatic hydrocarbon receptor, CYP1A induction, and mutagenicity of a series of diaminotoluenes: implications for their carcinogenicity. Toxicology and Applied Pharmacology 139: 203-211.

Cheung, Y-L., D.F.V. Lewis, T.I. Ridd, T.J.B. Gray, C. Ioannides. 1997. Diaminonaphthalenes and related aminocompounds: Mutagenicity, CYP1A induction and interaction with the Ah receptor. Toxicology 118: 115-127.

Christou, M., U. Savas, S. Schroeder, X. Shen, T. Thompson, M.N. Gould, C.R. Jefcoate. 1995. Cytochromes CYP1A1 and CYP1B1 in the rat mammary gland: Cell-specific expression and regulation by polycyclic aromatic hydrocarbons and hormones. Molecular and Cellular Endocrinology 115: 41-50.

Code, E.L., C.L. Crespi, B.W. Penman, F.J. Gonzalez, T.K.H. Chang, D.J. Waxman. 1997. Human cytochrome P4502B6 – Interindividual hepatic expression, substrate specificity, and role in procarcinogen activation. Drug Metabolism and Disposition 25: 985-993.

Danielson, P.B. 2002. The cytochrome P450 superfamily: Biochemistry, evolution and drug metabolism in humans. Current Drug Metabolism 3: 561-597.

Duescher, R.J., A.A. Elfarra. 1994. Human liver microsomes are efficient catalysts of 1,3-butadiene oxidation: evidence of major roles by cytochrome P4502A6 and 2E1. Archives of Biochemistry and Biophysics 311: 342-349.

Gardiner, S.J, E.J. Begg. 2006. Pharmacogenetics, drug-metabolizing enzymes, and clinical practice. Pharmacological Reviews 58: 521-590.

Guengerich, F.P. 1990. Characterization of roles in human cytochrome P-450 enzymes in carcinogen metabolism. 1990. Asia Pacific Journal of Pharmacology 5: 327-345.

Guengerich, F.P. 2002. Cytochrome P450. In: Enzyme Systems that Metabolise Drugs and Other Xenobiotics (ed. C. Ioannides), pp. 33-65. John Wiley & Sons, Chichester, UK.

Guengerich, F.P., T. Shimada. 1991. Oxidation of toxic and carcinogenic chemical by human cytochrome P-450 enzymes. Chemical Research in Toxicology 4: 391-407.

Hammons, G.J., F.P. Guengerich, C.C. Weis, F.A. Beland, F.F. Kadlubar. 1985. Metabolic oxidation of carcinogenic arylamines by rat, dog, and human hepatic microsomes and by purified flavin-containing and cytochrome P-450 monooxygenases. Cancer Research 45: 3578-3585.

Hayes, C.L., D.C. Spink, B.C. Spink, J.Q. Cao, N.J. Walker, T.R. Sutter. 1996. 17β-Estradiol hydroxylation catalysed by human cytochrome P4501B1. Proceedings of the National Academy of. Sciences USA 93: 9776-9781.

Ingelman-Sundberg, M. 2005. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics Journal 5: 6-13.

Ioannides, C. 1990. Induction of cytochrome P450 I and its influences in chemical carcinogenesis. Biochemical Society Transactions 18: 32-34.

Ioannides, C. 1999. Effect of diet and nutrition on the expression of cytochromes P450. Xenobiotica 29: 109-154.

Ioannides, C. (ed). 2002a. Enzyme Systems that Metabolise Drugs and Other Xenobiotics. 578 p. John Wiley & Sons, Chichester, UK.

Ioannides, C. 2002b. Pharmacokinetic interactions between herbal remedies and medicinal drugs. Xenobiotica 32: 451-478.

Ioannides, C., D.F.V. Lewis. 2004. Cytochromes P450 in the bioactivation of chemicals. Current Topics in Medicinal Chemistry 4: 1767-1788.

Ioannides, C., D.V. Parke, 1990. The cytochrome P450 I gene family of microsomal haemoproteins and their role in the metabolic activation of chemicals. Drug Metabolism Reviews 22: 1-85.

Ioannides, C., D.V. Parke. 1993. Induction of cytochrome P4501 as an indicator of potential chemical carcinogenesis. Drug Metabolism Reviews 25: 485-501.

Ioannides, C., Y-L. Cheung, J. Wilson, D.F.V. Lewis, T.J.G. Gray. 1993. The mutagenicity and interactions of 2- and 4-(acetylamino)fluorene with cytochrome P450 and the aromatic hydrocarbon receptor may explain the difference in their carcinogenic potency. Chemical Research in Toxicology 6: 535-541.

Ioannides, C., C.R. Barnett, A. Irizar, P.R. Flatt. 1996. Expression of cytochrome P450 proteins in disease. In: Cytochromes P450: Metabolic and Toxicological Aspects (ed. C. Ioannides), pp. 301-327. CRC Press Inc, Boca Raton, USA.

Kamataki, T., K-I. Fujita, K. Nakayama, Y. Yamazaki, M. Miyamoto, N. Ariyoshi. 2002. Role of human cytochrome P450 (CYP) in the metabolic activation of nitrosamine derivatives: application of genetically engineered *Salmonella* expressing human CYP. Drug Metabolism Reviews 34: 667-676.

Kim, J.H., K.G. Stansbury, N.J. Walker, M.A. Trush, P.T. Strickland, T.R. Sutter. 1998. Metabolism of benzo[*a*]pyrene and benzo-[*a*]pyrene-7,8-diol by human cytochrome P450 1B1. Carcinogenesis 19: 1847-1853.

Kitada, M., M. Taneda, K. Ohta, K. Nagashima, K. Itahashi, T. Kamataki. 1990. Metabolic activation of aflatoxin B_1 and 2-amino-3-methylimidazo[4,5-*f*]quinoline by human adult and fetal livers. Cancer Research 50: 2641-2645.

Lewis, D.F.V., C. Ioannides, D.V. Parke. 1986. Molecular dimensions of the substrate binding site of cytochrome P-448. Biochemical Pharmacology 35: 2179-2185.

Lewis, D.F.V., C. Ioannides, D.V. Parke. 1987. Structural requirements for substrates of cytochromes P-450 and P-448. Chemico-Biological Interactions 64: 39-60.

Lewis, D.F.V., C. Ioannides, D.V. Parke. 1993. Validation of a novel molecular orbital approach (COMPACT) for the prospective safety

evaluation of chemicals by comparison with rodent carcinogenicity and *Salmonella* mutagenicity data evaluated by the US NCI/NTP. Mutation Research 291: 61-77.

Liu, C., X. Zhuo, F.J. Gonzalez, X.X. Ding. 1996. Baculovirus-mediated expression and characterization of rat CYP2A3 and human CYP2A6: role in metabolic activation of nasal toxicants. Molecular Pharmacology 50: 781-788.

McKinnon, R.A., W.M. Burgess, M.P. Hall, S.J. Roberts-Thomson, F.J. Gonzalez, M.E. McManus. 1995. Characterization of CYP3A gene subfamily expression in human gastrointestinal tissues. Gut 36: 259-267.

Murray, G.I., M.C. Taylor, M.C.E. McFadyen, J.A. McKay, W.F. Greenlee, M.D. Burke, T.W. Melvin. 1997. Tumor-specific expression of cytochrome P-450 CYP1B1. Cancer Research. 57: 3026-3031.

Nebert, D.W. 1989. The Ah locus: genetic differences in toxicity, cancer, mutation and birth defects. CRC Critical Reviews in Toxicology 20: 153-174.

Ogawa, K., H. Tsuda, T. Shirai, T. Ogiso, K. Wakabayashi, D.W. Dalgard, U.P. Thorgeirsson, S.S. Thorgeirsson, R.H. Adamson, T. Sugimura. 1999. Lack of carcinogenicity of 2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline (MeIQx) in cynomolgus monkeys. Japanese Journal of Cancer Research 90: 622-628.

Rendic, S., F. Di Carlo. 1997. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. Drug Metabolism Reviews 29: 413-580.

Ronis, M.J.J., K.O. Lindros, M. Ingelman-Sundberg. 1996. The CYP2E subfamily. In: Cytochromes P450: Metabolic and Toxicological Aspects (ed. C. Ioannides), pp. 211-239. CRC Press Inc, Boca Raton, USA.

Roy, D., A. Bernhardt, H.W. Strobel, J.G. Liehr. 1992. Catalysis of the oxidation of steroid and stilbene estrogens to estrogen quinone metabolites by the β-naphthoflavone-inducible cytochrome P450IA family. Archives of Biochemistry and Biophysics 296: 450-456.

Ryan, D.E., W. Levin. 1990. Purification and characterization of hepatic cytochrome P-450. Pharmacology Therapeutics 45: 153-239.

Seeff, L.B., B.A. Cuccherini, H.J. Zimmerman, E. Adler, S.B. Benjamin, 1986. Acetaminophen toxicity in the alcoholic. A therapeutic misadventure. Annals of Internal Medicine 104: 399-404.

Shimada, T., S-I. Nakamura, S. Imaoka, Y. Funae. 1987. Genotoxic and mutagenic activation of aflatoxin B_1 by constitutive forms of cytochrome P-450 in rat liver microsomes. Toxicology and Applied Pharmacology 91: 13-21.

Shimada, T., M. Iwasaki, M.V. Martin, F.P. Guengerich. 1989. Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by *umu* gene response in *Salmonella typhimurium* TA1535/pSK1002. Cancer Research 49: 3218-3228.

Shimada, T., C.L. Hayes, H. Yamazaki, S. Amin, S.S. Hecht, F.P. Guengerich, T.R. Sutter, 1996. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. Cancer Research 56: 2979-2984.

Shimada, T., K. Inoue, Y. Suzuki, T. Kawai, E. Azuma, T. Nakajima, M. Shindo, K. Kurose, A. Sugie, Y. Yamagishi, Y. Fujii-Kuriyama, M. Hashimoto. 2002. Arylhydrocarbon receptor-dependent induction of liver and lung cytochromes P450 1A1, 1A2, and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in genetically engineered C57BL/6J mice. Carcinogenesis 23: 1199-1207.

Shimizu, Y., Nakatsuru, M. Ichinose, Y. Takahashi, H. Kume, J. Mimura, Y. Fujii-Kuriyama, T. Ishikawa. 2000. Benzo[*a*]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. Proceedings of the National Academy of Sciences USA 97: 779-782.

Shou, M., K.W. Krausz, F.J. Gonzalez, H.V. Gelboin. 1996. Metabolic activation of the potent carcinogen dibenzo[*a*]anthracene by cDNA-expressed human cytochromes P450. Archives of Biochemistry and Biophysics 328: 201-207.

Shu, L., P.F. Hollenberg. 1997. Alkylation of cellular macromolecules and target specificity of carcinogenic nitrosodialkylamines: metabolic activation by cytochromes P450 2B1 and 2E1. Carcinogenesis 18: 801-810.

Smith, T.J., Z. Guo, F.J. Gonzalez, F.P. Guengerich, G.D. Stoner, C.S. Yang. 1992. Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in human lung and liver microsomes and in human cells containing cDNA-expressed cytochromes P-450. Cancer Research 52: 1757-1763.

Sogawa, K., Y. Fujii-Kuriyama Y. 1997. Ah receptor, a novel ligand-activated transcription factor. Journal of Biochemistry 122: 1075-1079.

Sohn, O.S., E.S. Fiala, S.P. Requeijo, J.H. Weisburger, F.J. Gonzalez. 2001. Differential effects of CYP2E1 status on the metabolic activation of the colon carcinogens azoxymethane and methylazoxymethanol. Cancer Research 61: 8435-8440.

Su, T., Z. Bao, Q-Y. Zhang, T.J. Smith, J-Y. Hong, X. Ding. 2000. Human cytochrome P450 CYP2A3: Predominant expression in the respiratory tract and its high efficiency metabolic activation of a tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Cancer Research 60: 5074-5079.

Valentine, J.L., S.S.T. Lee, M.J. Seaton, B. Asgharian, G. Farris, J.C. Corton, F.J. Gonzalez, M. Medinsky. 1996. Reduction of benzene metabolism and toxicity in mice that lack CYP2E1 expression. Toxicology Applied Pharmacology 14: 205-213.

Wall, K.L., W. Gao, J.M. TeKoppele, G.Y. Kwei, F.C. Kauffman, R.G. Thurman. 1991. The liver plays a central role in the mechanism of chemical carcinogenesis due to polycyclic aromatic hydrocarbons. Carcinogenesis 12: 783-786.

Wong, F.W-Y., W-Y. Chan, S.S-T. Lee. 1998. Resistance to carbon tetrachloride-induced hepatotoxicity in mice which lack CYP2E1 expression. Toxicology Applied Pharmacology 153: 109-118.

Xu, G., C.Y.-T. Li, A.N.-T. Kong. 2005. Induction of Phase I, II and III drug metabolism/transport by xenobiotics. Archives of Pharmacological Research 28: 249-268.

Yamazaki, H., H. Inui, C.H. Yun, M. Mimura, F.P. Guengerich, T. Shimada. 1992. Cytochrome P4502E1 and 2A6 enzymes as major catalysts for metabolic activation of N-nitrosodialkylamines and tobacco-related nitrosamines in human liver microsomes. Carcinogenesis 13: 1789-1794.

Yamazaki, H., Y. Inui, S.A. Wrighton, F.P. Guengerich, T. Shimada. 1995. Procarcinogen activation by cytochrome-p450 3a4 and 3a5 expressed in *Escherichia coli* and by human liver-microsomes. Carcinogenesis 16: 2167-2170.

Yamazaki, H., P.M. Shaw, F.P. Guengerich, T. Shimada. 1998. Roles of cytochromes P450 1A2 and 3A4 in the oxidation of estradiol and estrone in human liver microsomes. Chemical Research in Toxicology 11: 659-665.

Yano, M., T. Higashi, S. Kano, N. Tateishi, Y. Kido, M. Takesada, K. Higashi, Y. Sakamoto. 1989. Decreased activation of carcinogens in the liver of carcinogen-resistant rats. Cancer Research 49: 5352-5375.

Yoshimoto, F., S. Masuda, T. Higashi, T. Nishii, A. Takamisawa, N. Tateishi, Y. Sakamoto. 1985. Comparison of drug metabolizing activities in the livers of carcinogen-sensitive parent drugs and carcinogen-resistant descendants. Cancer Research 45: 6155-6159.

Zenser, T.V., V.M. Lakshmi, B.B. Davis. 1999. Human and *Escherichia coli* β-glucuronidase hydrolysis of benzidine and 4-aminobiphenyl, and their hydroxymetabolites. Drug Metabolism and Disposition 27: 1064-1067.

Zhou, S., S.Y. Chan, B.C. Goh, E. Chan, W. Duan, M. Huag, H.L. McLeod. 2005. Mechanism-based inhibition of cytochrome P450 3A4 by therapeutic drugs. Clinical Pharmacokinetics 44: 279-304.

Zimmerman, H.J., W.C. Maddrey. 1995. Acetaminophen (paracetamol) hepatotoxicity with regular intake of alcohol: Analysis of instances of therapeutic misadventure. Hepatology 22: 767-773.