J Occup Health 1997; 39: 83–91

Journal of Occupational Health

Review

Cytochrome P450 Isoforms and the Metabolism of Volatile Hydrocarbons of Low Relative Molecular Mass

Tamie NAKAJIMA

Department of Hygiene, Shinshu University School of Medicine

Abstract: Cytochrome P450 Isoforms and the Metabolism of Volatile Hydrocarbons of Low Relative Molecular Mass: Tamie NAKAJIMA. Department of Hygiene, Shinshu University School of Medicine—Cytochrome P450 isoforms responsible for the metabolism of volatile hydrocarbons of low relative molecular mass are reviewed. Rat CYP2E1 catalyses the metabolism of all hydrocarbons with a low Km, whereas CYP1A1/2, CYP2B1/2 and CYP2C11/6 catalyse the metabolism of many hydrocarbons with a high Km, although the contribution of the first two is minimal. The metabolism of hydrocarbons is affected by physiological and environmental factors, changes in the expression of P450 isoforms and the affinity of the chemicals for the isoforms. Human CYP2E1 also catalysed all of the hydrocarbons investigated, with the same kinetics as that of rat CYP2E1. Human CYP2B6 and CYP2C8 catalyse the metabolism of some hydrocarbons, but with slightly different catalytic properties for the formation of *o*- and *p*-cresol from toluene. Although CYP2B1/2 is poorly expressed in liver microsomes from control rats, CYP2B6 is found immunochemically to be constitutive in human liver microsomes. Human CYP1A2 also catalyses the metabolism of some organic solvents, with varying kinetic and catalytic features. The contribution of human CYP3A3, CYP3A4 and CYP3A5 to metabolism is very low. In conclusion, CYP2E1 is an essential isoform for the metabolism of hydrocarbons in both rodents and humans, especially at low concentrations.
(*J Occup Health 1997; 39: 83–91*)

Key words: Metabolism, Cytochrome P450, Volatile hydrocarbons, Human, Rat, Mice, Age, Sex, Nutrition, Pregnancy

The metabolism of volatile hydrocarbons, including organic solvents, is receiving more and more attention because (i) it is essential as a biomarker for exposure in biological monitoring and (ii) metabolic activation often occurs during the metabolic process. The metabolism of xenobiotics can be divided roughly into two types: functional and conjugation reactions. The former are rate-limiting for the metabolism of xenobiotics and sometimes for the generation of active metabolites, suggesting that this group of enzymes is involved primarily in the toxicity of chemicals. The latter catalyse the reactions of chemicals or their metabolites with endogenous substrates such as uridine diphosphate-glucuronic acid, glutathione or glycine, but rarely activate chemicals.

Cytochrome P450 (P450)[1] is the primary group of enzymes involved in functional reactions, constituing an enzymatic interface between humans and a wide variety of chemicals. Many chemicals are metabolized by the P450 system[2]. We[3–5] investigated the metabolism of 27 organic solvents in liver microsomes from rat, with NADPH and oxygen (Table 1), and found that all were metabolized by this system, indicating the involvement of P450s. Since several forms of P450 have been isolated and characterized, it is difficult to distinguish the responsibility of individual isoenzymes in the metabolism of hydrocarbons. Studies using purified P450 systems[2] and monoclonal antibodies (mAbs)[6] to various P450s are useful in determining the contribution of specific isozymes to metabolism. For the study of human P450s, use of a cDNA expression system[7] developed at the US National Institutes of Health offers numerous advantages over the standard biochemical approach. In this review, the specific P450 isoforms involved in the metabolism of volatile hydrocarbons of low relative molecular mass are discussed.

P450 isoforms in rodents and the metabolism of hydrocarbons

Benzene

The metabolic pathway of benzene has been well documented in many laboratories[8]. It is first metabolized to phenol by the catalytic action of P450 via benzene epoxide. Some of the phenol is further metabolized to hydroquinone and catechol by the same enzyme, and the remainder is metabolized by UDP-gluchronylransferase and

Received May 22, 1996; Accepted July 9, 1996
Correspondence to: T. Nakajima, Department of Hygiene, Shinshu University School of Medicine, Matsumoto 390, Japan

Table 1. Effects of fasting and chronic ethanol intake on the metabolism of organic solvents (nmol/mg protein per min)

Solvent	Structure	Control	Fasting	Ethanol
Benzene	C_6H_6	0.61± 0.24	1.52± 0.12	3.43 ± 0.54
Toluene	$C_6H_5CH_3$	0.81 ± 0.22	1.68 ± 0.20	3.69 ± 0.42
Ethylbenzene	$C_6H_5CH_2CH_3$	1.03 ± 0.29	1.68 ± 0.04	2.75 ± 0.28
Propylbenzene	$C_6H_5CH_2CH_2CH_3$	2.09 ± 0.57	3.22 ± 0.73	ND
o-Xylene	$C_6H_4(CH_3)_2$	1.08 ± 0.25	1.72 ± 0.21	3.16 ± 0.47
m-Xylene	$C_6H_4(CH_3)_2$	0.94 ± 0.25	1.71 ± 0.08	3.00 ± 0.49
p-Xylene	$C_6H_4(CH_3)_2$	1.09 ± 0.19	2.11 ± 0.20	3.69 ± 0.56
Cumene	$C_6H_5CH(CH_3)_2$	1.64 ± 0.19	3.16 ± 0.70	ND
Styrene	$C_6H_5CH=CH_2$	1.28 ± 0.19	1.93 ± 0.21	3.54 ± 0.40
Dichloromethane	CH_2Cl_2	1.28 ± 0.07	2.54 ± 0.41	5.99 ± 0.86
Chloroform	$CHCl_3$	0.88 ± 0.12	2.30 ± 0.31	4.96 ± 0.41
Carbon tetrachloride	CCl_4	0.09 ± 0.01	0.25 ± 0.03	0.33 ± 0.06
1,1-Dichloroethane	CH_3CHCl_2	0.86 ± 0.15	2.33 ± 0.10	4.74 ± 0.29
1,2-Dichloroethane	CH_2ClCH_2Cl	1.06 ± 0.05	2.49 ± 0.13	5.04 ± 0.31
1,1,1-Trichloroethane	CH_3CCl_3	0.02 ± 0.01	0.05 ± 0.01	0.07 ± 0.02
1,1,2-Trichloroethane	$CH_2ClCHCl_2$	0.94 ± 0.09	2.33 ± 0.13	4.61 ± 0.38
1,1,1,2-Tetrachloroethane	$CHClCCl_3$	0.36 ± 0.03	1.37 ± 0.35	2.15 ± 0.22
1,1,2,2-Tetrachloroethane	$CHCl_2CHCl_2$	0.60 ± 0.03	1.67 ± 0.10	2.78 ± 0.33
1,1-Dichloroethylene	$CH_2=CCl_2$	1.39 ± 0.30	2.80 ± 0.28	3.95 ± 0.42
cis-1,2-Dichloroethylene	CHCl=CHCl	0.78 ± 0.23	1.67 ± 0.21	2.33 ± 0.39
trans-1,2-Dichloroethylene	CHCl=CHCl	<0.02	<0.02	0.45 ± 0.21
Trichloroethylene	$CHCl=CCl_2$	0.84 ± 0.33	2.38 ± 0.21	4.13 ± 0.26
Tetrachloroethylene	$CCl_2=CCl_2$	0.02 ± 0.01	0.08 ± 0.01	0.10 ± 0.02
1-Chloropropane	$CH_3CH_3CH_2Cl$	1.69 ± 0.26	4.17 ± 0.31	ND
Monoclorobenzene	C_6H_5Cl	0.37 ± 0.07	1.48 ± 0.25	2.79 ± 0.43
Boromobenzene	C_6H_5Br	0.61 ± 0.17	1.20 ± 0.20	2.81 ± 0.59
n-Hexane	C_6H_{14}	0.89 ± 0.18	1.58 ± 0.35	3.31 ± 0.31

ND, not determined. Substrate concentrations used were 0.09–0.13 mmol/*l*.

sulfotransferase. Benzene epoxide is metabolized to catechol and mercapturic acid by epoxide hydrolase and glutathione S-transferase, respectively. Catechol and benzene epoxide are metabolized to muconaldehyde, but this may not be a major route of benzene metabolism.

The P450 isoforms responsible for benzene metabolism are summarized in Table 2. Gorsky and Coon[9] reported that CYP2B4, an isoform found in liver microsomes from phenobarbital-treated rabbits, is involved in the step from benzene to phenol. It is characterized as a high-Km isoform for benzene oxidation. In contrast, Johansson and Ingelman-Sundberg[10] found a low-Km isoform that is involved in oxidation and is induced by ethanol and acetone. This isoform was subsequently identified as CYP2E1 by immunochemical analysis and purification. It is also responsible for the metabolism of phenol to hydroquinone[11]. We investigated the contribution of four P450 isozymes-CYP1A1, CYP2B1, CYP2C11 and CYP2E1-to the metabolism of benzene, using mAbs[12,13]. These isoforms are involved in the oxidation of benzene, CYP1A1/2, CYP2B1/2 and CYP2C11/6 being high-Km forms and CYP2E1 a low-Km form. Although CYP2E1 and CYP2C11/6 are both involved in metabolism in untreated rats, the contribution of the former is more than 50%, suggesting that it is a major isoform for benzene oxidation in rat liver, especially at low concentrations.

Toluene

Toluene is metabolized by P450s mainly to benzyl alcohol and marginally to o- and p-cresol, with occasional formation of *m*-cresol[14]. Only small percentages of *o*- and *p*-cresol are formed from toluene, although species differences exist[15]. Benzyl alcohol is further metabolized to benzoic acid via benzaldehyde by alcohol dehydrogenase and aldehyde dehydrogenase. The benzoic acid is conjugated with glycine to form hippuric acid, which is the major, final metabolite of the toluene side-chain reaction. Some of the benzoic acid is conjugated with glucuronic acid[16] or carnitine[17]. The concentration of benzoic acid in target tissue is important in glucuronyl conjugation of benzoic acid because of the catalytic nature of the high-Km enzyme for benzoic acid. Benzoyl glucuronide is therefore seen only after exposure to high concentrations of toluene[18].

The P450s responsible for toluene metabolism have been

Table 2. P450 isoforms involved in the metabolism of organic solvents in rodents

Substrate	Metabolites	CYP1A1/2[a] MC-inducible form	CYP2B1/2 PB-inducible form	CYP2C11/6 Constitutive form	CYP2E1 Constitutive form	CYP3A1/2 Constitutive form
Benzene	Phenol and hydroquinone	+	+	+	+	ND
Phenol	Hydroquinone and catecol	ND	+	ND	+	ND
Toluene	Benzyl alcohol	—	+	+	+	ND
	o-Cresol	+	+	+	—	ND
	p-Cresol	+	+	+	+	ND
Xylene	Methyl benzyl alcohol	ND	+	ND	+	ND
Styrene	Styrene glycol	+	+	+	+	+
Chloroform	SD	—	+	ND	+	ND
Carbon tetra-chloride	SD	—	+	+	+	ND
Trichloro-ethylene	Chloral hydrate	+	+	+	+	—
Acetone	Actol	—	—	ND	+	ND
Ethanol	Acetaldehyde	ND	ND	+	+	ND
Diethylether	Acetaldehyde	—	—	ND	+	ND
n-Pentane	SD	—	—	ND	+	ND
n-Hexane	1-hexanol, 2-hexanol and 3-hexanol	+	+	ND	+	ND

—, not involved in metabolism; +, involved in metabolism. ND, not determined; SD, substrate disappearance; MC, 3-methylcholanthrene; PB, phenobarbital. [a]These antibodies, except anti-CYP2E1, cross-reacted with the respective subfamily, CYP1A2, CYP2B2, CYP2C6 and CYP3A2, so that the expression of CYP1A1/2, CYP2B1/2, CYP2C11/6 and CYP3A1/2 was used.

studied using purified P450 isoforms and mAbs. Waxman *et al.*[19,20] investigated the contributions of five purified P450 isozymes to the formation of benzyl alcohol from toluene. The most active was CYP2B1, followed by CYP2C11 and CYP2C6, whereas the contributions of CYP2C12 and CYP2A1 were negligible. We[21] investigated four P450 isozymes-CYP1A1, CYP2B1, CYP2C11 and CYP2E1-involved in the formation of benzyl alcohol, *o*- and *p*-cresol from toluene in rat liver microsomes using the respective mAbs. CYP2B1/2, CYP2C11/6 and CYP2E1, but not CYP1A1/2, are involved in the formation of benzyl alcohol, the first two being high-Km and the last a low-Km. In contrast, CYP1A1/2, CYP2B1/2 and CYP2C11/6 are high-Km isoforms involved in the formation of *o*-cresol. All of the P450 isoforms investigated catalyse the formation of *p*-cresol, CYP2E1 being a low-Km and the others high-Km forms. Of the constitutive P450 isoforms, the male-specific CYP2C11 and CYP2E1 are the main types involved in toluene metabolism at high and low concentrations, respectively, and the female-specific form CYP2C12 is not.

Xylene

The metabolic pathway of xylene is very similar to that of toluene[22]. The P450 isozyme responsible for xylene metabolism has not been investigated using a reconstituted system with purified P450 and inhibition of catalytic activity by mAbs. Pretreatment with ethanol and phenobarbital[23–26], however, significantly enhanced the metabolism of xylene in rats, suggesting that at least CYP2E1 and CYP2B1/2 contribute to its metabolism.

Styrene

Styrene is activated metabolically by P450s to a toxic, mutagenic and potentially carcinogenic form, styrene 7,8-oxide, which is immediately converted to styrene glycol[27]. The P450 isozymes responsible for styrene metabolism were investigated in two laboratories. Fourman *et al.*[28] studied the contribution of purified P450 isoforms to styrene oxidation, and we[29] reported that at least four P450s, CYP2C11/6, CYP2E1, CYP2B1/2 and CYP1A1/2, contribute to the formation of styrene glycol in microsomes from untreated rat liver. CYP2C11 is the major isoform involved and is also the main form for styrene oxidation, even at low concentrations, in agreement with the findings for benzene and toluene. Thus, CYP2C11 may be a major isoform for the metabolism of hydrocarbons with longer

aliphatic carbon chains than styrene. Styrene causes lung tumors in rodents[30], but its metabolism in lung is catalysed mainly by a different form, CYP2B1.

Trichloroethylene

Trichloroethylene is metabolized by P450 to chloral hydrate, which is further converted by alcohol and aldehyde dehydrogenase to trichloroethanol and trichloroacetic acid, respectively. The majority of the trichloroethanol is conjugated with UDP-gluchronylransferase to form urochloral acid, some of which is converted by a microsomal alcohol oxidation enzyme to trichloroacetic acid via chloral hydrate[31].

Miller and Guengerich[32] investigated the contribution of P450 isoforms to the formation of chloral hydrate from trichloroethylene using eight purified P450s and found the following order of activity: CYP2B1>CYP1A2> CYP1A1>CYP2C11>CYP2C6; the contribution of CYP2B2, CYP2A1 and CYP3A1 was negligible. We[33] confirmed the findings of Fourman *et al.*[22] using mAbs. We also found that CYP2E1 is a major low-Km form for the metabolism of trichloroethylene in microsomes from untreated rat liver, whereas CYP1A1/2, CYP2B1/2 and CYP2C11/6 are high-Km forms, as for benzene. Thus, trichloroethylene and benzene have similar affinities for the four P450 isoforms, unlike those of the toluene side-chain.

Carbon tetrachloride

Although the use of carbon tetrachloride in industry is decreasing, it is often used as a model substrate for hepatotoxicity. P450s catalyse the reduction of carbon tetrachloride to the trimethyl radical[34]. The reductive reaction is enhanced by food deprivation[3] and ethanol[4], suggesting that CYP2E1 is involved. Johansson *et al.*[35,36] investigated the contribution of P450s to the metabolism of carbon tetrachloride using three purified P450 isoforms from rabbit liver microsomes. They confirmed the contribution of CYP2E1 and reported the formation of lipid peroxide but failed to detect the involvement of CYP2B4 and CYP1A2. The metabolism of carbon tetrachloride is enhanced by treatment with phenobarbital only when measured under anaerobic conditions[24], suggesting that CYP2B1/2 is also involved in reductive metabolism.

Chloroform

Chloroform not only damages the liver and kidneys but can also cause tumors[37]. It is generally accepted that the toxic effects of chloroform are associated with its activation by P450s[38]. At least two P450 isoforms, CYP2E1 and CYP2B1/2, are involved in its metabolism, the former having a low Km and the latter a high Km[39,40]. This kinetics reflects the hepatotoxicity of chloroform: CYP2E1 acts at low concentrations, whereas CYP2B1/2 acts effectively at high concentrations[41].

Ethanol

Three enzymes are involved in the metabolism of ethanol: alcohol dehydrogenase, ethanol P450 oxidation enzyme and catalase[42]. CYP2E1 is the main isoform involved in oxidation[43]. We investigated the contribution of two constitutive P450 isoforms to the formation of acetaldehyde from ethanol in mature male rats using mAbs. CYP2E1 is involved at low concentrations and CYP2C11/6 at high concentrations, as for toluene (Table 3).

Acetone

Acetone is widely used in the rubber and plastics industries[44] and is also an important endogenous substance,

Table 3. Inhibition of microsomal ethanol oxidase activity by monoclonal antibodies to CYP2E1 (clone 1-91-3) and CYP2C11/6 (clone 1-68-11)

Group	Ethanol	Hy-Hel		Anti-CYP2E1			Anti-CYP2C11/6		
		A	B	A	B	C	A	B	C
5 mmol/*l*									
Control	—	3.39	100	2.67	79	0.79	2.54	75	0.95
Control	+	4.39	100	3.39	77	1.00	3.51	80	0.88
Low CHO	—	3.85	100	2.66	69	1.19	2.96	77	0.88
Low CHO	+	6.25	100	2.27	36	3.98	4.96	79	1.29
50 mmol/*l*									
Control	—	14.71	100	13.30	90	1.44	7.19	49	7.55
Control	+	18.86	100	16.56	88	2.30	12.27	66	6.59
Low CHO	—	15.18	100	13.52	89	1.66	8.75	58	6.43
Low CHO	+	18.80	100	14.51	77	4.26	11.97	64	6.83

Hy-Hel, control monoclonal antibody; control, Wistar male rats fed a basal diet; low CHO, rats fed a low carbohydrate diet; A, remaining activity (nmol/mg protein per min); B, percentage of remaining activity (activity with each monoclonal antibody/activity with Hy-Hel × 100); C, actual inhibition (difference between A with Hy-Hel and A with anti-CYP2E1 or anti-CYP2C11/6, nmol/mg protein per min).

with elevated concentrations after fasting and in diabetes. Acetone is metabolized by P450 to acetol, which is further metabolized to D-glucose. Casazza *et al.*[45] first demonstrated that oxidation occurs in rat microsomes in the presence of NADPH and oxygen. Purified CYP2E1 from rabbit and rat liver microsomes was subsequently shown to be involved in the oxidation[36]. CYP2B4 and CYP1A1 were not found to be involved[46–48], but a low concentration of acetone was used in these experiments.

Ether

There is little information on the role of P450s in the metabolism of ether. Diethylether is de-ethylated by microsomes to produce acetaldehyde[47]. CYP2E1 is thought to be the major isoform involved in the metabolism of diethylether, as an mAb to this isozyme inhibited its de-ethylation by 45% in microsomes from untreated rats and by 78% in microsomes from acetone-treated rats[48]. This conclusion was confirmed by the finding that the rate at which CYP2E1 metabolizes diethylether is 10 times that of CYP2B1 at a low concentration. Furthermore, CYP2E1 is a low-Km isoform for ether metabolism.

Furan is a potent hepatocarcinogen in mice and rats[44]. The contribution of CYP2E1 to its oxidation was investigated in isolated acetone-treated rat hepatocytes. Acetone treatment increased oxidation by fivefold, suggesting an important role for CYP2E1[49].

Recently, the metabolism of a new ether, 1,1,2,3,3,3-hexafluoropropyl methyl ether, which is being developed as an alternative to ozone-depleting chlorofluorocarbons, was investigated in rat microsomes[50]. This chemical is metabolized to inorganic fluoride, formaldehyde, and 2,3,3,3-tetrafluoropropionic acid, and the rates of fluoride formation correlated with the relevant amount of rat CYP2E1.

The renal and hepatic toxicity of volatile fluorinated ether anesthetics is due to biotransformation to toxic metabolites[51]. Halothane was extensively metabolized by rat microsomes, but enflurane, isoflurane and sevoflurane underwent little metabolism (unpublished data). The metabolism of halothane was enhanced by food deprivation and ethanol treatment, suggesting a major role of CYP2E1[52].

n-Pentane

n-Pentane is used as a constituent of motor and aviation fuel and as a general laboratory solvent[53]. Like acetone, *n*-pentane is also produced endogenously during lipid peroxidation of polyunsaturated fatty acids. It is first metabolized to 2- and 3-pentanol by CYP2E1, with a negligible contribution of CYP2B4 and CYP1A2[54,55]. A low concentration of *n*-pentane was used in these studies, however, suggesting that further investigation may be required using high substrate concentrations.

n-Hexane

Many cases of *n*-hexane-induced polyneuropathy have been reported, which are strongly associated with its metabolism[56]. Morohashi *et al.*[57] investigated the contribution of P450 isoforms to *n*-hexane metabolism using purified CYP1A and CYP2B in liver microsomes from 3-methylcholanthrene- and phenobarbital-induced rats. Both isozymes catalysed the metabolism, but CYP2B1 was involved mainly in the formation of 2-hexanol, followed by 3-hexanol and 1-hexanol, with the metabolic rate of formation of 1-hexanol being one-tenth that of 2-hexanol, while CYP1A1 was involved mainly in the formation of 3-hexanol, followed by 2-hexanol, and did not catalyse the formation of 1-hexanol. The contribution of CYP2E1 to the metabolism of *n*-hexane has not been investigated with purified P450s and mAb-directed enzyme assay, but the metabolism of *n*-hexane is clearly enhanced by fasting and ethanol treatment (Table 1), suggesting that CYP2E1 is involved.

N,N-Dimethylformamide

N,N-Dimethylformamide is an industrial solvent with hepatotoxic properties[58]. The formation of N-(hydroxymethyl)-N-methylformamide from N,N-dimethylformamide was 175% greater in microsomes from acetone-treated rats than in control microsomes[59], suggesting the involvement of CYP2E1.

Regulation of P450 and the metabolism of hydrocarbons

Species

There have been many reports of species differences in the expression of P450 isoforms[60]. Generally speaking, mice have greater metabolic activity than rats; for example, they metabolize benzene[61] and trichloroethylene[62] to a greater degree than rats *in vivo*, resulting in a greater susceptibility to these chemicals. We[15] investigated differences in the expression of P450 isoforms between mature male B6C3F1 mice and Wistar rats of the same age and sex using mAbs. Mice constitutively have greater amounts of CYP2E1 and CYP1A1/2 than rats, whereas the expression of CYP2C11/6 is greater in rats. Since CYP2E1 is the major form in the catalysis of benzene and trichloroethylene, species differences in the metabolism of these chemicals are due to differences in the expression of CYP2E1. In contrast, toluene side-chain oxidation activity was greater in mice than rats at a low substrate concentration but greater in rats than mice at a high concentration. The difference between the two species is due to different contents of CYP2E1 at low concentration and of CYP2C11/6 at high concentration. Thus, species differences in the metabolism of hydrocarbons are closely related to the distribution of P450 isoforms and the affinity of the chemicals for each isoform.

Sex

A sex difference in the metabolism of many chemicals is seen in rats but not in humans or mice[60]. In rats, CYP2C11 is male-associated and CYP2C12 is female-associated[61]. A sex difference is also seen in the expression of CYP2E1, which is greater in mature female rats than in mature males[62]. Toluene is metabolized to a greater extent in males than in females[63], because of the higher affinity for CYP2C11 and the lower affinity for CYP2C12. In contrast, there is no sex difference in the metabolism of benzene and trichloroethylene. Since the contribution of CYP2C11 to the metabolism of these chemicals is much lower than that to toluene, the abundant expression of CYP2C11 may only contribute to offset the metabolic difference caused by a higher expression of CYP2E1 in female rats. A sex difference in the metabolism of organic solvents is common for aromatic hydrocarbons, except benzene[3], perhaps because of their high affinity for CYP2C11.

Age

The expression of P450s is low during fetal development in most animals but increases rapidly soon after birth. Some P450 isoforms are regulated developmentally in a sex-specific fashion. CYP2C11 is present at a significant level in immature male rats and shows more than 30-fold induction at puberty[61]. CYP2C12 is also induced developmentally in females. CYP2E1 occurs at a similar level in the livers of males and females at three weeks of age[64] but decreases more significantly in males than in females between three and six weeks of age. The expression of CYP2A1 decreases with age only in males. In contrast, the expression of CYP2C6 is not affected by age[65]. Thus, the metabolism of some organic solvents such as toluene and trichloroethylene is developmentally regulated, and the mode of the regulation is dependent on the affinity between these solvents and P450 isoforms.

Pregnancy

Pregnancy is known to decrease the overall content of the constitutive P450 isoforms in mice and rats[66]. We investigated the contribution of two P450 isoforms to the metabolism of some organic solvents in pregnant and non-pregnant rats[63]. The levels of CYP2E1 and CYP2C11/6 were slightly decreased during pregnancy, with a corresponding suppressesion of the metabolism of toluene and trichloroethylene.

Nutrition

Changes in nutritional status influence not only the metabolism of chemicals but also the induction of P450 isozymes by some chemicals[67]. As a lowered intake of carbohydrates induces CYP2E1, the metabolism of almost all organic solvent is increased[68]. Thus, fasting influences CYP2E1 due solely to the deficiency of carbohydrate intake and not to that of total energy or of nutrients other than carbohydrates[3,68]. Lowered carbohydrate intake also augments the induction of CYP2E1 by ethanol[23].

Environmental exposure to chemicals

The kinetics is biphasic when two or more chemicals are introduced into the body: one inhibits the interaction of the two metabolic pathways, and the other acts when one chemical is introduced after P450 induction by another chemical. Many chemicals induce P450 isoforms, including those associated with alcohol drinking and smoking. Ethanol induces CYP2E1 in both rodents[12] and human[69]; it also induces CYP2B1/2[10] but only at high doses. Smoking also influences some enzymes[70], but it is difficult to determine which chemicals are involved because of the large number that exist in tobacco smoke.

Human cytochrome P450 isoforms and the metabolism of organic solvents

Many biochemical approaches have been used to detect human P450 isoforms, and more than 20 have been characterized with regard to their properties and catalytic specificities[71,72]. The contribution of human P450 isoforms to the metabolism of xenobiotics was investigated by a variety of approaches, including (i) selective inhibition of catalytic activity in human liver microsomes by chemicals that are selective inactivators of target P450 isozymes, (ii) correlating the catalytic activity of one chemical and that of chemicals that are indicators of P450 isoforms in human liver microsomes, (iii) using a reconstituted system of purified human P450 isoforms, (iv) immunoinhibition of catalytic activity in human liver microsomes with antibodies and (v) identification of c-DNA-expressed human P450 isoforms in mammalian cell lines using vaccinia virus. Guengerich *et al.*[73] investigated the contribution of human CYP2E1 to the metabolism of 15 hydrocarbons (benzene, styrene, ethylene dibromide, ethylene dichloride, vinyl chloride, vinyl bromide, acetonitrile, vinyl carbamate, ethyl carbamate, carbon tetrachloride, chloroform, methyl chloride, 1,1-dichloropropane, 1,1,1-trichloromethane and trichloroethylene) using the first four methods. CYP2E1 is the main isoform involved in the metabolism of these hydrocarbons. Its involvement in the metabolism of N,N-dimethylformamide[59], 1,1,2,3,3,3-hexafluoropropyl methyl ether[50], phenol[74] and anesthetic chemicals[75] has been confirmed by several means.

We characterized the contribution to the metabolism of toluene (formation of benzyl alcohol, *o*- and *p*-cresol)[76], and styrene (formation of styrene glycol)[77] of 10 human hepatic P450 isoforms expressed by cDNA in hepatoma G2 cells using vaccinia virus. CYP2E1 was most effective, followed by CYP2B6, CYP2C8 and CYP1A2. The contribution of CYP3A3, 3A4 and 3A5 was small, and that of CYP2A6, CYP2D6 and CYP2C9 was negligible. CYP1A2 catalysed the formation of *o*- and *p*-cresol from toluene, whereas CYP2B6, CYP2C8 and CYP2E1 catalysed

only the formation of *p*-cresol. CYP2E1 has a low Km for the metabolism of these hydrocarbons, but CYP2B6 and/or CYP2C8 have a high Km.

The catalytic and kinetic properties of CYP2E1 are similar to those of rat CYP2E1. The properties of human CYP2C8 are also similar to those of rat CYP2C11, except that CYP2C8 does not catalyse the formation of *o*-cresol. Of the CYP2B family, rat CYO2B1 is expressed constitutively at such a low rate that it does not contribute to metabolism. In contrast, human CYP2B6 is expressed significantly in liver microsomes. The catalytic properties of the two subforms differ in terms of aromatic hydroxylation of toluene: CYP2B1 catalyses the formation of benzyl alcohol, *o*- and *p*-cresol from toluene, whereas CYP2B6 catalyses benzyl alcohol and *p*-cresol and not *o*-cresol. For the formation of *o*-cresol from toluene, the catalytic properties of human CYP1A1/2 are similar to those of rat CYP1A1/2, but the kinetics is different: rat CYP1A1/2 is a high-Km isoform and human CYP1A1/2 is a low-Km form[76]. Thus, measurement of urinary *o*-cresol is a significant marker of exposure to toluene in biological monitoring.

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