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CYTOCHROME P450-RELATED DIFFERENCES BETWEEN RATS AND MICE IN THE METABOLISM OF BENZENE, TOLUENE AND TRICHLOROETHYLENE IN LIVER MICROSOMES

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Abstract—In evaluating the risks to humans of exposure to chemicals, the results of studies in rodents are sometimes used as a basis for extrapolation. It is therefore important to elucidate differences in metabolism among species. Differences in cytochrome P450-catalysed oxidation of benzene, toluene and trichloroethylene (TRI) between male Wistar rats and male B6C3F1 mice were investigated by immunoblot and immunoinhibition assays using monoclonal antibodies (MAbs) to cytochrome P450 (CYP1A1/2, CYP2B1/2, CYP2E1 and CYP2C11/6). Immunoblot analysis showed that anti-CYP2B1/2 did not detect any protein in either untreated rat or mouse liver microsomes, whereas with anti-CYP2E1 and/or anti-CYP1A1/2 a clear-cut band was seen more in liver microsomes from mice than from rats. Mouse liver microsomes had a greater monooxidation activity for benzene and TRI than rat liver microsomes; mice also had a higher rate of aromatic hydroxylation of toluene at low substrate concentration, but a low rate of side-chain oxidation when a high concentration of toluene was used. The metabolism of benzene was saturated in mice at around 0.23 mM, but the metabolism of the other two solvents was not saturated in either rats or mice at the low concentrations used. Anti-CYP2E1 inhibited the metabolism of benzene, toluene and TRI in microsomes from mice to a greater extent than in rats, while anti-CYP2C11/6 inhibited their metabolism in rats to a greater extent than in mice; anti-CYP1A1/2 inhibited the metabolism of TRI only in microsomes from mice. These results indicate that (i) male B6C3F1 mice have more CYP2E1 and 1A1/2 than male Wistar rats, whereas rats have more CYP2C11/6 than mice; (ii) rats and mice express CYP2B1/2 but they are not immunochemically detectable; (iii) CYP2E1 and 2C11/6 in both species are responsible for the metabolism of benzene, toluene and TRI, whereas CYP1A1/2 in mice catalyses the oxidation of TRI. The differences in the metabolism of benzene, toluene and TRI in rats and in mice may therefore depend, at least in part, on differences in the distribution of P450 isozymes between the two species.

Several studies indicate that mice are more sensitive to the hematotoxic and leukemogenic effects of benzene [1, 2] and hepatotoxicity and carcinogenicity of trichloroethylene (TRI¶) than rats [3, 4], perhaps owing to the more active production of toxic metabolites of benzene [5, 6] and TRI [7, 8]. The metabolism of benzene and TRI, like other hydrocarbons, such as toluene, is catalysed primarily by cytochrome P450-mediated oxidation. This enzyme system consists of multiple isozymes which are expressed differently among the species [9]. Therefore, it is very important to characterize differences in the distribution of isozymes between rats and mice in relation to the metabolic profile of chemicals.

In a previous paper, we demonstrated that the affinity of benzene for P450 isozymes in rat liver is very similar to that of TRI, but widely different from that of toluene: in intact rat liver, CYP2E1 and CYP2C11/6 [9] contribute mainly to the metabolism of benzene, toluene and TRI; CYP2E1 is the isozyme primarily responsible for the metabolism of benzene and TRI; CYP2C11/6 is associated mainly with toluene metabolism [10]. The problem is to determine how species differences in toluene metabolism occur between rats and mice.

The objective of the present study was to examine the distribution of CYP2E1, CYP2C11/6, CYP1A1/2 and CYP2B1/2 in rats and mice in relation to differences in their metabolism of benzene, toluene and TRI.

MATERIALS AND METHODS

Animals. Five male Wistar rats and five male B6C3F1 mice (6 weeks of age) were obtained from Nippon SLC Inc. (Shizuoka, Japan), and used throughout. Rats were housed individually in stainless steel wire-bottom cages, and mice were housed together in aluminum cages in a room with

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¶ Abbreviations: TRI, trichloroethylene; MAb, monoclonal antibody; BAH, benzene aromatic hydroxylase; BA, benzyl alcohol.

controlled temperature (20 ± 2°), relative humidity and light (12 hr light/dark cycle). They were maintained on rodent chow (Clea CE-2, Nippon Clea, Tokyo) and tap water *ad lib.* for 2 weeks to allow for acclimatization. At 8 weeks of age, all animals were killed by decapitation at 10.00 a.m.

Preparation of microsomes. Livers were removed promptly and perfused with cold 1.15% (w/v) KCl solution. After homogenization in the salt solution, 25% (w/v) each liver homogenate was centrifuged at 10,000 *g* for 10 min, and the supernatant was further centrifuged at 105,000 *g* for 60 min. The microsomal pellets were washed in the salt solution, suspended in 50 mM phosphate buffer containing 10% glycerin and then stored frozen at −85° until use.

Enzyme and protein assays. The activity of benzene aromatic hydroxylase (BAH) was assessed by measuring the rate of formation of phenol and hydroquinone, by the method of Nakajima *et al.* [11] with slight modification, as follows: 200 μg of microsomal protein, 1.0 mM NADP, 20 mM glucose 6-phosphate, 50 mM magnesium chloride, 2 IU glucose 6-phosphate dehydrogenase, 50 mM potassium phosphate buffer (pH 7.4) and 0.23 or 6.26 mM of benzene in 0.5 mL. The reaction mixture was incubated at 37° for 10 min. After the reaction was stopped by adding 0.1 mL of 15% $ZnSO_4$, followed by 0.1 mL of saturated $Ba(OH)_2$, the mixture was centrifuged at 1500 *g* for 15 min; 50 μL of supernatant were injected into an HPLC. The HPLC conditions were as follows for assay of phenol: 4.0 mm i.d. × 250 mm stainless steel column packed with Unisil C18 (Gasukurokogyo, Inc., Tokyo); mobile phase, 30% acetonitrile solution; flow speed, 1.0 mL/min; wavelength, 200 nm. For measurement of hydroquinone: 4.0 mm i.d. × 150 mm stainless steel column packed with Hitachi gel 3056 (Hitachi, Inc., Tokyo); mobile phase, 5% acetonitrile containing 0.2% phosphoric acid; flow speed, 1.2 mL/min; wavelength, 200 nm.

The metabolism of toluene was assessed by measuring the rate of benzyl alcohol (BA), *o*- and *p*-cresol formation. The same medium as benzene metabolism was used except that 0.20 and 5.0 mM of toluene were used as the substrate. The metabolites of toluene were measured by the method of Nakajima *et al.* [12] using the same HPLC condition as phenol.

The metabolism of TRI was assessed by measuring the rate of chloral hydrate, according to the method reported previously [13]. The same medium as benzene metabolism was used except that 600 μg of microsomal protein and 0.20 and 5.9 mM of TRI were in the incubation mixture. Chloral hydrate concentration was measured according to the method in the measurement of hydroquinone.

The microsomal protein content was measured by the method of Lowry *et al.* [14] and the concentration of cytochrome P450 by the spectrophotometric method of Omura and Sato [15].

Study of immunoinhibition. Monoclonal antibodies (MAbs) and Hy-Hel were produced by the hybridoma technique at the U.S. National Cancer Institute Laboratory of Molecular Carcinogenesis (Bethesda, MD, U.S.A.) using a modification of the method of Koehler and Milstein [16], tested and characterized as described previously. In the present study, four MAbs specific for different cytochrome P450 were used as follows: anti-CYP1A1 (clone 1-7-1) MAb, cross-reaction with CYP1A2 [17], anti-CYP2C11 (clone 1-68-11), cross-reacted with CYP2C6 [18] and anti-CYP2E1 (clone 1-91-3) [19]. As a control MAb, Hy-Hel against chicken lysozyme was used to determine any non-specific reaction. Each MAb was reacted with microsomes in buffer solution at room temperature for 30 min, and the assay for metabolism was started by adding an NADPH-generating system and substrate (benzene, toluene and TRI) at 37°. In preliminary assays, maximal inhibition by each MAb was attained at a MAb protein:microsomal protein ratio of less than 0.5.

We reported previously that CYP2E1 acts as a low-K_m isozyme and the other isozymes as high-K_m isozymes in benzene [11, 20], toluene [12] and TRI metabolism [10]. Therefore, inhibition by anti-CYP2E1 was conducted only at low concentrations (0.23 mM for benzene and 0.20 mM for toluene and TRI) and the inhibition by anti-CYP1A1/2 and anti-CYP2C11/6 only at high concentrations (6.26 mM for benzene, 5.0 mM for toluene and 5.9 mM for TRI).

Immunoblot analysis. After 40 μg of rat and mouse liver microsomes were separated electrophoretically on standard Laemmli [21] sodium dodecyl sulfate–polyacrylamide gel (10%), electrophoretic transfer of microsomal protein to 0.45 μm nitrocellulose sheets (Bio-Rad) was done according to Towbin *et al.* [22]. The nitrocellulose sheets were incubated in 50 mM Tris–200 mM NaCl (pH 7.4) containing 0.05% Tween 20 for 30 min followed by anti-CYP2E1 (clone 1-98-1, 0.06 mg/mL) [19], anti-CYP1A1/2 (0.015 mg/mL) and anti-CYP2B1/2 (clone 2-66-3, 0.03 mg/mL) [23] for 1 hr, respectively. Then, cytochrome P450 isozymes were analysed by

Table 1. Protein and cytochrome P450 content (mean ± SD) of microsomes from male Wistar rats and male B6C3F1 mice

Species	Number	Body weight (g)	Liver weight (g)	Microsomal protein (mg/g liver)	Cytochrome P450 (nmol/mg protein)
Rat	5	195 ± 5	8.62 ± 0.42	25.2 ± 1.9	0.85 ± 0.10
Mouse	5	27 ± 1*	2.34 ± 0.16*	22.8 ± 3.5	0.89 ± 0.05

* Significant difference between rats and mice ($P < 0.05$).

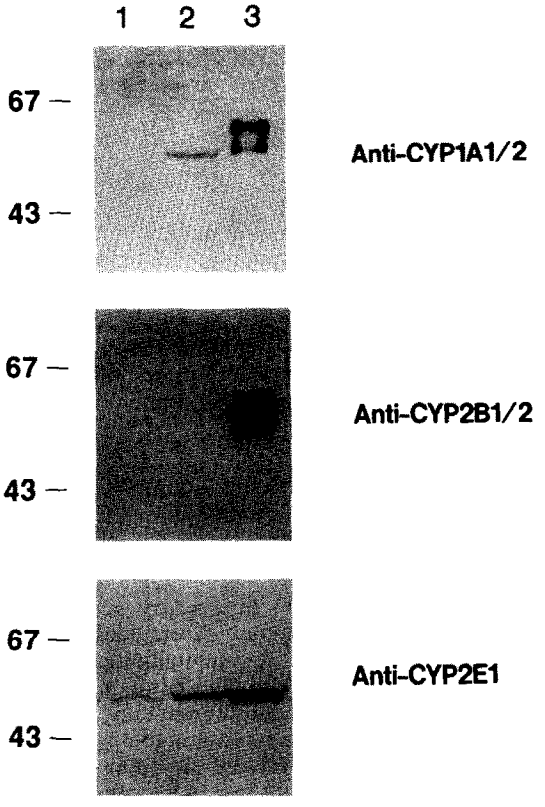

Fig. 1. Immunoblotting and immunodetection of cytochrome P450s with anti-CYP1A1/2, anti-CYP2B1/2 and anti-CYP2E1 in liver microsomes from male Wistar rats (lane 1, 40 μg protein) and male B6C3F1 mice (lane 2, 40 μg protein). Each lane 3 contained liver microsomes prepared previously [12]: 3-methylcholanthrene-treated rats (20 mg/kg for 4 days, i.p.) (3 μg protein) for anti-CYP1A1/2, phenobarbital-treated rats (80 mg/kg for 4 days, i.p.) (20 μg protein) for anti-CYP2B1/2, and ethanol-induced rats (2 g/rat for 3 weeks, gavage) (40 μg protein) for anti-CYP2E1.

immunodetection using Vectastain ABC kit [anti-mouse immunoglobulin G, Burlingame, CA, U.S.A.]. Anti-CYP2B1/2 was used only to detect the CYP2B1/2 isozymes, not in the immunoinhibition because of the non-detection of this isozyme in liver microsomes. Anti-CYP2C11/6 was used in immunoinhibition analysis and not in immunoblot analysis because it has a much lower binding affinity for sodium dodecyl sulfate-denatured cytochrome P450s than for undenatured cytochromes [24].

Statistics. Analysis of variance was performed. Student's *t*-test or a paired *t*-test was employed under the null-hypothesis that there is no significant difference between groups. The 0.05 level of probability was the criterion of significance.

RESULTS

Microsomal protein and cytochrome P450

There was no difference between the species in the hepatic microsomal protein and cytochrome P450 (Table 1), so that the incubations employing the same amount of microsomal protein from the two species contained the same amount of cytochrome P450.

Immunodetection of cytochrome P450 isozymes by MAbs

In examination of rat and mouse liver microsomal proteins by immunoblot analysis, anti-CYP2B1/2 did not detect anything in untreated rat and mouse liver microsomes (Fig. 1). Anti-CYP2E1 gave a clear-cut band in liver microsomes, which was more pronounced in mice than in rats. Anti-CYP1A1/2 bound microsomal protein in the region of cytochrome P450s only in mice; the band was in the area of lower molecular mass of standard CYP1A1/2 staining, suggesting that constitutively expressed P450 isozymes may be CYP1A2 but not CYP1A1. Although Waxman *et al.* [25] reported that rat also has CYP1A2 constitutively, we could not detect the protein in untreated rat microsomes. The amount of CYP1A2 in rats seems to be as low as it could not be detected by our condition of western blot analysis.

Table 2. Metabolism of benzene, toluene and TRI (nmol/mg protein/min, mean ± SD) in liver microsomes from male Wistar rats and male B6C3F1 mice

		Toluene			
Species	Benzene	BA	*o*-Cresol	*p*-Cresol	TRI
	(0.23 mM)		(0.20 mM)		(0.20 mM)
Rat	0.53 ± 0.07	1.05 ± 0.19	ND	ND	0.62 ± 0.07
Mouse	1.36 ± 0.08*	2.06 ± 0.25*	0.30 ± 0.07*	0.10 ± 0.01*	1.84 ± 0.24*
	(6.26 mM)		(5.0 mM)		(5.9 mM)
Rat	0.98 ± 0.08†	10.07 ± 1.53†	0.16 ± 0.03†	0.12 ± 0.01†	1.51 ± 0.27†
Mouse	1.40 ± 0.15*	4.06 ± 0.26*†	0.69 ± 0.12*†	0.17 ± 0.03*†	2.98 ± 0.42*†

The percentage conversion of each substrate in rat and mouse liver microsomes was as follows: 0.9 and 2.4% at 0.23 mM, and 0.6 and 0.09% at 6.26 mM of benzene; 2.1 and 4.9% at 0.2 mM, and 0.8 and 0.4% at 5.0 mM of toluene; 3.7 and 11.0% at 0.2 mM, and 0.3 and 0.4% at 5.9 mM of TRI.

* Significant difference ($P < 0.05$) between rats and mice.

† Significant difference ($P < 0.05$) between low and high substrate concentrations.

ND, not detected.

Table 3. Inhibition of benzene metabolism by MAbs

MAbs	Rat	Mouse
0.23 mM		
Hy-Hel	0.52 ± 0.15* (100)†	1.48 ± 0.18§ (100)
Anti-CYP2E1	0.17 ± 0.03‡ (33 ± 6)‡	0.67 ± 0.13‡§ (45 ± 5)‡§
6.26 mM		
Hy-Hel	1.09 ± 0.11 (100)	1.45 ± 0.10 (100)
Anti-CYP2C11/6	0.77 ± 0.12‡ (71 ± 7)‡	1.37 ± 0.08§ (94 ± 3)§
Anti-CYP1A1/2	1.06 ± 0.13 (98 ± 7)	1.39 ± 0.07§ (96 ± 3)

* Mean ± SD of remaining activity (nmol/mg protein/min) for five animals.
† Mean ± SD of percentages of remaining activity expressed as activity with MAbs/activity with Hy-Hel × 100.
‡ Significantly different ($P < 0.05$) from respective Hy-Hel value.
§ Significantly different ($P < 0.05$) from respective rat value.

Metabolic rate of benzene, toluene and TRI in rat and mouse liver microsomes

The metabolic rate of benzene in rats doubled when a high substrate concentration was used, whereas the rate in mice at the high concentration was the same as that at the low concentration (Table 2). The metabolic rate of benzene in mouse liver was 2.6 times higher than that in rats at the low concentration and 1.4 times higher at the high substrate concentration.

In toluene metabolism, the rate of BA formation was much greater at a high than at a low substrate concentration in rats; a similar, but less marked increase was seen in mice. The rate of BA formation in mice was double that in rats at low substrate concentration, but the difference in the rate was reversed at high substrate concentration. As for the BA formation, the rates of *o*- and *p*-cresol formation were greater at high than at low substrate concentration in both rat and mouse liver microsomes. In contrast to the result obtained for BA formation, however, the rates of *o*- and *p*-cresol formation in mice were always higher than those in rats. Although the ratio of *o*- and *p*-cresol formation to that of total metabolites was almost the same in rats, the ratio of *o*-cresol to total metabolite formation in mice was four times higher than that of *p*-cresol.

The metabolic rate of TRI in rat and mouse liver microsomes was increased with the high substrate concentration and was consistently higher in mice than in rats.

Immunoinhibition of benzene metabolism

At low substrate concentration, anti-CYP2E1 inhibited the activity of BAH by 67% in rat liver microsomes and 55% in mouse (Table 3). Thus, although the inhibitable percentage was slightly higher in rats than in mice, the inhibitable activity (net inhibition, difference between activity with Hy-Hel and that with the MAb) in rat liver microsomes was about half that of mice. In contrast, anti-CYP2C11/6 inhibited BAH activity only in rat liver microsomes, by 29%. Anti-CYP1A1/2 did not inhibit BAH activity in microsomes from either rats or mice.

Immunoinhibition of toluene metabolism

Anti-CYP2E1 inhibited BA formation from toluene by 19% and 25% in terms of percentage inhibition in rat and mouse liver microsomes, respectively; the degree of inhibitable activity in mice was twice that in rats (Table 4). At a high toluene concentration, anti-CYP2C11/6 strongly inhibited BA formation in rat liver microsomes, and less so in mice; it was therefore surprising that the activity remaining in rat liver microsomes after immunoinhibition by this MAb was similar to that of mice. Anti-CYP1A1/2 did not inhibit BA formation from toluene in liver microsomes from rats or mice.

In both species, anti-CYP2E1 did not inhibit the formation of *o*-cresol from toluene at low concentration; anti-CYP1A1/2 also did not inhibit the formation at high concentration. Anti-CYP2C11/6 inhibited *o*-cresol formation in liver microsomes from rats but not in those from mice.

Similarly, anti-CYP2E1 slightly inhibited *p*-cresol formation in liver microsomes from rats but not in those from mice, and neither anti-CYP2E1 nor anti-CYP1A1/2 inhibited *p*-cresol formation.

Immunoinhibition of TRI metabolism

Anti-CYP2E1 strongly inhibited the formation of chloral hydrate from TRI in terms of percentage inhibition in liver microsomes from rats, but only mildly in liver microsomes from mice (Table 5). The inhibitable activity was, however, greater in liver microsomes from mice than in those of rats. Anti-CYP2C11/6 inhibited the formation of chloral hydrate in liver microsomes from both rats and mice, but the degree and percentage of inhibitable activity were greater in rats than in mice. Anti-CYP1A1/2 moderately inhibited chloral hydrate formation only in liver microsomes from mice.

DISCUSSION

The present study showed a marked difference in the metabolism of benzene and TRI *in vitro* between male Wistar rats and male B6C3F1 mice. Mice metabolized more benzene and TRI than rats at low and high substrate concentrations, and more toluene

Table 4. Inhibition of toluene metabolism by MAbs

MAbs	Rat	Mouse
Benzyl alcohol formation		
0.20 mM		
Hy-Hel	1.00 ± 0.13* (100)†	1.97 ± 0.27§ (100)
Anti-CYP2E1	0.80 ± 0.11‡ (81 ± 8)‡	1.50 ± 0.31‡§ (75 ± 9)‡
5.0 mM		
Hy-Hel	9.40 ± 1.54 (100)	3.97 ± 0.63§ (100)
Anti-CYP2C11/6	2.67 ± 0.05‡ (29 ± 5)‡	2.88 ± 0.32‡ (72 ± 11)‡
Anti-CYP1A1/2	9.73 ± 1.47 (104 ± 12)	3.95 ± 0.60§ (100 ± 7)
o-Cresol formation		
0.20 mM		
Hy-Hel	ND	0.31 ± 0.03§ (100)
Anti-CYP2E1	ND	0.30 ± 0.06§ (97 ± 9)
5.0 mM		
Hy-Hel	0.15 ± 0.02 (100)	0.61 ± 0.06§ (100)
Anti-CYP2C11/6	0.04 ± 0.01‡ (29 ± 5)‡	0.60 ± 0.04§ (97 ± 9)§
Anti-CYP1A1/2	0.15 ± 0.02 (101 ± 6)	0.60 ± 0.04§ (98 ± 5)
p-Cresol formation		
0.20 mM		
Hy-Hel	ND	0.10 ± 0.02§ (100)
Anti-CYP2E1	ND	0.09 ± 0.02§ (90 ± 6)
5.0 mM		
Hy-Hel	0.11 ± 0.02 (100)	0.16 ± 0.02§ (100)
Anti-CYP2C11/6	0.09 ± 0.02‡ (89 ± 4)‡	0.14 ± 0.01§ (98 ± 9)
Anti-CYP1A1/2	0.11 ± 0.01 (101 ± 8)	0.15 ± 0.02§ (97 ± 4)

* Mean ± SD of remaining activity (nmol/mg protein/min) for five animals.
† Mean ± SD of percentages of remaining activity expressed as activity with MAbs/activity with Hy-Hel × 100.
‡ Significantly different ($P < 0.05$) from respective Hy-Hel value.
§ Significantly different ($P < 0.05$) from respective rat value.
ND, not detected.

Table 5. Inhibition of trichloroethylene metabolism by MAbs

MAbs	Rat	Mouse
0.20 mM		
Hy-Hel	0.40 ± 0.06* (100)†	1.76 ± 0.28§ (100)
Anti-CYP2E1	0.13 ± 0.03‡ (32 ± 4)‡	1.33 ± 0.23‡§ (76 ± 1)‡
5.9 mM		
Hy-Hel	1.16 ± 0.16 (100)	2.36 ± 0.39§ (100)
Anti-CYP2C11/6	0.65 ± 0.08‡ (57 ± 10)‡	1.95 ± 0.37‡§ (83 ± 9)‡
Anti-CYP1A1/2	1.16 ± 0.09 (101 ± 7)	1.81 ± 0.26‡§ (77 ± 4)‡

* Mean ± SD of remaining activity (nmol/mg protein/min) for five animals.
† Mean ± SD of percentages of remaining activity expressed as activity with MAbs/activity with Hy-Hel × 100.
‡ Significantly different ($P < 0.05$) from respective Hy-Hel value.
§ Significantly different ($P < 0.05$) from respective rat value.

than rats only at a low concentration; in contrast, rats metabolized toluene in a higher rate than mice at a high substrate concentration.

This species difference may depend, at least in part, on the different distribution of P450 isozymes and on the affinity of these solvents for P450 isozymes in the two species. The rate of benzene metabolism in mice was consistently higher than in rats, in good agreement with results obtained *in vivo* [5, 6]. Although rats have much more CYP2C11/6 than mice (Table 4), the affinity of benzene for this isozyme is relatively low compared with that of toluene [10] (Tables 3 and 4). Therefore, CYP2C11/6 does not contribute to the greater activity for BAH in rats than in mice. Since CYP2E1 contributed mainly to the metabolism of benzene in liver microsomes from both rats and mice (Table 3), the greater metabolic activity for BAH in mice than in rats may be due mainly to the high level of CYP2E1 in mice liver microsomes, as shown by immunoblot analysis (Fig. 1) and the MAb-directed inhibitable activity of BAH (Table 3). The difference, however,

may not be due only to different amounts of this enzyme, because the activity remaining after immunoinhibition by the relevant MAb is also different. P450 isozymes other than CYP2E1, not identified by us, may also contribute to the observed metabolic difference for benzene.

Numerous studies suggested that metabolism of benzene is required for hematopoietic toxicity, and muconaldehyde and benzoquinone may be responsible for a portion of the toxic effects of benzene [1]. Since mice have a higher capacity for the first step of benzene metabolism, they might have higher concentrations of these toxic metabolites in target tissues, and have higher susceptibility to the toxic effects of benzene. Thus, CYP2E1 may be involved in the higher susceptibility to benzene hematopoietic toxicity in mice.

The rate of metabolism of TRI was two to four times higher in mice than in rats, in good agreement with observations *in vivo* [7, 8, 26]. One reason for this species difference may be differential contents of CYP2E1 and CYP1A2, which also contribute to TRI metabolism [10, 27]. On the other hand, CYP2C11/6 contributed more to TRI metabolism in rats than in mice. Nevertheless, mice have more active TRI metabolism than rats, suggesting that enzymes other than CYP2E1 and CYP1A2 are involved in the higher activity of TRI metabolism in mice. In fact, the activities remaining after immunoinhibition by these MAbs were higher in mice than in rats.

Mice metabolized more TRI to a hepatic macromolecular binding metabolite *in vivo* than rats; high doses of TRI caused histopathological changes which lead to an increase in DNA synthesis and incidence of mitotic figures in mouse liver, but not in rat liver [7]. One reason that mice are more susceptible to the hepatotoxicity of TRI than rats may be due to the prevailing existence of CYP2E1 and CYP1A2.

The species difference in benzene metabolism was similar to that for TRI. The metabolism of benzene was, however, saturated at a concentration less than 0.23 mM in mouse but not in rat liver microsomes. This finding is in concordance with the report that the apparent K_m for benzene is five times lower in mice than in rats [6]. The explanation may be in differences in the relative contributions of CYP2C11/6 and CYP1A2 to the metabolism of the two chemicals: these isozymes contribute to the metabolism of TRI but not of benzene. This seems to be inconsistent with the fact that CYP2C11/6 and CYP1A2 contribute to the metabolism of benzene in control and 3-methylcholanthrene-treated microsomes, respectively [12]. Mice may have insufficient CYP2C11/6 and CYP1A2 to catalyse the oxidation of benzene but enough to catalyse the metabolism of TRI. In fact, the amount of CYP1A2 in untreated mouse liver microsomes was less than 10% of that in 3-methylcholanthrene-treated rat liver microsomes (Fig. 1).

Species differences in the metabolism of toluene depend on the metabolic route. Metabolic activity for toluene side-chain oxidation was greater in mice than rats at low substrate concentration but greater in rats than mice at high substrate concentration, whereas the activity for toluene aromatic hydroxylation was consistently higher in mice than in rats. The difference in BA formation between the two species may be due to different contents of CYP2E1 at low substrate concentration and of CYP2C11/6 at high concentration. In particular, the activity remaining after inhibition by anti-CYP2C11/6 in rats was similar to that in mice, suggesting that the species difference in the activity for BA formation at high substrate concentration is only due to the different expression of CYP2C11/6 between rats and mice. We found no P450 isozymes with high activity for the formation of *o*-cresol in mice; only CYP2C11/6 contributed to formation of this metabolite in rats but not in mice. We reported previously [12] that anti-CYP1A1/2 strongly inhibits *o*-cresol formation from toluene in 3-methylcholanthrene-treated rat liver microsomes. Even this MAb, however, did not inhibit *o*-cresol formation in mouse liver microsomes, suggesting either that mouse CYP1A2 is inactive in the formation of *o*-cresol from toluene, or that the amount of this enzyme in mice is insufficient to catalyse the formation. P450 isozymes other than those investigated here may contribute to *o*-cresol formation in mouse liver microsomes. Thus, not only quantitative but also qualitative differences in toluene metabolism were seen between the two species. This may be due to the different contribution of P450 isozymes to three pathways of toluene metabolism between mouse and rat liver microsomes.

In conclusion, a different distribution of P450 isozymes was seen between male Wistar rats and male B6C3F1 mice. The species differences in the metabolism of benzene, toluene and TRI were therefore dependent on their affinities for different P450 isozymes. Of course, it is prudent to point out that the manifestation of the toxicity of these chemicals require even chronic administration in which case species differences in the induction of P450 protein by these compounds may be more relevant than the basal enzyme levels. In order to evaluate the risk of exposure to chemicals, the results from studies in rodents are sometimes extrapolated to humans. It is, therefore, very important to know the differences in the metabolism between species. In general, the mouse has a greater metabolic activity for chemicals than the rat; however, while this is true for benzene and TRI, it may not hold for toluene.

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