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METABOLIC ACTIVATION OF ENVIRONMENTAL CARCINOGENS AND MUTAGENS BY HUMAN LIVER MICROSOMES

ROLE OF CYTOCHROME P-450 HOMOLOGOUS TO A 3-METHYLCHOLANTHRENE-INDUCIBLE ISOZYME IN RAT LIVER

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Abstract—The metabolic activation of procarcinogens and promutagens by human liver microsomal cytochrome P-450 has been investigated by means of a newly developed method measuring the induction of *umu* gene in *Salmonella typhimurium* TA1535/pSK1002 [T. Shimada and S. Nakamura, *Biochem. Pharmac.* **36**, 1979 (1987)]. The chemicals examined were aflatoxin B_1 (AFB_1), eight carcinogenic heterocyclic aromatic amines isolated from protein and amino acid pyrolysates, and 2-aminoanthracene. Liver microsomes from six patients catalyzed the metabolic activation of these chemicals; 2-amino-3,5-dimethylimidazo[4,5-*f*]quinoline (MeIQ) and AFB_1 were most actively bioactivated, followed by 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-aminoanthracene (2-AA) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline. At least two forms of human cytochrome P-450 may be involved in the activation of these procarcinogens. This suggestion was supported by the following lines of evidence: (a) addition of non-ionic detergent Emulgen 913 to the incubation mixture caused a more profound inhibition of microsome-catalyzed activation of AFB_1 than of MeIQ, IQ and 2AA, (b) 7,8-benzoflavone stimulated the activation of AFB_1 by about 2.5-fold, whereas it inhibited significantly the reactions with MeIQ, IQ and 2AA, and (c) polyclonal antibodies against a 3-methylcholanthrene-inducible form of rat cytochrome P-450 (P-450d) caused a marked inhibition of the metabolic activation of MeIQ, IQ and 2-AA by human liver microsomes though they did not show any effects on the microsomal activation of AFB_1. Data are also presented showing that none of the reactions catalyzed by human liver microsomes were inhibited by antibodies to a phenobarbital-inducible form of rat cytochrome P-450 (P-450b). These results suggest that the human cytochrome P-450 isozyme that is immunochemically similar and, thus, homologous to rat P-450d plays a major role in the metabolic activation of several procarcinogens examined, and that the activation of AFB_1 is catalyzed by another and, possibly, not phenobarbital-inducible form(s) of human cytochrome P-450.

Several classes of environmental chemicals are carcinogenic and mutagenic only after metabolic activation by a cytochrome P-450-linked monooxygenase system [1–3]. Carcinogenic polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene, benz[*a*]anthracene and chrysene, are bioactivated to DNA-damaging products by a form of cytochrome P-450 (P-450c)† which is inducible by MC in rat liver microsomes [1–3]. On the other hand, a variety of heterocyclic aromatic amines including Trp-P-1, Trp-P-2, Glu-P-1, IQ and MeIQ have been reported to be highly mutagenic after activation by another MC-inducible form of cytochrome P-450 (P-450d) that is more active than P-450c [4–6]. Although a potent hepatocarcinogen, AFB_1 is also transformed by cytochrome P-450 in a variety of animals examined, it is not clear which form(s) of cytochrome P-450 is (are) actually involved in the activation process [6–10]. Our recent investigations employing induction of *umu* gene in *Salmonella typhimurium* TA1535/pSK1002 by activated carcinogens have suggested that constitutive forms of cytochrome P-450 have more important roles for the activation of AFB_1 than MC- and PB-inducible isozymes in rat liver microsomes [6]. Thus, the importance of individual forms of cytochrome P-450 in the bioactivation of environmental carcinogens and mutagens has been suggested.

Human liver microsomes also contain multiple forms of cytochrome P-450, and individual isozymes have recently been purified and characterized [11–15]. Although polymorphic variations of human cytochrome P-450 species in some of the drug oxidation

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† Abbreviations: MC, 3-methylcholanthrene; PB, phenobarbital; AFB_1, aflatoxin B_1; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ, 2-amino-3,5-dimethylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; AαC, 2-amino-9*H*-pyrido[2,3-*b*]indole; MeAαC, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; 2-AA, 2-aminoanthracene; P-450b, a major form of rat cytochrome P-450 induced by PB; P-450c, a low spin form of rat cytochrome P-450 induced by MC; P-450d, a high spin form of rat cytochrome P-450 induced by MC; and IgG, immunoglobin G.

reactions are important for elucidating the mechanism of bioactivation and detoxication of xenobiotics, Wolff *et al.* [16] have reported recently that a human cytochrome P-450 isozyme ($P\text{-}450_{DB}$), a polymorphic debrisoquine 4-hydroxylase, does not participate in the metabolic activation of a number of carcinogens including 2-aminofluorene, benzo[*a*]pyrene and AFB_1. However, very recently several laboratories have identified cytochrome P-450 species that are homologous to rat cytochromes P-450 including P-450c and P-450d in human liver microsomes [17–21]. These results suggest that human liver microsomes also contain several cytochrome P-450 species that are active in transforming procarcinogens and promutagens.

In this paper, we show that a variety of chemicals including AFB_1, eight carcinogenic heterocyclic aromatic amines, and 2-AA are metabolized by human liver microsomes to genotoxic intermediates which cause induction of *umu* gene in *S. typhimurium* TA1535/pSK1002. The potent genotoxic compounds after the activation by liver microsomes are AFB_1 and MeIQ, followed by IQ, 2-AA and MeIQx, and data are also presented that at least two forms of cytochrome P-450 in human liver microsomes may be involved in the activation of these compounds. This conclusion is supported by the experiments employing the effects of cytochrome P-450 modifiers on the activities of human liver microsomes, and the inhibitory effects of some of the polyclonal antibodies raised against cytochromes P-450 isolated from rat liver microsomes.

MATERIALS AND METHODS

Materials. AFB_1 was purchased from the Makor Chemical Co. (Israel), and PB and MC from the Katayama Chemical Co. (Osaka). Trp-P-1, Trp-P-2, Glu-P-1, IQ, MeIQ, MeIQx, AαC and MeAαC were gifts of Drs. M. Nagao and S. Sato of the National Cancer Center Research Institute. 2-AA, 7-ethoxyresorufin, $NADP^+$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from the Sigma Chemical Co. (St. Louis, MO), and the enzyme substrate *o*-nitrophenyl-β-D-galactopyranoside for the assay of β-galactosidase activity was from the Wako Pure Chemical Co. (Osaka). All the other chemicals and reagents used were of the highest quality commercially available.

Preparation of liver microsomes. The liver tissues from four cancer patients (HL-CP1, HL-CP2, HL-CP3, and HL-CP4) undergoing liver resection were obtained from Dr. K. Inui of the Center for Adult Disease and were selected from areas of the liver that were visually free of tumors. Liver samples from organ donors (HL-OD1 and HL-OD2) who met accidental deaths were donated by Dr. C. von Bahr of the Karolinska Institute to Dr. Y. Funae of the Osaka City University Medical School, and we obtained these liver microsomes from Dr. Y. Funae. Liver microsomes were prepared by differential centrifugation, as described by Guengerich [22], and were stored frozen at −80° in 10 mM Tris–acetate buffer (pH 7.4) containing 1 mM EDTA and 20% (v/v) glycerol.

Liver microsomes from untreated, and PB- and MC-treated rats (Sprague–Dawley strain, weighing about 150 g) were also obtained by the same methods.

Purification of rat liver cytochromes P-450 and preparation of antibodies. Three forms of rat liver cytochrome P-450, termed P-450b, P-450c, and P-450d* according to the nomenclature by Levin and associates [23], were purified to apparent homogeneity by the methods described previously [6, 24]. P-450b is a major isozyme induced in rats by PB, and P-450c and P-450d are two types of cytochrome P-450 induced by MC. The specific contents of these cytochrome P-450 isozymes were greater than 15 nmol/mg protein.

Rabbit antisera against these cytochromes P-450 were obtained, and the IgG fractions were prepared by the methods described previously [6, 13].

Analytical and assay methods. The induction of *umu* gene in *S. typhimurium* TA1535/pSK1002 by activated metabolites of procarcinogens and promutagens was determined as described previously [6]. Briefly, the standard reaction mixture (final volume, 1.0 ml) contained: 50 mM potassium phosphate buffer (pH 7.25), 0.25 mM $NADP^+$, 5 mM glucose-6-phosphate, 0.5 unit of glucose-6-phosphate dehydrogenase, 3 mM $MgCl_2$, 0.1 mM EDTA, 0.01 nmol equivalent of human liver microsomal cytochrome P-450, 0.01 mM substrates (except that the concentrations of AαC and MeAαC were 0.08 mM) dissolved in 10 μl of dimethyl sulfoxide, and 0.75 ml of the bacterial tester strain *S. typhimurium* TA1535/pSK1002 (the bacterial density at 600 nm was about 0.28). The reaction was run at 37° for 30, 60, and 90 min, and then was terminated by rapid cooling in an ice-water bath. The expressed β-galactosidase activity was determined by the method of Miller [25], and the induction of *umu* gene expression was represented as specific β-galactosidase activity/min/nmol cytochrome P-450 [6]. One unit (U) of activity was arbitrarily defined as 1000 units of β-galactosidase activity determined by the method of Miller [25].

7-Ethoxyresorufin *O*-deethylase activity was determined by the method of Pohl and Fouts [26]. Cytochrome P-450 and protein contents in liver microsomes were assayed by the methods of Omura and Sato [27] and Lowry *et al.* [28] respectively.

RESULTS

Case histories. The case histories, when available, the concentration of cytochrome P-450, and the activity of 7-ethoxyresorufin *O*-deethylase in liver microsomes from six human samples are shown in Table 1. The mean cytochrome P-450 concentration was 0.26 nmol/mg protein, and some of the preparations also contained considerable amounts of cytochrome P-420, a denatured form of cytochrome P-450. From these reasons the induction of *umu* gene expression by activated carcinogens was expressed on the basis of cytochrome P-450 content. 7-Ethoxyresorufin *O*-deethylase activities by liver microsomes

* In our previous paper, these three forms of rat cytochrome P-450 have been described as PB-1, MC-1, and MC-2 respectively [6].

Table 1. Case histories of six human subjects: cytochrome P-450 content and 7-ethoxyresorufin *O*-deethylase activity in liver microsomes

Subject	Sex	Age	Cytochrome P-450 (nmol/mg protein)	Ethoxyresorufin O-deethylation (nmol/min/nmol P-450)	Case history
HL-CP1	M	33	0.21	1.3	Hepatic tumor
HL-CP2	M	54	0.12	0.90	Hepatic tumor
HL-CP3	M	48	0.24	0.83	Hepatic tumor
HL-CP4	F	53	0.16	2.4	Hepatic tumor
HL-OD1	M	24	0.31	3.8	Accidental death
HL-OD2	F	44	0.56	0.83	Accidental death

Each value represents the mean of duplicate determinations.

from six patients ranged between 0.8 and 3.8 nmol/min/nmol P-450.

Effects of concentrations of cytochrome P-450 and substrates on the induction of umu *gene expression in* S. typhimurium TA1535/pSK1002. Human liver microsomes catalyzed the metabolic activation of several procarcinogens and promutagens in the present assay system. Figure 1 shows the effects of concentrations of cytochrome P-450 and substrates on the expression of *umu* gene in *S. typhimurium* TA1535/pSK1002. Increasing the concentrations of cytochrome P-450 in the reaction mixture caused an enhancement of the gene expression; the linear increase was observed in concentrations of cytochrome P-450 up to 0.01 μM (Fig. 1A). The highly effective inducers were MeIQ and AFB_1, followed by IQ and 2-AA. As shown in Fig. 1B, the maximum activities of *umu* gene expression were obtained with substrate concentrations between 0.01 and 0.02 mM, except for AαC and MeAαC for which a 0.08 mM concentration was required for exerting full activities (data not shown). From these results the following experiments were done with 0.01 μM for the concentration of cytochrome P-450 and 0.01 mM for the concentration of substrate except for AαC and MeAαC in which 0.08 mM substrate was used.

Figure 2 shows the effects of metabolic activation of MeIQ, AFB_1, IQ and 2-AA on the cell growth of *S. typhimurium* TA1535/pSK1002 in human liver microsomes. As can be seen, the cell growth rate was retarded by the activated metabolites of the chemicals; the order of inhibitory actions of these chemicals was MeIQ, AFB_1, IQ, and 2-AA.

Metabolic activation of ten procarcinogens and promutagens by liver microsomes from six patients. Table 2 shows the activation by liver microsomes from six patients of ten procarcinogens and promutagens to genotoxic metabolites which caused induction of an *umu* gene in *S. typhimurium* TA1535/pSK1002. As mentioned above, MeIQ and AFB_1 were efficiently bioactivated by all of the human liver microsomal preparations used, and IQ, MeIQx and 2-AA were intermedially genotoxic. The other five compounds were not so potent inducers of *umu* gene in the present assay system.

Since we obtained human liver samples from four surgery patients under anesthesia, these liver microsomes may have different isozyme compositions of

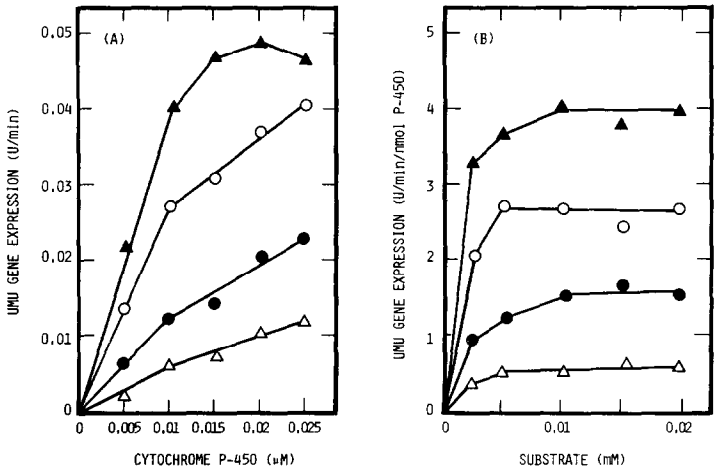

Fig. 1. Effects of concentrations of cytochrome P-450 (A) and substrates (B) on the induction of *umu* gene by MeIQ (▲), AFB_1 (○), IQ (●), and 2-AA (△) in liver microsomes from human sample HL-OD1. The substrate concentration used in Fig. 1A was 0.01 mM, and the concentration of cytochrome P-450 in Fig. 1B was 0.01 μM. Experimental values from Fig. 1 to Fig. 8 are represented as means of duplicate determinations.

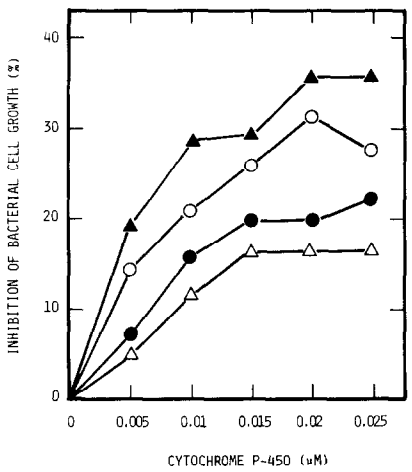

Fig. 2. Effect of concentration of cytochrome P-450 on the inhibition of bacterial growth by MeIQ (▲), AFB_1 (○), IQ (●), and 2-AA (△) in liver microsomes from human sample HL-OD1. The inhibition of cell growth was determined at 600 nm using the growth rate without an NADPH-generating system as control. Other details are given in the legend to Fig. 1.

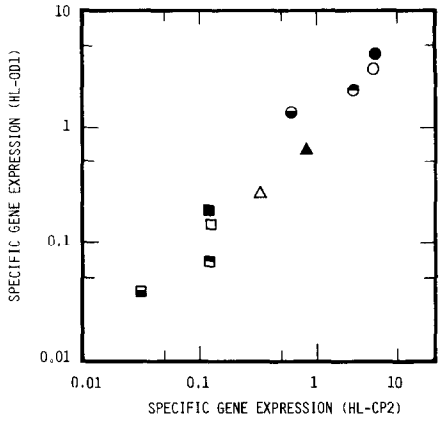

Fig. 3. Correlation among activities of liver microsomes between human sample HL-CP2 and HL-OD1 on the induction of *umu* gene expression by ten chemicals. The chemicals used were: AFB_1 (○), Trp-P-1 (△), Trp-P-2 (□), Glu-P-1 (■), IQ (◓), MeIQ (●), MeIQx (◒), AαC (▬), MeAαC (▬) and 2-AA (▲).

cytochrome P-450 from those of normal individuals such as organ donors. To show that no differences exist in monooxygenase activities of liver microsomes between surgery patients and organ donors, we compared the relative activation by these liver microsomes of ten procarcinogens and promutagens to genotoxic metabolites. As shown in Fig. 3, the activities of liver microsomes with ten chemicals from a surgery patient (HL-CP2) were closely correlated ($r = 0.98$) with those from an organ donor (HL-OD1). When the comparison was also made between each of the liver microsomes from six human samples, the correlation coefficients in all of the cases exceeded over 0.88 (data not shown).

Effects of Emulgen 913 *and* 7,8-*benzoflavone on the liver microsome-catalyzed activation of several procarcinogens.* To show the possible roles of individual forms of human cytochrome P-450 in the metabolic activation of procarcinogens, we examined the effects of Emulgen 913 and 7,8-benzoflavone on the metabolic activation of chemicals, since these two agents have been reported to be activators or inhibitors of monooxygenase activities depending upon the uses of individual forms of cytochrome P-450 in the reaction mixture [29, 30]. As shown in Fig. 4, Emulgen 913 inhibited the activation of AFB_1 by human liver microsomes very markedly, whereas it did not affect the activation of MeIQ, IQ, and 2-AA at the concentrations within 0.008%. When the higher concentrations of the detergent were used, the lower activities with the latter three chemicals were also observed.

Figure 5 shows the effects of 7,8-benzoflavone on the activation of four procarcinogens by human liver microsomes. In contrast to the effects of Emulgen 913, 7,8-benzoflavone inhibited markedly the microsomal activities with MeIQ, IQ, and 2-AA, whereas it enhanced the activation of AFB_1 by human liver microsomes markedly.

Effects of antibodies raised against rat cytochromes P-450 on the metabolic activities of human liver microsomes. Previously we have shown that antibodies to rat P-450d inhibit most actively the meta-

Table 2. Induction of *umu* gene expression by ten procarcinogens and promutagens in liver microsomes from six human samples

	umu Gene expression (U/min/nmol P-450)					
	HL-CP1	HL-CP2	HL-CP3	HL-CP4	HL-OD1	HL-OD2
AFB_1	1.3	3.2	1.7	2.0	3.1	2.1
Trp-P-1	0.19	0.32	0.20	0.10	0.28	0.05
Trp-P-2	0.05	0.13	0.03	0.11	0.15	0.05
Glu-P-1	0.03	0.13	0.07	0.30	0.18	0.07
IQ	0.79	2.1	1.2	2.5	2.0	0.51
MeIQ	2.9	3.4	3.0	4.6	4.1	3.1
MeIQx	0.23	0.62	0.38	1.2	1.4	0.14
AαC	0.12	0.13	0.04	0.14	0.07	0.06
MeAαC	0.05	0.03	0.06	0.08	0.04	0.04
2-AA	0.41	0.83	0.41	1.5	0.65	0.44

The induction of *umu* gene expression by activated metabolites of procarcinogens and promutagens in liver microsomes from six human samples was determined as described in Materials and Methods. Each value represents the mean of duplicate determinations.

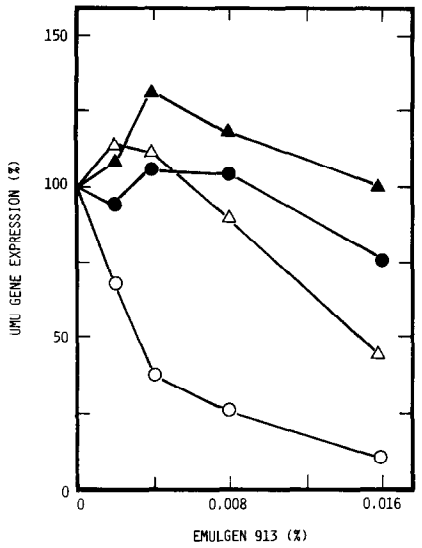

Fig. 4. Effect of Emulgen 913 on the induction of *umu* gene expression by MeIQ (▲), IQ (●), 2-AA (△), and AFB_1 (○) in liver microsomes from human sample HL-CP4. The control activities without Emulgen 913 are given in Table 2.

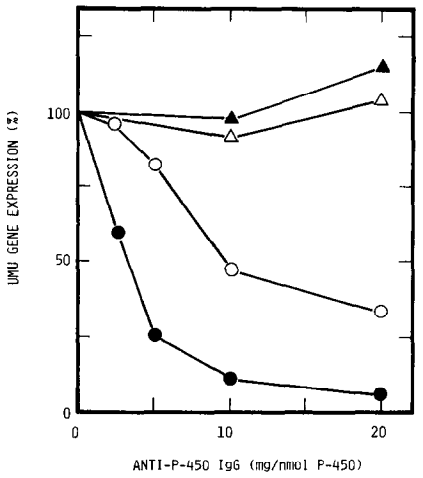

Fig. 6. Effects of preimmune IgG (△), anti-P-450b IgG (▲), anti-P-450c IgG (○), and anti-P-450d IgG (●) on the induction of *umu* gene expression by MeIQ in liver microsomes from untreated male rats. The specific activity without antibodies was 1.80 U/min/nmol P-450.

bolic activation of MeIQ catalyzed by liver microsomes from polychlorinated biphenyl-treated rats [6]. As shown in Fig. 6, the antibodies to P-450d also inhibited completely, and anti-P-450c IgG partially, the genotoxic activation of MeIQ by liver microsomes from untreated male rats. As expected, the anti-P-450b IgG did not affect the microsomal activation of MeIQ. Figure 7A shows the effects of these antibodies on the 7-ethoxyresorufin *O*-deethylase activity catalyzed by liver microsomes from MC-treated rats. The reaction was inhibited more strongly by anti-P-450c IgG than by anti-P-450d IgG. Anti-P-450b IgG did not affect the *O*-deethylase activity of rat liver microsomes. On the other hand, 7-ethoxyresorufin *O*-deethylase activity of liver microsomes from human sample HL-CP4 was inhibited markedly by anti-P-450d IgG, while both antibodies against P-450c and P-450b were less active (Fig. 7B).

Figure 8 shows the effects of these antibodies on the metabolic activation of AFB_1, MeIQ, IQ, and 2-AA by human liver microsomes. Activation of AFB_1 was unaffected by antibodies to three forms of rat cytochrome P-450. On the other hand, the addition of antibodies against P-450c and P-450d in the reaction mixture caused an inhibition of metabolic activation of MeIQ, IQ, and 2-AA by human liver microsomes; the latter antibodies were more effective than the former. The antibodies to P-450b did not inhibit the genotoxic activation of these procarcinogens by human liver microsomes.

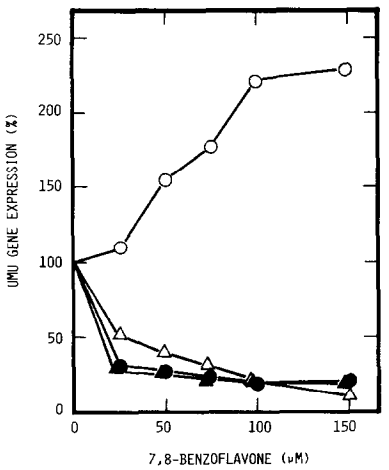

Fig. 5. Effect of 7,8-benzoflavone on the induction of *umu* gene by AFB_1 (○), MeIQ (▲), IQ (●) and 2-AA (△) in liver microsomes from human sample HL-CP4. The control activities without 7,8-benzoflavone are shown in Table 2.

DISCUSSION

Recent numerous studies have shown that two MC-inducible forms of cytochrome P-450 in liver microsomes from mice, rats or rabbits have important roles in the metabolic activation of a wide variety of environmental chemicals to carcinogenic and mutagenic metabolites [1–10]. Several classes of carcinogenic polycyclic aromatic hydrocarbons including benzo[*a*]pyrene, benz[*a*]anthracene and chrysene are bioactivated extensively by a low spin type of the MC-inducible cytochrome P-450, namely P_1-450 in mice, P-450c in rats, and P-450LM6 in rabbits [1–3]. Additional studies have also established that a high spin form of cytochrome P-450, which is called P_3-450 in mice, P-450d in rats and P-450LM4 in rabbits, has a major role in the carcinogenic and mutagenic activation of many other chemicals including 2-AA, 2-aminofluorene, and carcinogenic heterocyclic aromatic amines from amino acid and protein pyrolysates [4, 5]. Using polyclonal and monoclonal antibodies against these forms of cytochrome P-450, several laboratories have recently isolated cDNA

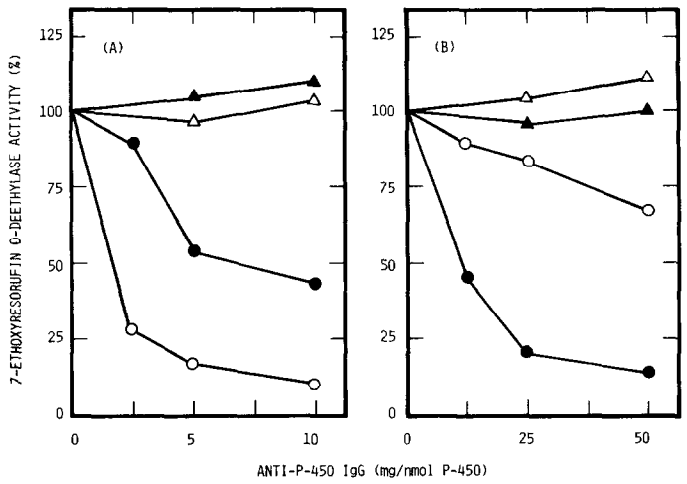

Fig. 7. Effects of preimmune IgG (△), anti-P-450b IgG (▲), anti-P-450c IgG (○), and anti-P-450d IgG (●) on the 7-ethoxyresorufin *O*-deethylase activity catalyzed by liver microsomes from MC-treated rats (A) and from human sample HL-CP4 (B). The specific activities without antibodies were 11.9 nmol/min/nmol P-450 for rat liver microsomes, and 3.81 nmol/min/nmol P-450 for human liver microsomes.

clones complementary to human cytochromes P-450 which are homologous to two MC-inducible forms in mice, rats, or rabbits [17–21]. Very recently, Wrighton *et al.* [21] reported that human liver microsomes contain considerable amounts of cytochrome P-450 immunochemically similar to rat P-450c and P-450d. They also showed that, although a type of cytochrome P-450 related to rat P-450d can be detected in all of the human liver preparations examined, a P-450c type of the cytochrome is detectable in only one of the fifteen human preparations.

The present investigation was undertaken to determine whether the individual forms of human cytochrome P-450 could also participate in the metabolic activation of several procarcinogens and promutagens in a newly developed method by measuring the induction of *umu* gene expression in *S. typhimurium* TA1535/pSK1002 [6]. Although we used human liver samples from four surgery patients under anesthesia and two organ donors who died accidentally, the relative activations by these liver microsomes of ten chemicals were highly correlated (cf. Fig. 3). These results suggest that surgical treatment and liver tumors may not affect the isozyme compositions of cytochrome P-450 in human liver microsomes.

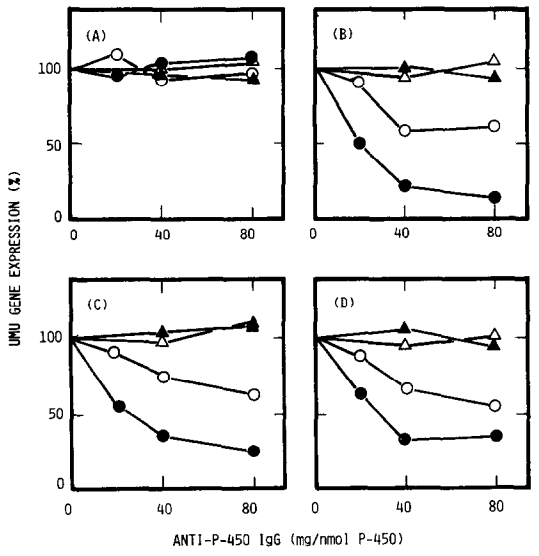

Fig. 8. Effects of preimmune IgG (△), anti-P-450b IgG (▲), anti-P-450c IgG (○), and anti-P-450d IgG (●) on the induction of *umu* gene expression by AFB_1 (A), IQ (B), MeIQ (C), and 2-AA (D) in liver microsomes from human sample HL-CP4. The specific activities in the absence of antibodies are given in Table 2.

The following lines of evidence indicate that a human cytochrome P-450 homologous to rat P-450d plays an important role in the genotoxic activation of several chemical carcinogens. First, all of the liver microsomes from six human samples catalyzed efficiently the genotoxic activation of MeIQ, IQ, MeIQx and 2-AA; these reactions have been reported to be mediated most actively by a form of cytochrome P-450 (P-450d) in rat liver microsomes [4–6]. Second, 7,8-benzoflavone, a known inhibitor of monooxygenase activities associated with MC-inducible forms of cytochrome P-450 [30], inhibited very markedly the genotoxic activation of MeIQ, IQ, and 2-AA by human liver microsomes. Finally, polyclonal antibodies against rat P-450d affected the activities of human liver microsomes to catalyze MeIQ, IQ and 2-AA more profoundly than those against rat P-450c. Data were also presented that anti-P-450b IgG did not cause an inhibition of the reaction with these procarcinogens in human liver microsomes. These results are compatible with the findings of Wrighton *et al.* [21] and led us to conclude that a form of human cytochrome P-450 homologous to rat P-450d can actually participate in the metabolic activation of several classes of environmental carcinogens and mutagens.

7-Ethoxyresorufin *O*-deethylation has been shown to be a typical monooxygenase activity associated with an MC-inducible form of cytochrome P-450 (P-450c) in rat liver microsomes [26]. The present results

support this view, since anti-P-450c IgG strongly inhibited the 7-ethoxyresorufin *O*-deethylase activity catalyzed by liver microsomes from MC-treated rats. On the other hand, in human liver microsomes the polyclonal antibodies to rat P-450c were weakly effective in the metabolism of 7-ethoxyresorufin, although anti-P-450d IgG inhibited markedly the O-deethylation when an excess amount of the antibodies was added to the reaction mixture. These results suggest that in human liver microsomes the cytochrome(s) P-450 homologous to rat P-450d has (have) more important roles for the oxidative metabolism of a number of xenobiotics than those homologous to rat P-450c.

AFB_1 is a potent hepatocarcinogenic mycotoxin and has been shown to be catalyzed to a putative reactive electrophile AFB_1-2,3-epoxide by cytochrome P-450 [31–33]. Our previous studies have indicated that constitutive forms of cytochrome P-450 have important roles for the genotoxic activation of AFB_1 in rat liver microsomes (unpublished results) [6]. The present results show that all of the human liver microsomes could catalyze the activation of AFB_1 at relatively high rates, suggesting the possibility that the specific, possibly constitutive, forms of human cytochrome P-450 may also be involved in this reaction. The possible roles of human cytochrome P-450 isozymes homologous to rat P-450b, P-450c, and P-450d in the activation of AFB_1 may be excluded, because polyclonal antibodies raised against rat P-450b, P-450c and P-450d did not show any effects on the reactions catalyzed by human liver microsomes. The different effects of Emulgen 913 and 7,8-benzoflavone on the reactions with AFB_1 and other heterocyclic aromatic amines by human liver microsomes supported the above suggestion.

In conclusion, the present study shows that there are at least two cytochrome P-450 species that are involved in the metabolic activation of environmental carcinogens and mutagens in human liver microsomes. The significance of the major roles of a form of human cytochrome P-450 homologous to rat P-450d in the metabolic activation of several heterocyclic aromatic amines and 2-AA is suggested in the present study. The search for a specific form(s) of cytochrome P-450 involved in the bioactivation of AFB_1 is currently underway in this laboratory.

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