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Metabolic Activation of Pyrolysate Arylamines by Human Liver Microsomes; Possible Involvement of a P-448-H Type Cytochrome P-450

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Metabolic activating capacity of human livers for carcinogenic heterocyclic arylamines has been studied using a *Salmonella* mutagenesis test. A large individual variation was observed among 15 liver samples in the capacities of activation of Glu-P-1 (2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole), IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline) and MeIQx (2-amino-3,8-dimethyl-3*H*-imidazo[4,5-*f*]quinoxaline). The average numbers of revertants induced by the three heterocyclic arylamines were nearly the same or rather higher in the presence of hepatic microsomes from human than those from rat. In high-performance liquid chromatography, formation of N-hydroxy-Glu-P-1 was detected and accounted for more than 80% of the total mutagenicity observed in the human microsomal system with Glu-P-1, indicating that, similarly to experimental animals, N-hydroxylation is a major activating step for heterocyclic arylamines in human. Addition of flavone or 7,8-benzoflavone to human liver microsomes showed effective inhibition of the mutagenic activation of Glu-P-1, although the treatment rather enhanced microsomal benzo[*a*]pyrene hydroxylation in human livers. Mutagenic activation of Glu-P-1 by human liver microsomes was also decreased by the inclusion of anti-rat P-448-H IgG, and was well correlated with the content of immunoreactive P-448-H in livers, suggesting the involvement of a human cytochrome P-450, which shares immunochemical and catalytic properties with rat P-448-H, in the metabolic activation of heterocyclic arylamines in human livers.

Key words: Human P-448-H — Glu-P-1 — IQ — MeIQx

Dietary factors are believed to be a major contributor to cancer incidence in humans.[2–5] Most carcinogens which are present in foodstuffs require metabolic activation for their carcinogenic and mutagenic effects, and the activating capacity is dependent on the hepatic activities of carcinogen-metabolizing enzymes.[6–13] In experimental animals, heterocyclic arylamine pyrolysates such as Glu-P-1,*2 IQ and MeIQx undergo N-hydroxylation, as a main activating step, by hepatic cyt P-450s. Studies in our laboratory indicate that a high-spin form of cyt P-450, P-448-H, mainly catalyzes the metabolic activation of these pyrolysates in rat livers.[10–12] Using a partially purified preparation, a specific form(s) of cyt P-450 was shown to catalyze the activation of Trp-P-1 (3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole) and Trp-P-2 (3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole) in human liver.[7] Recent progress on cyt P-450 genes also suggests the existence of a P-448-H type P-450 in human liver.[14] However, the chemical and substrate specificities of this form are still unknown.

Recent reports on extensive inter-individual variations in *in vitro* drug-metabolizing activities have suggested the existence of variations in cyt P-450 isozyme compositions in human livers.[15–18] Knowledge of the hepatic P-450 isozyme composition and of the rela-

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*2 Abbreviations used: cyt P-450, microsomal cytochrome P-450; P-448-H, a high-spin form of cyt P-450 which probably corresponds to P-450d,[1] as judged from the spectral and catalytic properties, and electrophoretic comparison in SDS gel; AHH, aryl hydrocarbon hydroxylase; BP, benzo[*a*]pyrene; Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethyl-3*H*-imidazo[4,5-*f*]quinoxaline; N-OH-Glu-P-1,2-N-hydroxyamino-6-methyl-dipyrido[1,2-*a*:3′,2′-*d*]-imidazole; HPLC, high-performance liquid chromatography; IgG, immunoglobin G; rev., revertants.

tive concentrations in an individual would provide valuable information on the potential susceptibility of that individual to chemical carcinogens.[18-20] In the present study, the human activating capacity for pyrolysates has been investigated in relation to human P-448-H level as detected on the basis of its immunocrossreactivity with IgG of rat P-448-H antibody.

Materials and Methods

Chemicals IQ hydrobromide monohydrate salt, MeIQx bromide salt and Glu-P-1 were supplied under the Research Resource Program for Cancer Research of the Ministry of Education, Science, and Culture, Japan. Flavone and 7,8-benzoflavone were obtained from Wako Pure Chemicals, Osaka. Benzo[*a*]pyrene, dithiothreitol and ellipticine were from Sigma Chemical Co., St. Louis, Mo. $NADP^+$, glucose-6-phosphate and glucose 6-phosphate dehydrogenase purified from yeast were purchased from Oriental Yeast Co., Osaka. *Salmonella typhimurium* TA98 was kindly provided by Dr. M. Nagao, National Cancer Center Research Institute, Tokyo. DE-52 was purchased from Whatman, Kent, England. All the other chemicals used for chromatographic and immunological studies were of analytical grade and were obtained from the sources described elsewhere.[21-24]

Human Liver Samples Autopsy liver samples of fifteen patients were obtained within 3 hr after clinical death and stored at $-80°$ until use. The case histories of these patients are summarized in Table I. Physically normal portions of livers were used in this study.

Preparation of Rat Liver Microsomes Adult male Sprague-Dawley rats were used to prepare hepatic microsomes as described previously.[24] Washed microsomes were stored at $-80°$ until used and microsomal protein concentration was determined by the method of Lowry *et al.*[25]

Preparation of Human Liver Microsomes Microsomal fractions were prepared from 5-g portions of human liver samples in a mixture of potassium chloride (0.1*M*) and potassium phosphate buffer (0.1*M*) containing 1m*M* dithiothreitol (DTT), by the same method as employed in the preparation of rat liver microsomes. The contents of cyt P-450 and b_5 were determined according to the methods of Omura and Sato,[26] and Omura and Takesue,[27] respectively. NADPH-cyt P-450 reductase was measured spectrophotometrically using a Beckman DU-65 spectrophotometer by the method of Phillips and Langdon.[28] Microsomal benzo[*a*]-pyrene hydroxylation (AHH) was quantitated by the method reported previously[29] using a Hitachi 650-60 fluorescence spectrophotometer (Hitachi, Japan).

Microsomal Activation of Pyrolysates Glu-P-1, IQ and MeIQx were activated in incubation mixtures consisting of 0.25 mg protein of microsomes, an NADPH generating system (1.6*M* $NADP^+$, 16 m*M* glucose 6-phosphate, 6m*M* $MgCl_2$ and 1.0 IU of glucose 6-phosphate dehydrogenase), 50m*M* potassium phosphate buffer (pH 7.4) and 0.1m*M* Glu-P-1, IQ or MeIQx in a final volume of 0.5 ml. The reaction was started by the addition of the NADPH generating system, which had been preincubated for 3 min at 37°. The mixture was incubated at 37° for 10 min.

Mutation Assay Ames mutation assay[30] was done with modifications for comparison of microsomal activating capacities.[31] A two hundred microliter volume of the reacted mixture was filtered by using a 0.45 μm HAWP 01300 millipore filter for sterilization and termination of the microsomal activating reaction. When necessary, flavone (50μM), 7,8-benzoflavone[11, 12, 24, 32] (50 μM) or ellipticine[11, 33] (1 μM) was added. More than 90% of the mutagenicity was recovered in the filtrate by using this filtration procedure. A ten microliter volume (rat microsomal incubation mixture) or 25 μl volume (human microsomal incubation mixture) of the filtrate was transferred to a tube containing 400 μl of 100m*M* sodium phosphate (pH 7.4) and 100 μl of the bacterial suspension. The mixture was incubated at 37° for 10 min to ensure the contact of the mutagen and bacteria; and after mixing with 2 ml of molten soft agar, it was poured gently onto minimal-glucose agar plates containing 0.1 μmol of L-histidine and 0.1 μmol of biotin. The His^+ revertants were counted after incubation at 37° for 48 hr. The number of spontaneous revertants (25–30 revertants/plate) was subtracted from each count. The data are the mean values of at least triplicate determinations from individual samples.

Analysis of N-Hydroxy-Glu-P-1 Separation of N-OH-Glu-P-1 from Glu-P-1 was carried out by HPLC; a Jasco Tri-Rotar V pump and UVIDEC 100-III UV absorbance monitor (254 nm) connected with a Nucleosil C_{18} reverse-phase column (0.39×30 cm) were used. A precolumn (0.39×2.5 cm) packed with Nucleosil C_{18} was used to minimize the loss of separation efficiency of the analytical column. The reaction mixture was eluted with a mobile phase of acetonitrile: 20m*M* sodium acetate buffer pH 5.3 (25:75, v/v) at a flow rate of 1.0 ml/min as described previously.[10] The reaction was stopped by the addition of an equal volume of acetonitrile:water (50:50, v/v). The resultant supernatant, after centrifugation at 2,500*g* for 5 min, was injected onto the column by means of the automatic sample injector. The amount of N-OH-Glu-P-1 was determined from the area under the

peak using a calibration curve prepared with synthetic N-OH-Glu-P-1 as mentioned previously.[34]

Immunochemical Studies A polyclonal antibody was prepared from goat immunized with a purified preparation of rat P-448-H according to a previously described schedule, and IgG fraction was prepared by ammonium sulfate fractionation followed by DEAE-cellulose column chromatography.[35] For immunochemical inhibition, goat anti rat P-448-H IgG was used at different concentrations (25, 50, 100 and 200 μg of the protein) with 100 μg protein of a human microsomal preparation (sample number 12), which showed the highest activating rate with Glu-P-1 among those examined. AHH activity was also examined in the presence of the antibody to check the specificity of the antibody used.[29] All the other experimental conditions were exactly the same as mentioned in the descriptions of the determinations of mutagenic and AHH activities. In a Western blot, the same polyclonal antibody of rat P-448-H as described above was used on the basis of its cross reaction with P-448 type cyt P-450 in the human samples. Other procedures were done as described previously.[29, 36]

Results

Source and Hepatic Levels of Microsomal Electron-transferring Components of Individuals Contents of cyt P-450 and b_5, and activity of NADPH-cyt P-450 reductase in hepatic microsomes prepared from apparently normal portions of livers of 15 individuals are

Table I. Case Histories, cyt P-450 and b_5 Contents, and Microsomal Activities of Human Subjects in Comparison with Sprague-Dawley Rats

Sample	Sex	Age	cyt P-450[a]	cyt b_5[a]	NADPH-cyt P-450-reductase[b]	AHH (BP) activity[c]	Case history
Human							
01	F	72	0.23	0.23	0.20	6.3	Unstable angina (pectoris)
02	F	38	0.05	0.01	0.06	ND[d]	Lymphoma
03	M	62	0.25	0.20	0.03	ND[d]	Lung cancer
04	F	36	0.23	0.21	0.10	7.4	Chronic renal failure (malignant hypertension)
05	M	66	0.21	0.29	0.11	11.5	Renal failure (lung emphysema)
06			0.32	0.10	0.05	0.7	Unidentified
07	M	18	0.24	0.36	0.20	27.7	Cerebral tumor
08	F	63	0.21	0.20	0.12	11.4	Cerebral aneurysm explosion
09			0.22	0.08	0.10	2.0	Unidentified
10	F	64	0.35	0.28	0.20	27.2	Duodenal ulcer
11			0.02	0.12	0.03	0.6	Unidentified
12	M	58	0.37	0.25	0.31	38.5	Cancer of lachrymal gland (liver metastasis)
13	M	46	ND[d]	0.01	0.03	1.2	Dissecting aneurysm
14	M	82	0.01	0.12	0.13	9.3	Ischemic heart disease (mitral valve disease)
15	F	63	0.27	0.20	0.16	16.9	Liver left lobe hemangioma
Mean			0.20	0.18	0.12	10.7	
±SE			±0.03	±0.03	±0.02	±2.96	
Rat[e]							
Male			0.72	0.41	0.27	231.4	
			±0.05	±0.05	±0.01	±10.7	
Female			0.54	0.43	0.26	42.3	
			±0.03	±0.02	±0.09	±3.0	

a) nmol/mg of protein.
b) U/mg of protein/min.
c) pmol/mg of protein/min.
d) ND, Not detected (cyt P-450, less than 0.01 nmol/mg of protein; AHH, less than 0.6 pmol/mg of protein/min).
e) Mean of five animals used per group±SE.

described in Table I. The average content of cyt P-450 in human livers was 0.20 nmol/mg protein, which was 3.6- or 2.7-fold lower than those in male and female rat livers, respectively. The microsomal hemoprotein, cyt b_5, was also 2-fold lower in livers of human than rats. The activity of NADPH-cyt P-450 reductase was also measured and was nearly half in human as compared to those of male and female rats. Activity of benzo[*a*]pyrene hydroxylase (AHH) is often used as a probe to assess the susceptibility to chemical-induced carcinogenesis in human. In the present study, the rate of microsomal benzo[*a*]-pyrene hydroxylation differed markedly among individuals and no significant correlation was found between the contents of cyt P-450 and b_5, and the reductase activity (data not shown). The mean value of AHH activity in human was four- or twenty-fold less than that of female or male rats, respectively, although some individuals showed nearly the same activity as that of female rats.

Mutagenic Activation of Pyrolysates In this experiment, a *Salmonella* mutagenesis test with a modified procedure using a filtration step to terminate the microsomal activation at an appropriate time was used to compare quantitatively the microsomal activating capacity of each sample. As described in Table II, heterocyclic arylamines, Glu-P-1, IQ and MeIQx, were activated to their mutagens by human liver microsomes. For Glu-P-1, a 13-fold difference was observed in the rate of mutagenic activation among human microsomes. The mean value (3.0×10^3 rev./mg protein/min) was slightly higher than the rates observed with microsomes of male and female rats. Similar results were also detected with IQ and MeIQx. Also, in accordance with our previous results,[12] the activating rates of IQ and MeIQx were higher in male than female rats. However, no significant sex difference was observed in the activation of both compounds in human. Among human individuals, samples 2 and 12 showed higher activating capacities for mutagenic activation of

Table II. Mutagenic Activation of Pyrolysates by Human and Rat Liver Microsomes

Sample	Mutagenicity ($\times10^{-3}$ rev./mg of protein/min)		
	Glu-P-1	IQ	MeIQx
Human			
01	1.5	1.9	1.4
02	6.9	4.8	4.1
03	1.1	1.3	2.0
04	1.9	1.6	1.9
05	1.3	1.7	1.3
06	2.8	4.4	1.9
07	1.2	1.7	1.7
08	1.4	1.6	1.7
09	1.7	1.6	1.5
10	1.2	1.9	1.7
11	1.4	1.9	1.2
12	14.4	7.8	4.5
13	1.5	1.5	2.0
14	2.8	1.8	1.7
15	3.5	2.1	2.3
Mean±SE	3.0±0.9	2.5±0.5	2.1±0.2
Rat[a]			
Male	2.3±0.2	2.2±0.3	1.7±0.05
Female	1.9±0.1	1.3±0.2	1.1±0.05

a) Mean of five animals used per group±SE.

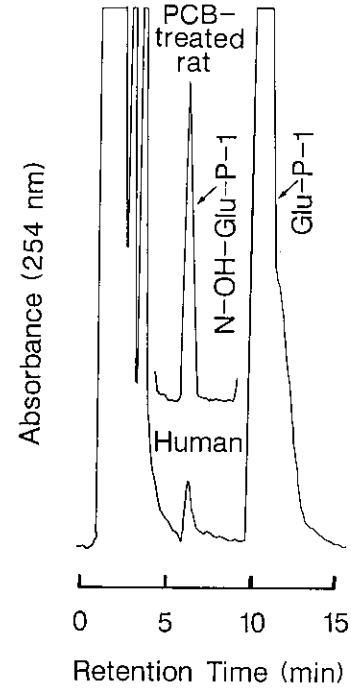

Fig. 1. Separation of the mutagenic metabolite of Glu-P-1 by HPLC. Metabolites formed by human liver microsomes and by PCB-treated rat liver microsomes (upper inset) are shown as detected by UV absorbance.

Table III. The Rate of Glu-P-1 N-Hydroxylation in Human Liver Microsomes

Sample	N-Hydroxy-Glu-P-1 (nmol/mg of protein/min)
Human	
04	0.14
09	0.39
10	0.21
12	0.64
14	0.16
15	0.24
Rat (mean±SE)	
Male	0.32±0.06
Female	0.35±0.09

All the other human samples showed no detectable activity (less than 0.1 nmol/mg of protein/min).

Table IV. Effect of Known Rat P-448 Inhibitors on the Human Liver Microsomal Activation Capacity

Chemical	Concentration (μM)	Mutagenicity Glu-P-1[a]	AHH activity BP[b]
None		6.5 (100)[c]	35.5 (100)
Ellipticine	1	0.3 (4.6)	5.9 (16.6)
Flavone	50	0.8 (12.3)	73.9 (208)
7,8-Benzoflavone	50	2.4 (36.9)	105.1 (296)

a) Number of revertants $\times 10^3$/mg of protein/min.
b) pmol/mg of protein/min.
c) Relative percent.

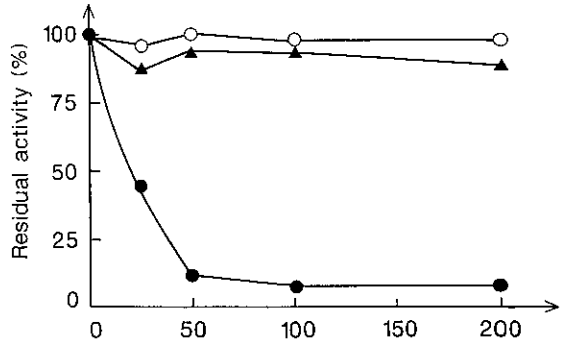

Fig. 2. Effect of anti-P-448-H IgG on the microsomal activation of Glu-P-1 (determined by Ames test) and AHH (determined fluorimetrically) in human liver. Assay was done by incubating anti P-448-H IgG or IgG fraction of non immunized rabbit serum with human microsomes and Glu-P-1 or BP as mentioned in "Materials and Methods." The microsomal protein used in this experiment was 100 μg/incubation mixture. Open symbols show the effect of non-immune IgG (○), and closed symbols indicate the effect of the antibody on benzo[*a*]pyrene (▲) and Glu-P-1 (●) activation.

all these heterocyclic arylamines, although sample 2 had no detectable benzo[*a*]pyrene hydroxylase activity (Table I).

N-Hydroxylation of Glu-P-1 To assess the mechanism of mutagenic activation of heterocyclic arylamines in human livers, microsomal metabolism of Glu-P-1 was studied by HPLC. As shown in Fig. 1, a metabolite which comigrated with synthetic N-hydroxy-Glu-P-1 was detected in the reaction mixture containing human or PCB-treated rat liver microsomes. The isolated metabolite from HPLC of the human sample showed direct mutagenicity to *Salmonella* at around 8.0×10^3 rev./nmol, which is comparable to the specific activity of the synthetic standard, N-hydroxy-Glu-P-1. In addition, there is a good correlation ($r=0.828$) between the number of revertants induced and microsomal N-hydroxylation rate of Glu-P-1 with 7 individuals from the data shown in Table III.

Characteristics of Microsomal Activating Enzyme in Human Liver Heterocyclic arylamines have been shown to be activated by microsomal cyt P-450, particularly the P-448-type forms and to some extent by a constitutive form, P-450-male, in rat liver.[11,12] To understand the characteristics of the enzyme catalyzing the activation of heterocyclic arylamines in human liver, the effects of chemicals which modify the activity of cyt P-450 were examined using liver microsomes of human sample 12. Similar to the results with rat liver, addition of 1μM ellipticine effectively decreased both the mutagenic activation of Glu-P-1 and activity of AHH in human liver (Table IV). Flavone and 7,8-benzoflavone have also been shown to inhibit the activation of heterocyclic arylamines catalyzed by P-448-type cyt P-450 in rats.[11,12,24] Mutagenic activations of Glu-P-1 in human liver

Fig. 3. Immunoblot of the human liver microsomes against goat anti-rat P-448-H IgG. Rat P-448-H was used as a standard at concentrations of 0.75 (a), 0.5 (b), 0.05 (c) and 0.15 (d) pmol per 80 μl, which was applied to SDS gel as same as the human samples.

microsomes were also reduced to 12% and 37% of the untreated control by the addition of 50μM flavone or 7,8-benzoflavone, respectively. However, microsomal benzo[*a*]pyrene hydroxylation in human liver was rather enhanced by the addition of these compounds as previously described.[37] These results suggest that a form(s) of P-448-type cyt P-450 mediates the activation of Glu-P-1 in human liver. Therefore, the effect of antibody raised against a rat cyt P-450, P-448-H, was examined on the microsomal activation of Glu-P-1 to assess the role of human orthologue P-448-H. As shown in Fig. 2, the number of revertants was decreased by the addition of anti-P-448-H IgG in a dose-dependent manner and was less than 20% of the control at the concentration of 50 μg anti-P-448-H IgG/100 μg human microsomal protein. On the other hand, AHH activity was not appreciably inhibited by the addition of the antibody even at the highest concentration used with Glu-P-1. These results suggest that the activation of Glu-P-1, but not benzo[*a*]pyrene hydroxylation, in human liver is catalyzed mainly by the orthologue P-448-H.

Immunoquantitation of the Orthologue P-448-H As described above, human microsomal activation of Glu-P-1 was inhibited by anti P-448-H IgG. Thus, by the use of the immunocross reactivity between anti-P-448-H IgG and human microsomes, the hepatic level of P-448 type cyt P-450 in human was quantitated by a Western blot method. The immunoblots of the fifteen samples are shown in Fig. 3. Immunoreactive human P-448-H was detected in 7 samples (Table V) in the range of 2.9−59.6 pmol equivalent/mg of microsomal protein (21.6±6.8 pmol equivalent/mg of microsomal protein as the mean of 7 samples±SE). On the other hand, P-448-H levels in male and female rats are 17.4±1.3 and 28.2±0.8 pmol/mg of microsomal protein, respectively. The average level in human is less than that in rats, but some individuals, especially number 12, showed fairly high

Table V. Immunoquantitation of P-448-H Level in Human Liver Microsomes

Sample	Immunoreactive P-448-H level (pmol/mg of protein)
Human	
01	ND[a]
02	3.4
03	ND
04	23.5
05	ND
06	ND
07	ND
08	11.8
09	ND
10	24.4
11	14.8
12	59.6
13	ND
14	2.9
15	ND
Mean±SE	21.6±6.8 (7 samples)
Rat[b]	
Male	17.4±1.3
Female	28.2±0.8

a) ND, not detected (less than 1 pmol/mg of protein).
b) Mean of five animals used per group±SE.

levels. These results indicate that a cyt P-450, which shares the immunochemical and catalytic properties of P-448-H, exists in human livers. Among the 7 individuals, good correlations were found between the determined immunoreactive P-448-H content and N-hydroxylation ($r=0.787$) or mutagenicity of Glu-P-1 ($r=0.702$).

Discussion

Heterocyclic arylamines produced in the pyrolysis of proteins or foods are considered to be one of the main causative factors in human environmental carcinogenesis. In the present study, human liver has been shown to catalyze the metabolic activation of three carcinogenic heterocyclic arylamines, Glu-P-1, IQ and MeIQx, which were recently reported to be accumulated in plasma of uremic patients.[38,39] Among 15 human samples, a large individual variation was detected in the microsomal activation of these heterocyclic arylamines (Table II). The average rates of the mutagenic activation in human liver were nearly the same as or rather higher than those in rat liver. A large individual difference was also observed in microsomal benzo[*a*]-pyrene hydroxylation (Table I) but no appreciable correlation exists between the two reactions in human microsomes. The available data on medical history and microsomal components in each individual also did not afford any explanation for the large individual difference. These results suggest that the inter-individual difference exists in the activating capacity of human livers for these heterocyclic arylamines, although a deteriorative change before preparation of microsomes may also contribute to the difference.

Concerning the activation mechanism of these heterocyclic arylamines, human livers catalyzed the mutagenic activation of Glu-P-1 mainly through N-hydroxylation, as is the case with experimental animal species (Fig. 1, Table III).

Heterocyclic arylamines have been shown to be activated by microsomal cyt P-450, particularly the P-448-type forms,[10-12] and to some extent by a constitutive form, P-450-male, in rat liver.[12] Recent investigations using molecular cloning techniques indicate that cyt p-450 comprises a large family of hemoproteins and, within a sub-family, their primary structures are fairly well conserved among animal species.[14] In fact, several human cyt P-450 genes corresponding to animal counterparts have been demonstrated using cDNA of cyt P-450 in experimental animals. The existence of a cyt P-450 orthologous to rat P-448-H (P-450d) in human liver is also suggested by the results of molecular cloning and immunoblotting experiments.[14,40,41] As shown in Table IV, microsomal activation of Glu-P-1 in human liver was decreased by the addition of known inhibitors of rat P-448-H, ellipticine, flavone or 7,8-benzoflavone, and also by the addition of anti P-448-H, in a dose-dependent manner (Fig. 2). The involvement of P-448-H type of cyt P-450 was further supported by the results of immunoblotting, since microsomal contents of immunoreactive P-448-H in human livers were correlated with the rate of N-hydroxylation of Glu-P-1. These results indicate that, as in other animal species, P-448-H orthologue catalyzes the metabolic activation of heterocyclic arylamines in human liver, and the large individual differences in P-448-H orthologue content may be a factor affecting the susceptibility to chemical-induced carcinogenesis. In the present study, the cause of the individual differences in P-448-H orthologue content remained unresolved, but our recent study has indicated that P-448-H content in rat liver is increased in diabetes.[42] These results may imply that we need to consider the large variation in P-448-H orthologue content in association with the patho-physiological condition of human individuals, as well as chemicals ingested.

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References

1) Kamataki, T., Maeda, K., Yamazoe, Y., Matsuda, N., Ishii, K. and Kato, R. A high spin form of cytochrome P-450 highly purified from polychlorinated biphenyl-

treated rats: catalytic characterization and immunochemical quantitation in liver microsomes. *Mol. Pharmacol.*, **24,** 146–155 (1983).
2) Sugimura, T. and Sato, S. Mutagens-carcinogens in foods. *Cancer Res.* (Suppl.), **43,** 2415(s)–2421(s) (1983).
3) Doll, R. and Peto, R. Quantitative estimates of available risks of cancer in the United States today. *J. Natl. Cancer Inst.*, **66,** 1191–1308 (1981).
4) Nagao, M., Wakabayashi, K. and Sugimura, T. Mutagens in food and drinks, and their carcinogenicity. *In* "Mutagenicity Testing in Environmental Pollution Control," 19th Ed., ed. F. K. Zimmermann and R. E. Taylor-Mayer, pp. 69–85 (1985). Ellis Horwood Ltd., Chichester.
5) Sugimura, T., Sato, S., Ohgaki, H., Takayama, S., Nagao, M. and Wakabayashi, K. Mutagens and carcinogens in cooked foods. *In* "Genetic Toxicology of the Diet," ed. I. Knudsen, pp. 85–107 (1986). Alan R. Liss Inc., New York.
6) Kato, R. Metabolic activation of mutagenic heterocyclic aromatic amines from protein pyrolysates. *CRC Crit. Rev. Toxicol.*, **16,** 307–348 (1986).
7) Kato, R., Yamazoe, Y. and Kamataki, T. Metabolic activation of tryptophan pyrolysates, Trp-P-1 and Trp-P-2 by hepatic cytochrome P-450. *In* "Genetic and Environmental Factors in Experimental and Human Cancer," ed. H. V. Gelboin, B. Macmahon, T. Matsushima, T. Sugimura, S. Takayama and H. Takebe, pp. 79–87 (1980). Japan Sci. Soc. Press, Tokyo.
8) Wislocki, P. G., Miwa, G. T and Lu, A. Y. H. Reactions catalyzed by the cytochrome P-450 system. *In* "Enzymatic Basis of Detoxication," ed. W. B. Jakoby, Vol. 1, pp. 135–182 (1980). Academic Press, New York.
9) Kato, R., Kamataki, T. and Yamazoe, Y. Hydroxylation of carcinogenic and mutagenic aromatic amines. *Environ. Health Perspect.*, **49,** 21–25 (1983).
10) Ishii, K., Yamazoe, Y., Kamataki, T. and Kato, R. Metabolic activation of glutamic acid pyrolysis products, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole and 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole, by purified cytochrome P-450. *Chem.-Biol. Interact.*, **38,** 1–13 (1981).
11) Yamazoe, Y., Shimada, M., Kamataki, T. and Kato, R. Microsomal activation of 2-amino-3-methylimidazo[4,5-*f*]quinoline, a pyrolysate of sardine and beef extracts, to mutagenic intermediate. *Cancer Res.*, **43,** 5768–5774 (1983).
12) Yamazoe, Y., Abu-Zeid, M., Manabe, S., Toyama, S. and Kato, R. Metabolic activation of a protein pyrolysate promutagen 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) by rat liver microsomes and purified cytochrome P-450. *Carcinogenesis,* **9,** 105–109 (1988).
13) Kato, R. and Yamazoe, Y. Metabolic activation and covalent binding to nucleic acids of carcinogenic heterocyclic amines from cooked foods and amino acid pyrolysates. *Jpn. J. Cancer Res. (Gann),* **78,** 297–311 (1987).
14) Quattrochi, L. C., Pendurthi, U. R., Okino, S. T., Potenza, C. and Tukey, R. H. Human cytochrome P-450 4 mRNA and gene: part of a multigene family that contains Alu sequences in its mRNA. *Proc. Natl. Acad. Sci. USA,* **83,** 6731–6735 (1986).
15) Ekstrom, G., Bahr, C. V., Glaumann, H. and Ingelman-Sundberg, M. Interindividual variation in benzo[*a*]pyrene metabolism and composition of isoenzymes of cytochrome P-450 as revealed by SDS-gel electrophoresis of human liver microsomal fractions. *Acta Pharmacol. Toxicol.*, **50,** 251–260 (1982).
16) Kremers, P., Beaune, P., Cresteil, T., De Graeve, J., Columelli, S., Leroux, J. P. and Gielen, J. E. Cytochrome P-450 monooxygenase activity in human and rat liver microsomes. *Eur. J. Biochem.*, **118,** 599–606 (1981).
17) Jakobsson, S. W., Okita, R. T., Mock, N. J., Masters, B. S. S., Buja, L. M. and Prough, R. A. Monooxygenase activities of human liver, lung and kidney microsomes: a study of 42 post-mortem cases. *Acta Pharmacol. Toxicol.*, **50,** 332–341 (1982).
18) Davies, D. S., Kahn, G. C., Murray, S., Brodie, M. J. and Boobis, A. R. Evidence for an enzymatic defect in the 4-hydroxylation of debrisoquine by human liver. *Br. J. Clin. Pharmacol.*, **11,** 89–91 (1981).
19) Eichelbaum, M., Spannbrucker, N. and Dengler, H. J. A probable genetic defect of the metabolism of sparteine. *In* "Biological Oxidation of Nitrogen," ed. J. W. Gorrod, pp. 113–118 (1977). Elsevier/North-Holland Biomedical Press, Amsterdam.
20) Eichelbaum, M. Defective oxidation of drugs: pharmacokinetic and therapeutic implications. *Clin. Pharmacokinet.*, **7,** 1–22 (1982).
21) Kato, R., Yamazoe, Y., Shimada, M., Murayama, N. and Kamataki, T. Effect of growth hormone and ectopic transplantation of pituitary gland on sex-specific forms of cytochrome P-450 and testosterone and drug

oxidation in rat liver. *J. Biochem.*, **100,** 895–902 (1986).
22) Kamataki, T., Maeda, K., Yamazoe, Y., Nagai, T. and Kato, R. Partial purification and characterization of cytochrome P-450 responsible for sex difference in drug metabolism in the rat. *Biochem. Biophys. Res. Commun.*, **103,** 1–7 (1981).
23) Kamataki, T., Maeda, K., Shimada, M., Kitani, K., Nagai, T. and Kato, R. Age-related alteration in the activities of drug-metabolising enzymes and components of sex-specific forms of P-450 in liver microsomes from male and female rats. *J. Pharmacol. Exp. Ther.*, **233,** 222–228 (1985).
24) Yamazoe, Y., Kamataki, T. and Kato, R. Species difference in N-hydroxylation of a tryptophan pyrolysis product in relation to mutagenic activation. *Cancer Res.*, **41,** 4518–4522 (1981).
25) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193,** 265–275 (1951).
26) Omura, T. and Sato, R. The carbon monoxide-binding pigment of liver microsomes: solublization, purification and properties. *J. Biol. Chem.*, **239,** 2379–2385 (1964).
27) Omura, T. and Takesue, S. A new method for simultaneous purification of cytochrome b_5 and NADPH-cytochrome *c* reductase from rat liver microsomes. *J. Biochem.*, **67,** 249–257 (1970).
28) Phillips, A. V. and Langdon, R. G. Hepatic triphosphopyridine nucleotide cytochrome *c* reductase: isolation, characterization and kinetic studies. *J. Biol. Chem.*, **237,** 2652–2660 (1962).
29) Nebert, D. W. and Gelboin, H. V. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture: assay and properties of induced eyzyme. *J. Biol. Chem.*, **243,** 6242–6249 (1968).
30) Ames, B. N., McCann, M. and Yamasaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsomes mutagenicity test. *Mutat. Res.*, **31,** 347–363 (1975).
31) Yamazoe, Y., Ishii, K., Kamataki, T., Kato, R. and Sugimura, T. Isolation and characterization of active metabolite of tryptophan-pyrolysate mutagen, Trp-P-2 formed by rat liver microsomes. *Chem.-Biol. Interact.*, **30,** 125–138 (1980).
32) Diamond, L. and Gelboin, H. V. Alpha-naphthoflavone: an inhibitor of hydrocarbon cytotoxicity and microsomal hydroxylase. *Science*, **168,** 1023–1025 (1969).
33) Lesca, P., Rafidinarivo, E., Lecointe, P. and Mansuy, D. A class of strong inhibitors of microsomal monooxygenases. The ellipticines. *Chem.-Biol. Interact.*, **24,** 189–198 (1979).
34) Saito, K., Yamazoe, Y., Kamataki, T. and Kato, R. Syntheses of hydroxyamino, nitroso and nitro derivatives of Trp-P-2 and Glu-P-1, amino acid pyrolysate mutagens, and their direct mutagenicities towards *Salmonella typhimurium* TA98 and TA98NR. *Carcinogenesis*, **4,** 1547–1550 (1983).
35) Noshiro, M. and Omura, T. Immunochemical study on the electron pathway from NADH to cytochrome P-450 of liver microsomes. *J. Biochem.*, **83,** 61–77 (1978).
36) Yamazoe, Y., Shimada, M., Murayama, N. and Kato, R. Suppression of levels of phenobarbital-inducible rat liver cytochrome P-450 by pituitary hormone. *J. Biol. Chem.*, **262,** 7423–7428 (1987).
37) Kapitulnik, J., Poppers, P. J., Buening, M. K., Fortner, J. B. and Conney, A. H. Activation of monooxygenases in human liver by 7,8-benzoflavone. *Clin. Pharmacol. Ther.*, **22,** 475–484 (1978).
38) Manabe, S., Yanagisawa, H., Ishikawa, S., Kitagawa, Y., Kanai, Y. and Wada, O. Accumulation of 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole and 2-aminodipyrido-[1,2-*a*:3′,2′-*d*]imidazole, carcinogenic glutamic acid pyrolysis products, in plasma of patients with uremia. *Cancer Res.*, **47,** 6150–6155 (1987).
39) Yanagisawa, H., Manabe, S., Kitagawa, Y., Ishikawa, S., Nakajima, K. and Wada, O. Presence of 2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline (MeIQx) in dialysate from patients with uremia. *Biochem. Biophys. Res. Commun.*, **138,** 1084–1089 (1986).
40) McManus, M. E., Stupans, I., Ioannoni, B., Burgess, W., Robson, R. A. and Birkett, D. J. Identification and quantitation in human liver of cytochrome P-450 analogous to rabbit cytochromes P-450 forms 4 and 6. *Xenobiotica*, **18,** 207–216 (1988).
41) Jaiswal, A. K., Nebert, D. W. and Gonzalez, F. J. Human $P_3$450: cDNA and complete amino acid sequence. *Nucleic Acids Res.*, **14,** 6773–6774 (1986).
42) Yamazoe, Y., Abu-Zeid, M., Yamauchi, K., Murayama, N., Shimada, M. and Kato, R. Enhancement by alloxan-induced diabetes of the rate of metabolic activation of three pyrolysate carcinogens via increase in the P-448-H content in rat liver. *Biochem. Pharmacol.*, **37,** 2503–2506 (1988).