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Serum aminotransferase activity as a predictor of clearance of drugs metabolized by CYP isoforms in rats with acute hepatic failure induced by carbon tetrachloride

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Abstract

The values of serum aminotransferase activity (AST) in untreated rats and rats with acute hepatic failure at 24 h after an oral administration of CCl_4 (0.5 ml/kg) were 85 ± 9 IU/l and 4260 ± 620 IU/l (mean ± S.D., $n = 6$), respectively. The values of total clearance (CL_{tot}) after intravenous administration of caffeine, tolbutamide, chlorzoxazone or lidocaine (as probe drugs for various CYP isoforms) to CCl_4-treated rats were decreased to about 1/8, 1/3, 1/3 or 1/2 compared with those in untreated rats. Good correlations were observed between mRNA expression and enzyme activity of CYP2C11, CYP2E1, CYP3A2 and CYP1A2 in livers of rats given various doses of CCl_4. There was also a good negative correlation between serum AST activity and hepatic enzyme activity of each CYP. The serum AST activities corresponding to a 50% decrease of CYP2C 11, CYP2E1, CYP3A2 and CYP1A2 activities were about 710, 780, 1030 and 1300 IU/l, respectively. In conclusion, when the serum AST value in CCl_4-treated rats reached about 4000 IU/l, the hepatic CYP activities were one-tenth or less of the control, although the degree of decrease of the CL_{tot} values varied markedly. Nevertheless, the AST value appears to be a promising candidate for an indicator to predict appropriate dose modification of drugs for patients with acute hepatic failure.

Keywords: Cytochrome P450; Metabolic activity; Liver function; Acute hepatic failure; Disposition kinetics

1. Introduction

During drug therapy, it is important to take into account changes of disposition kinetics, in order to obtain optimum efficacy. However, when predicting drug disposition kinetics in patients with hepatic failure, there is no useful indicator corresponding to the creatinine clearance in patients with renal failure. Decrease in hepatic functions in patients with conditions such as hepatitis, hepatocirrhosis, and hepatocarcinoma is expected to influence the clearance of drugs which are metabolized and excreted by the liver. In particular, drug-metabolizing ability may affect drug disposition kinetics, and cytochrome P450 (CYP) plays a major role in drug metabolism in the liver. In humans, there are four predominant families of CYP species,

Abbreviations: CYP, cytochrome P450; RT-PCR, reverse transcription-polymerase chain reaction; AST, aspartate aminotransferase

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CYP1, CYP2, CYP3 and CYP4, and all the isoforms differ in their patterns of drug-metabolizing activity (Kerremans, 1996). It has also been reported that the amounts of CYP isoforms in the liver are altered in conditions involving hepatic failure (Guengerich and Turvy, 1991).

Clinically, it is important to establish appropriate drug therapy by predicting the metabolic ability in individual patients with hepatic failure. Wensing et al. (1997) proposed that the aminopyrine breath test, measurement of the elimination capacity for indocyanine green or galactose, or measurement of hepatic elimination of sorbitol can provide a quantitative measure of hepatic function. Other authors have proposed pharmacokinetic analysis after administration of various probe drugs for CYP isoforms to elucidate this problem (Tanaka et al., 1992; Huang et al., 1993; Girre et al., 1994; Sotaniemi et al., 1995; Horsmans et al., 1996; Tanaka, 1998). However, it is not easy to utilize these procedures to determine the degree of decrease in hepatic function in patients.

We considered that laboratory data might be useful to evaluate hepatic function and be available as a predictor of hepatic drug clearance. Here, we describe a study of the relationships among disposition kinetics, drug-metabolizing activities of CYP isoforms and laboratory data in rats treated with CCl_4 as a model of acute hepatic failure, and we propose that serum aminotransferase activity would be useful as an indicator to predict appropriate dose modification of drugs for patients with acute hepatic failure.

2. Methods

2.1. Materials

Ethoxyresorufin and 6-hydroxychlorzoxazone were purchased from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). Resorufin, 2α-testosterone, 6β-testosterone, 11β-testosterone, chlorzoxazone, 7-(β-hydroxy)theophylline and lidocaine hydrochloride were purchased from Sigma-Aldrich Co., Ltd. (MO, USA). CCl_4, caffeine and tolbutamide were purchased from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). Oligonucleotide primers were custom-synthesized by Amersham Pharmacia Biotech (UK). Other chemicals were of reagent grade.

2.2. Preparation of model rat for acute hepatic failure

Model rats for acute hepatic failure were prepared by oral administration of CCl_4 and used 24 h after the administration. CCl_4 was dissolved in corn oil and administered at a dose of 0.01, 0.025, 0.1, 0.25 or 0.5 ml/kg in a volume of 2 ml/kg to male Wistar rats (8 weeks old, Nippon SLC Co., Ltd., Hamamatsu, Japan). The untreated control rats received corn oil alone.

2.3. Determination of laboratory data

Blood samples were collected from the jugular vein under light ether anesthesia, and the plasma was separated by centrifugation and stored at −30 °C. Measurements of laboratory data were conducted by SRL Co. Ltd. (Tokyo, Japan).

2.4. Preparation of rat liver microsomes

For preparation of rat liver microsomes, the liver was homogenized in three volumes of 100 mM Tris–HCl buffer (100 mM KCl, 1 mM EDTA, pH 7.4). Microsomes were prepared as reported previously (Kamataki and Kitagawa, 1974) and stored at −80 °C until use. Protein contents were measured according to the method of Lowry et al. (1951).

2.5. Measurement of metabolic activities of CYP isoforms

The microsomal activity of CYP1A2 was measured in terms of *O*-dealkylation of ethoxyresorufin, essentially according to Burke et al. (1985). The reaction mixture (180 μl) contained ethoxyresorufin (final concentration 2 μM) and microsomal protein (final concentration 25 μg/ml) in Tris–HCl buffer diluted with distilled water (final concentration 100 mM, pH 7.4). After pre-incubation for 2 min at 37 °C, the reaction was started by adding NADPH (20 μl of a 50 mM solution). After incubation for 5 min, the reaction was stopped by adding 100 μl of ice-cold acetonitrile. Then, the solution was centrifuged at 12,000 × *g* for 5 min and 100 μl of the supernatant was injected into the HPLC column. All samples were analyzed on an HPLC system (Shimadzu, Kyoto, Japan) equipped with a Shim-pack CLC-ODS column

(4.6 mm × 250 mm, Shimadzu, Kyoto, Japan). Fluorescence was detected at an excitation wavelength of 574 nm and an emission wavelength of 596 nm. The mobile phase of 45% acetonitrile was pumped at a rate of 1 ml/min.

The microsomal activity of CYP2E1 was measured essentially according to Court et al. (1997). The reaction solution (180 μl) contained chlorzoxazone as a substrate of CYP2E1 (final concentration 50 μM) and microsomal protein (final concentration 500 μg/ml) in phosphate buffer diluted with distilled water (final concentration 50 mM, pH 7.4). Subsequent procedures were as described above. For HPLC, the mobile phase (20% acetonitrile in 50 mM phosphate buffer, pH 4.0) was pumped at a rate of 1 ml/min and the detection wavelength was 295 nm.

The microsomal activity of CYP2C11 or CYP3A2 was measured in terms of conversion of testosterone to 2α- or 6β-hydroxytestosterone, essentially according to Arlotto et al. (1991). The reaction solution (180 μl) contained testosterone (final concentration 50 μM) and microsomal protein (final concentration 500 μg/ml) in phosphate buffer diluted with distilled water (final concentration 100 mM, pH 7.4). Subsequent procedures were as described above. For HPLC, the mobile phase consisted of 60% methanol pumped at a rate of 1 ml/min, and the detection wavelength was 240 nm.

2.6. Reverse transcription-polymerase chain reaction (RT-PCR) assay

Total RNA was isolated from the liver by using an Isogen Kit (Wako, Osaka). Synthesis of cDNA from the isolated total RNA was carried out using RNase H-reverse transcriptase (GIBCO BRL, Rockville, MD). Reverse transcription (RT) reaction mixtures consisted of 40 mM KCl, 50 mM Tris–HCl (pH 8.3), 6 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM each of dATP, dCTP, dGTP, and dTTP, 10 units of RNase inhibitor (Promega, Madison, WI), 100 pmol of random hexamer, total RNA and 200 units of the Moloney murine leukemia virus reverse transcriptase (Gibco-BRL, Berlin, Germany) in a final volume of 50 μl, and the reaction was conducted at 37 °C for 60 min.

Polymerase chain reaction (PCR) was carried out in a final volume of 20 μl, containing 1 μl of RT reaction mixture, 50 mM KCl, 20 mM Tris–HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 10 μM each of the mixed oligonucleotide primers, and 1 unit of Taq DNA polymerase (GIBCO BRL). Primers used for rat *CYP1A2* were 5′-CTT GGA GAA GCG CCA GG-3′ and 5′-CTA CAA AGA CAA CGG TGG TCT-3′ (664 bp) (Geng and Strobel, 1993), those for rat *CYP2C11* were 5′-GTA TCG CTG TCA TCC ATA C-3′ and 5′-GGA AAT GGG GAT ATG TG-3′ (297 bp) (Pampori and Shapiro, 2000), those for rat *CYP2E1* were 5’-CTG ATT GGC TGC GCA CCC TGC-3′ and 5′-GAA CAG GTC GGC CAA AGT CAC-3′ (363 bp) (Jiang et al., 1998), those for rat *CYP3A2* were 5′-AGT AGT GAC GAT TCC AAC ATA T-3′ and 5′-TCA GAG GTA TCT GTG TTT CCT-3′ (252 bp) (Oinonen and Lindros, 1995), and those for rat *β-actin* were 5′-TTC TAC AAT GAG CTG CGT GTG GC-3′ and 5′-CTC (A/G)TA GCT CTT CTC CAG GGA GGA-3′ (456 bp), as previously reported by Waki et al. (1995). Each cycle consisted of 45 s at 94 °C, 60 s at 64 °C, and 75 s at 72 °C for *CYP1A2*, 45 s at 94 °C, 60 s at 56 °C, and 75 s at 72 °C for *CYP2C11* and *CYP3A2*, 45 s at 94 °C, 60 s at 66 °C, and 75 s at 72 °C for *CYP2E1*, and 45 s at 94 °C, 60 s at 60 °C, and 75 s at 72 °C for *β-actin*. PCR reaction was run for 26 cycles for *CYP1A2*, 25 cycles for *CYP2C11*, 23 cycles for *CYP2E1*, 24 cycles for *CYP3A2*, and 19 cycles for *β-actin.*

2.7. Animal experiments

A 100 μl aliquot of caffeine (5 mg/kg), tolbutamide (10 mg/kg), chlorzoxazone (10 mg/kg) or lidocaine (10 mg/kg) in distilled water was injected *via* the femoral vein. Blood samples (350 μl each) were collected at designated time intervals from the jugular vein under light ether anesthesia. Plasma was separated by centrifugation at 3000 × *g* for 10 min and stored at −30 °C. The HPLC assays for caffeine (Tanaka, 1992), tolbutamide (Matunaga et al., 2001), chlorzoxazone (Chen and Yang, 1996; Mizuno et al., 2000) or lidocaine (Shibasaki et al., 1988; Tanaka et al., 1994) were carried out by means of the cited methods.

2.8. Data analysis

The pharmacokinetic parameters were estimated according to model-independent moment analysis as

described by Yamaoka et al. (1978). The data were analyzed by using Student's *t*-test to compare the unpaired mean values of two sets of data. The number of determinations is noted in each table and figure. A value of $P < 0.05$ or 0.01 was taken to indicate a significant difference between sets of data. The electrophoresis results were analyzed by using NIH Image software.

3. Results

3.1. Laboratory data in rats after CCl_4 treatment

Table 1 shows the physical and biochemical data in rats treated with 0.5 ml/kg CCl_4. At 24 h after treatment with CCl_4, the body weight was decreased and the liver weight was significantly increased. Serum biochemical parameters associated with liver function, except for serum albumin, were markedly changed at 24 h after CCl_4, but subsequently recovered nearly to untreated control levels. The serum concentration of albumin was hardly changed after CCl_4 treatment. Pathological observations revealed severe fatty degradation of the liver at 24 h after CCl_4 and almost full recovery by 7 days (data not shown). These results indicated that rats at 24 h after CCl_4 administration are a good model of acute hepatic failure.

3.2. Changes in enzyme activity and mRNA expression of CYP isoforms in the liver

Figs. 1 and 2 show the changes in the activity and mRNA expression of CYP isoforms in the liver of rats with acute CCl_4 (0.5 ml/kg)-induced hepatic failure. The enzyme activities of all the CYP isoforms were

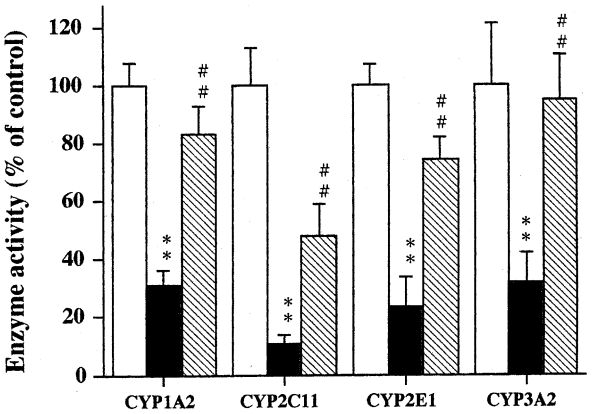

Fig. 1. Relative enzyme activities of CYP isoforms in the liver of rats with acute hepatic failure rats. The activities were determined in vehicle control rats (□) and rats with acute hepatic failure at 24 h (■) and 7 days (▨) after an oral administration of CCl_4 (0.5 ml/kg). **Significantly different from the control at $P < 0.01$. ##Significantly different from the value at 24 h after CCl_4 treatment at $P < 0.01$. Each column with bar represents the mean ± S.D. of six rats.

Table 1
Physical and biochemical data in rats with acute hepatic failure

	Control	CCl_4 treatment	
		After 24 h	After 7 days
Bodyweight (g)	226 ±5	212 ± 8**	210 ± 12
Liver weight (g/100 g body weight)	2.9 ± 0.2	4.7 ± 0.3**	3.7 ± 0.6[a]
AST (IU/l)	83 ± 5	3840 ± 520**	215 ± 65[a]
ALT (IU/l)	39 ± 8	826 ± 185**	71 ± 14[a]
ALP (IU/l)	682 ± 93	822 ± 96*	674 ± 62[a]
LDH (IU/l)	233 ± 70	5920 ± 830**	312 ± 91[a]
Albumin (g/dl)	3.65 ± 0.21	3.60 ± 0.16	3.75 ± 0.48
A/G ratio	3.23 ± 0.36	2.83 ± 0.29	2.56 ± 0.42
Total bilirubin (mg/dl)	0.10 ± 0.02	1.51 ± 0.35**	0.14 ± 0.04[a]

Data were measured at 24 h and 7 days after an oral administration of CCl_4 (0.5 ml/kg) in rats. Each value represents the mean ± S.D. of six rats.

[a] Significantly different from the value at 24 h after CCl_4 treatment at $P < 0.01$.

* Significantly different from the vehicle control at $P < 0.05$.

** Significantly different from the vehicle control at $P < 0.01$.

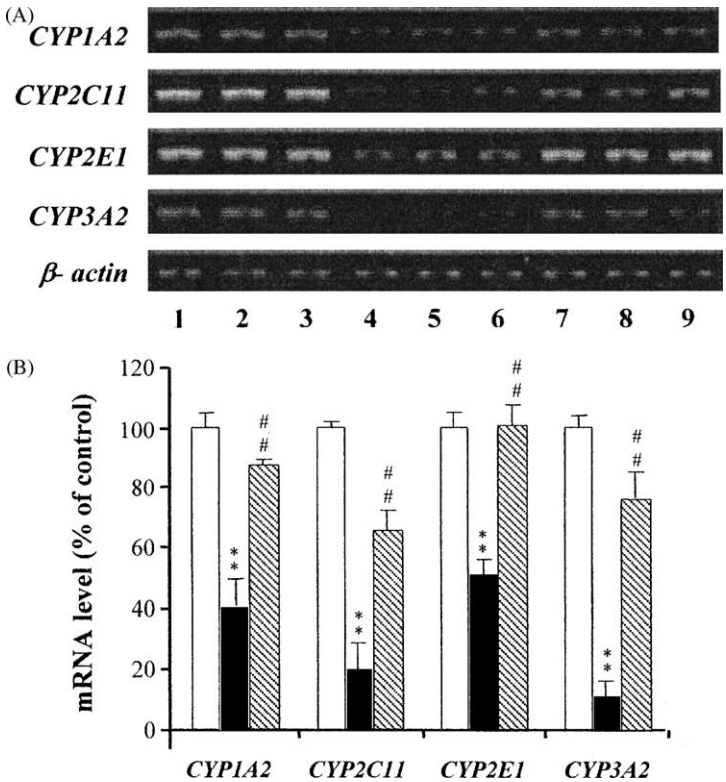

Fig. 2. Relative mRNA expressions of CYP isoforms in the liver of rats with acute hepatic failure. (A) RT-PCR of CYP mRNAs of vehicle control rats and rats with acute hepatic failure at 24 h and 7 days after an oral administration of CCl_4 (0.5 ml/kg). Lanes 1–3, vehicle control; Lanes 4–6, at 24 h after treatment of CCl_4. Lanes 7–9, at 7 days after treatment of CCl_4. The sizes of the RT-PCR products are 644 bp (*CYP1A2)*, 297 bp (*CYP2C11*), 363 bp (*CYP2E1*) and 252 bp (*CYP3A2*). (B) Relative levels of CYP mRNAs. Each level of CYP mRNA was corrected on the basis of β-actin mRNA and is indicated as a percentage of the control level. Each column with a bar represents the mean ± S.D. of six rats. **Significantly different from the vehicle control at $P < 0.01$. $^{\#\#}$Significantly different from the value at 24 h after treatment of CCl_4 at $P < 0.01$. (□), Vehicle control; (■), at 24 after CCl_4; (▨), at 7 days after CCl_4.

significantly decreased at 24 h after CCl_4 treatment, but their patterns of recovery were different (Fig. 1). The recovery of enzyme activity was fast in the case of CYP3A2, intermediate for CYP1A2 and 2E1, and slow for CYP2C11. CYP3A2 mRNA was most sensitive to acute hepatic failure and CYP2E1 mRNA was comparatively resistant, but both mRNA levels had significantly recovered by 7 days after CCl_4 treatment (Fig. 2).

3.3. Plasma concentration–time courses of various drugs

Untreated rats and rats at 24 h after oral administration of 0.5 ml/kg CCl_4 were intravenously given caffeine (5 mg/kg), tolbutamide (10 mg/kg), chlorzoxazone (10 mg/kg) or lidocaine (10 mg/kg). As shown in Fig. 3, the plasma concentrations of these drugs were all significantly higher in the CCl_4-treated group than in the untreated control group. The pharmacokinetic parameters of these drugs are listed in Table 2. The values of the area under the plasma concentration–time curve from 0 time to infinity (AUC) in CCl_4-treated rats were significantly larger for all the drugs than those in untreated rats. The values of total clearance (CL_{tot}) of all the drugs in CCl_4-treated rats were significantly smaller than those in untreated rats, whereas the values of distribution volume at the steady-state (Vd_{ss}) were not significantly different between untreated and treated rats.

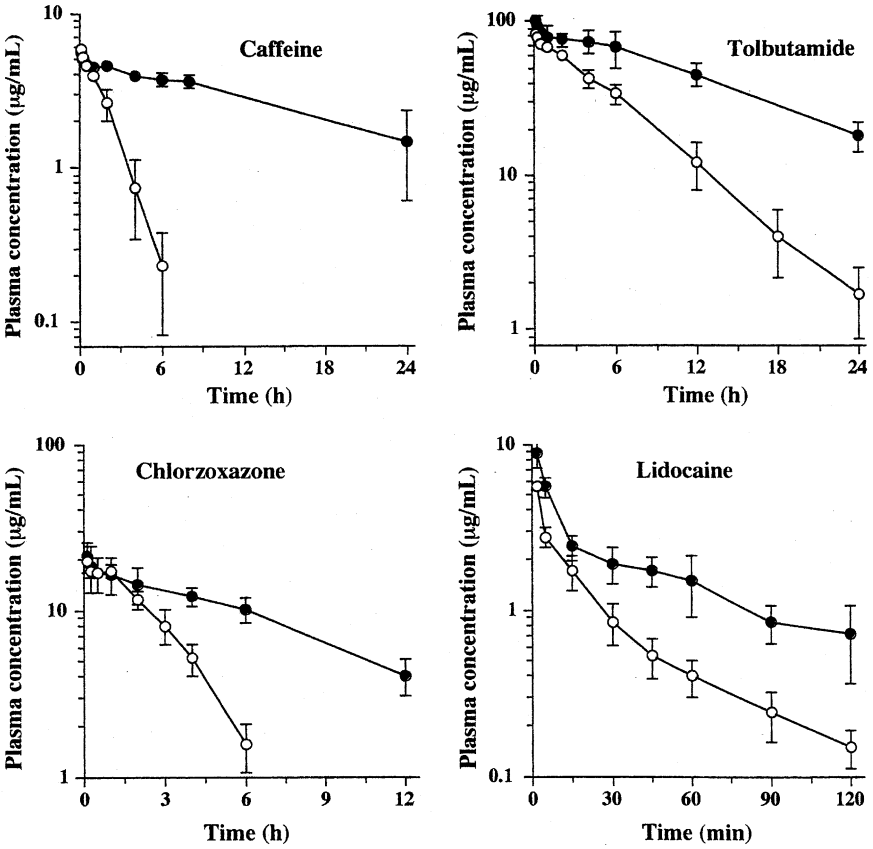

Fig. 3. Plasma concentration–time courses of caffeine, tolbutamide, chlorzoxazone and lidocaine after intravenous administration in vehicle (○) and CCl_4-treated (●) rats. Rats were orally treated with CCl_4 (0.5 ml/kg) at 24 h prior to caffeine (5 mg/kg), tolbutamide (10 mg/kg), chlorzoxazone (10 mg/kg) or lidocaine (10 mg/kg) administration. Each point with a bar represents the mean ± S.D. of four rats.

The values of CL_{tot} of caffeine, tolbutamide, chlorzoxazone and lidocaine in CCl_4-treated rats were decreased to about 1/8, 1/3, 1/3 or 1/2 of those in untreated rats.

3.4. Correlation between mRNA expression and enzyme activity of CYP isoforms in the liver

The mRNA expression and the metabolic activity of CYP isoforms were measured in rat liver at 24 h after treatment with CCl_4 (0.01–0.5 ml/kg). The decreases in mRNA expression and activity of each CYP isoform tended to be larger as the dose of CCl_4 was increased. A good correlation was observed between mRNA expression and enzyme activity for each CYP isoform (Fig. 4).

3.5. Correlation between enzyme activity of CYP isoforms and serum aspartate aminotransferase activity

As shown in Table 1, values of most laboratory data, including markers of hepatic function, were decreased after CCl_4 treatment, but their relationship to the severity of hepatic failure was variable. For example, serum albumin content is a good marker of hepatocyte function, but its value was hardly changed after CCl_4 treatment. On the other hand, serum aminotransferase leakage from the liver is a marker of hepatocyte destruction, and the changes in serum aminotransferase activity (AST and ALT) were well related to the changes of hepatic enzyme activities of CYP isoforms. Fig. 5 shows the correlations between

Table 2
Pharmacokinetic parameters of various drugs after an intravenous administration in untreated and CCl_4-treated rats

Parameter	Caffeine		Tolbutamide		Chlorzoxazone		Lidocaine	
	No treatment	CCl_4 treatment	No treatment	CCl_4 treatment	No treatment	CCl_4 treatment	No treatment	CCl_4 treatment
AUC (μg/h/ml)	12.6 ± 1.4	104 ± 19**	531 ± 81	1420 ± 110**	57.8 ± 6.6	155 ± 36**	2.95 ± 0.61	5.48 ± 1.25*
MRT (h)	1.72 ± 0.49	19.1 ± 6.9**	5.84 ± 0.81	14.5 ± 2.4**	2.25 ± 0.22	7.26 ± 1.09**	0.407 ± 0.086	1.00 ± 0.24**
Vd_{ss} (ml/kg)	683 ± 273	919 ± 440	110 ± 12	95 ± 11	378 ± 15	476 ± 91	1380 ± 185	1820 ± 543
CL_{tot} (ml/h/kg)	397 ± 45	48.1 ± 9.2**	19.1 ± 2.8	7.1 ± 0.6**	175 ± 20	67.5 ± 16.7**	3380 ± 520	1820 ± 350**
$T_{1/2}$ (h)	0.99 ± 0.66	14.2 ± 7.1*	4.89 ± 1.54	9.35 ± 0.42**	1.23 ± 0.08	4.87 ± 0.75**	0.492 ± 0.095	0.926 ± 0.181**

Rats were orally treated with CCl_4 (0.5 ml/kg) at 24 h prior to administration of each drug. The AST values of the end of experiments were 85 ± 9 IU/l in untreated rats and 4260 ± 620 IU/l in CCl_4-treated rats. Each value represents the mean ± S.D. of four rats.
* Significantly different from the untreated rats at $P < 0.05$.
** Significantly different from the untreated rats at $P < 0.01$.

the hepatic enzyme activities of CYP isoforms and the serum aspartate aminotransferase (AST) activities in rats at 24 h after several doses of CCl_4. There was a good negative correlation between serum AST activity and hepatic activity of each CYP isoform. Decreases of the CYP2C11, CYP2E1, CYP3A2 and CYP1A2 activities to half of untreated control levels corresponded to serum AST activities of about 710, 780, 1030 and 1300 IU/l, respectively.

4. Discussion

Although several investigators have examined hepatic metabolic ability and clearance of drugs in hepatic failure (Tanaka et al., 1992; Huang et al., 1993; Girre et al., 1994; Sotaniemi et al., 1995; Horsmans et al., 1996; Wensing et al., 1997; Tanaka, 1998), no simple and practical method is yet available for predicting changes of drug clearance in patients with acute hepatic failure. In this study, we investigated whether laboratory data of CCl_4-treated rats with acute hepatic failure can be used as predictors of drug clearance. The drug-metabolizing activity and mRNA expression of four isoforms of CYP in the liver were significantly decreased in the acute phase and recovered within 7 days after CCl_4 treatment, with some variation among the isoforms. The enzyme activities were closely correlated with the mRNA levels, suggesting that gene expression of CYPs was suppressed in CCl_4-induced acute hepatic failure. The order of susceptibility of CYP isoforms was CYP2C11 > CYP2E1 > CYP3A2 > CYP1A2, and the CYP enzyme activities were well correlated with serum aminotransferase activities, including AST, among laboratory data from the rats with acute hepatic failure.

In general, it is thought that serum albumin content is a marker of hepatocyte function, while serum activities of enzymes, such as aminotransferases, that are leaked from the liver are markers of hepatocyte destruction. In rats treated with CCl_4, serum albumin concentration was hardly changed, while the activities of AST, ALT, ALP and LDH and total bilirubin concentration in serum were markedly increased during the acute phase and had almost recovered to the control values by 7 days after CCl_4 treatment. Therefore, the decrease in CYP isoform activities may reflect the contents of active enzymes in living

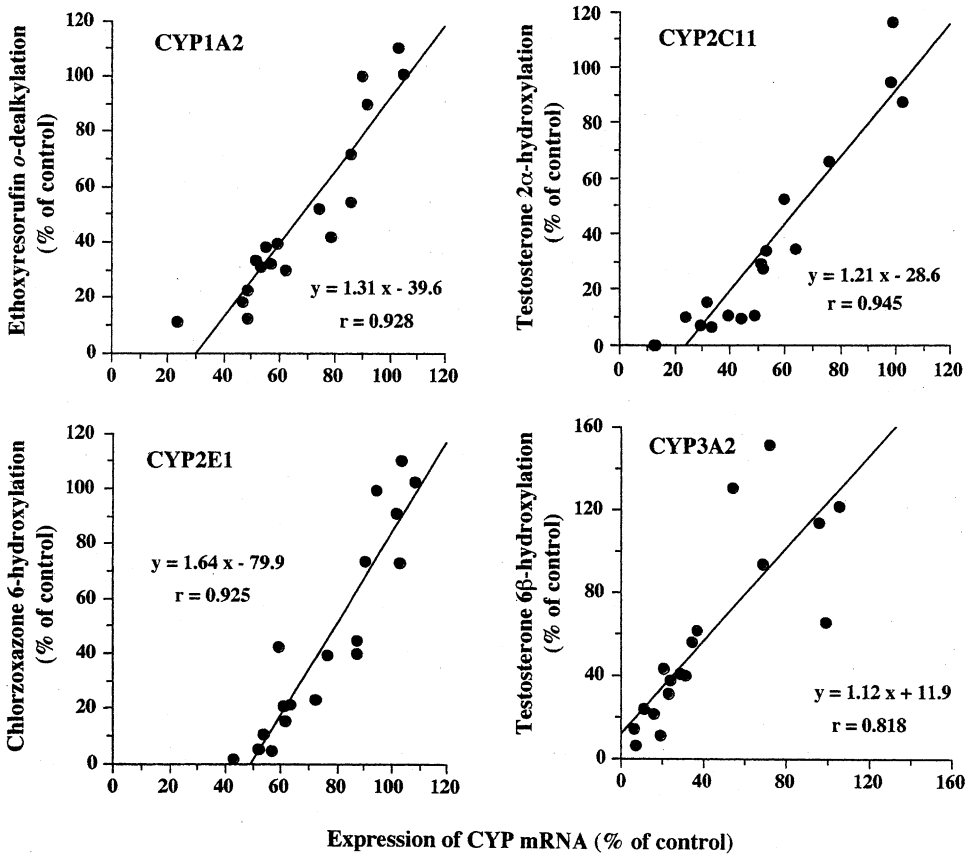

Fig. 4. Correlations between mRNA levels and enzyme activities of CYP isoforms in rat liver at 24 h after treatment with several doses of CCl_4 (0.01–0.5 ml/kg). Each level of CYP mRNA was corrected on the basis of the β-actin mRNA level.

hepatocytes, rather than the function of CCl_4-injured hepatocytes, whereas expression of these mRNAs was decreased. Based on these results, in acute hepatic failure, we propose that serum transaminase activity is a good candidate as an indicator of hepatic drug-metabolizing ability. Indeed, when we examined the plasma concentration–time courses of four specific probe drugs, caffeine for CYP1A2, tolbutamide for CYP2C11, chlorzoxazone for CYP2E1 and lidocaine for CYP3A2, in rats treated with 0.5 ml/kg of CCl_4, we found that the serum AST value was increased to 4260 ± 620 IU/l from 85 ± 9 IU/l in untreated control rats, and the AUC and CL_{tot} values of these drugs were markedly decreased, without any change in the Vd_{ss} (Fig. 3, Table 2).

Because three drugs except for lidocaine show hepatic metabolism-limited elimination, their clearance may be dependent upon hepatic CYP activity. However, when the serum AST level in rats was at about 4000 IU/l, and the hepatic CYP activities were one-tenth or less of the control values, the degree of decrease of the CL_{tot} values showed marked variation. The elimination from the body of drugs metabolized in the liver is considered to be dependent upon hepatic blood flow, bile excretion, renal excretion of the metabolites, and other factors. Matunaga et al. (2001) reported that the hepatic blood flow was decreased by about 10% from the control value, without statistical significance, in CCl_4-treated rats with an AST level of about 2000 IU/l. When we measured the hepatic blood flow rate in the CCl_4-treated rats, it was about 60 ml/min/kg and there were no significant differences in the degree of acute hepatic failure (data not shown). There, we thought that lidocaine shows

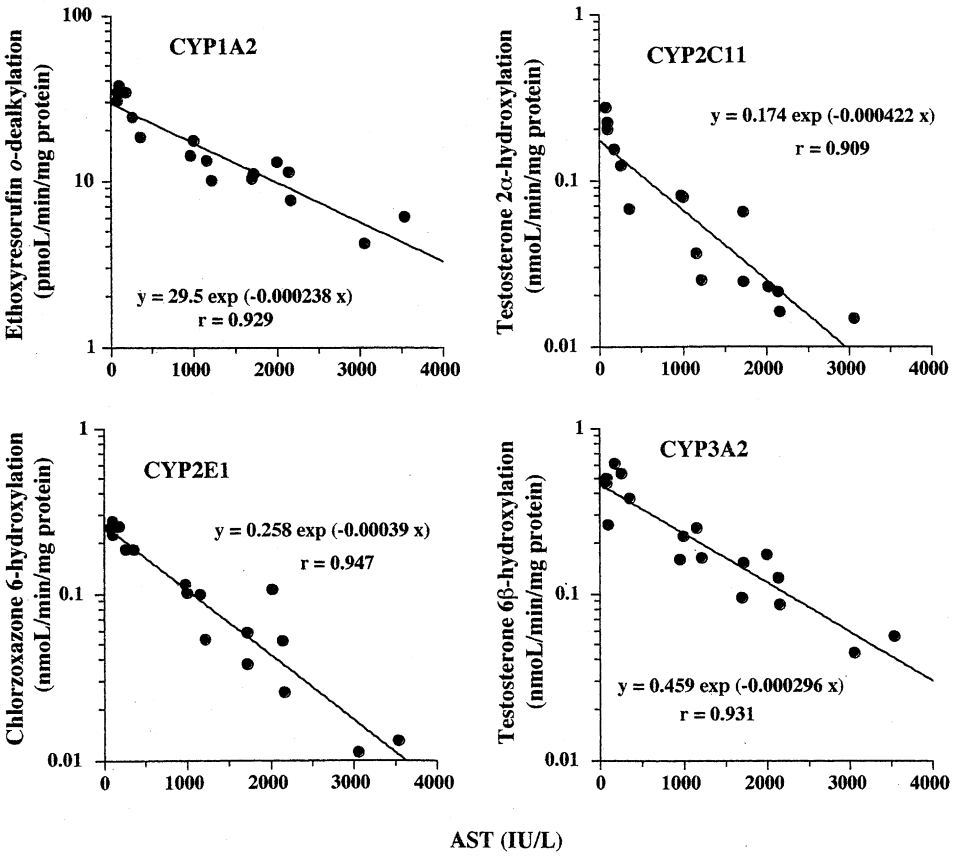

Fig. 5. Correlations between serum AST values and CYP activities in the liver of rats at 24 h after treatment with several doses of CCl_4 (0.01–0.5 ml/kg).

hepatic blood flow-limited elimination, resulting the decreased ratio of CL_{tot} of lidocaine in CCl_4-treated rats is the smallest of four probe drugs. In this study, the acute hepatic failure rats also exhibited hyperbilirubinemia. Therefore, the CL_{tot} value might also be influenced by changes in bile excretion. It has been reported that the expression of P-glycoprotein in liver, kidney and brain is increased in CCl_4-treated rats (Nakatsukasa et al., 1993; Huang et al., 2001). We confirmed in a preliminary study that the expression of *mdr1b* mRNA was induced, but that of other transporters, including *mdr1a* mRNA, was unchanged by CCl_4 treatment (data not shown). Therefore, it seems reasonable to assume that the hepatic clearance of drugs in acute hepatic failure mainly reflects the hepatic metabolizing ability. However, because in this study the activity of each CYP isoform was measured in vitro using microsomes from liver of CCl_4-treated rats, it is likely that the results do not filly reflect the in vivo activity. Bergquist et al. (1999) reported that the AUC of a drug metabolized by CYP2C9 was not correlated with AST or ALT in patients with hepatic cirrhosis, but found a good correlation with serum albumin concentration. The reason for this may be that AST or ALT leaks into the blood from hepatocytes damaged acutely by CCl_4, whereas the serum albumin concentration may depend on the decrease of hepatocyte function in chronic hepatic failure. Therefore, serum albumin concentration may be a candidate to predict the hepatic drug-metabolizing ability and drug disposition kinetics in patients with chronic hepatic failure.

In conclusion, our results show that the activities of the CYP isoforms, CYP1A2, CYP2C11, CYP2E1,

and CYP3A2, are negatively correlated with the serum AST value and exhibit different sensitivities in CCl_4-induced acute hepatic failure. Although the CL_{tot} for drugs metabolized by CYP isoforms cannot be precisely predicted from the serum AST values, serum AST may be useful for the estimation of dose modification of drug for patients with acute hepatic failure.

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