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Expression and function of P-glycoprotein in rats with carbon tetrachloride-induced acute hepatic failure

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Abstract

Acute hepatic failure was induced experimentally in rats by intraperitoneal injection of 2.5 mL kg^{-1} carbon tetrachloride (CCl_4), and the effects on the expression and function of P-glycoprotein in the liver, kidney and brain were evaluated. The CCl_4 injection significantly increased the indicators of hepatic function (glutamate oxaloacetate transaminase, glutamate pyruvate transaminase), but not of renal function (blood urea nitrogen, glomerular filtration rate). In rats with acute hepatic failure, the hepatic P-glycoprotein concentration increased 1.5-fold and the ATP concentration decreased to approximately 40 % that in control rats. In contrast, P-glycoprotein concentrations in the kidney and brain and ATP concentrations in the kidney remained unchanged. The in-vivo P-glycoprotein function in these tissues was suppressed as evaluated by biliary and renal secretory clearances and brain distribution of rhodamine 123, a P-glycoprotein substrate. These findings suggest that factors other than P-glycoprotein concentration are involved in the systemic suppression of P-glycoprotein function in diseased rats. In Caco-2 cells, plasma collected from CCl_4-treated rats exhibited a greater inhibitory effect on P-glycoprotein-mediated transport of rhodamine 123 than that from control rats, suggesting the accumulation of an endogenous P-glycoprotein substrate/inhibitor in the plasma of diseased rats. In fact, the plasma concentration of corticosterone, an endogenous P-glycoprotein substrate, increased 2-fold in CCl_4-treated rats compared with control rats. It was demonstrated that P-glycoprotein function is systemically suppressed in rats with CCl_4-induced acute hepatic failure, not only in the target organ (liver), but also in other organs (kidney and brain), although the P-glycoprotein concentration remained unchanged in the kidney and brain, and increased in the liver. In the systemic suppression of the P-glycoprotein function in the diseased state, the alteration of plasma concentrations or components of endogenous P-glycoprotein-related compounds, such as corticosterone, would likely be involved.

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Introduction

Acute hepatic failure induced experimentally with CCl_4 in animals is accompanied by a reduction in the renal tubular secretion of cefpiramine and impaired intestinal sugar transport, in addition to the suppression of various hepatic functions (Li et al 1990; Sugihara et al 1992; Castilla-Cortazar et al 1997). Acute renal failure induced by glycerol or uranyl nitrate also reduces the hepato-biliary transport of certain drugs, modulates the distribution of drugs into the central nervous system and decreases the activity of various hepatic microsomal enzymes (Bowmer & Yates 1984; Naora et al 1999). Thus, dysfunction of the liver or kidney, both

important organs in the detoxification of xenobiotics, exerts a major influence on the pharmacokinetics of various drugs not only in the target organ but also in other organs. However, the mechanisms underlying such systemic influences of disease states are not well understood.

P-Glycoprotein, an ATP-dependent efflux pump, is widely expressed in normal tissues including the bile canalicular membrane of hepatocytes, brush-border membrane of renal proximal tubules, and capillary endothelial cells of brain (Thiebaut et al 1987). Recently, we analysed the effect of glycerol-induced acute renal failure on the expression and function of P-glycoprotein in rats, and found that P-glycoprotein function was systemically suppressed even though the concentration of P-glycoprotein remained unchanged in the liver and brain, and even increased in the kidney (Huang et al 2000). Regarding the effect of CCl_4-intoxication on P-glycoprotein, an increase in the expression of P-glycoprotein mRNA (mdr1a and mdr1b) in rat liver has been reported (Nakatsukasa et al 1993). However, the actual in-vivo P-glycoprotein function in such diseased states has not been analysed. In the present study, the expression and function of P-glycoprotein in the liver, kidney and brain were evaluated in rats with CCl_4-induced acute hepatic failure. P-glycoprotein function in these tissues was evaluated by measuring the biliary and renal secretory clearances and brain distribution of rhodamine 123, a P-glycoprotein substrate, under steady-state plasma concentrations.

Rhodamine 123 has been often used as a probe to assess P-glycoprotein function in-vitro and in-vivo, and to examine whether a test compound is a P-glycoprotein inhibitor (Lee et al 1994; Scala et al 1997). There are strong overlapping substrate specificities between cytochrome P450 3A and P-glycoprotein (Wacher et al 1995; Schuetz et al 1996). However, rhodamine 123 is not a substrate of cytochrome P450 3A, which would also be a suitable characteristic for the assessment of P-glycoprotein function. In addition, the mechanism underlying the systemic suppression of P-glycoprotein function in rats with acute hepatic failure was investigated from the viewpoint of tissue ATP concentration and the contribution of putative endogenous P-glycoprotein-related compounds in the plasma.

Materials and Methods

Materials

Rhodamine 123 was obtained from Kanto Chemical Co. (Tokyo, Japan). Corticosterone and 4-androsten-11 β-ol-3,17-dione were from Sigma Chemical Co. (MO). A monoclonal antibody for P-glycoprotein, C219, was from Signet Laboratories, Inc. (MA) and a secondary antibody, peroxidase-labelled affinity purified antibody to mouse IgG (H + L), was from Kirkegaard & Perry Laboratories, Inc. (MD). All other chemicals used were of the highest purity available.

Animal treatment

Animal experiments were performed in accordance with the Guide for Animal Experimentation from the Committee of Research Facilities for Laboratory Animal Sciences, Faculty of Medicine, Hiroshima University. Acute hepatic failure was induced in male Wistar rats (260–320 g) by intraperitoneal injection of a mixture of CCl_4 and olive oil (50 % v/v) at a dose of 5 mL kg^{-1}. Control rats received the same volume of olive oil alone. These rats were used for experiments 24 h after CCl_4 injection. Acute hepatic failure was confirmed by measuring plasma glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activity, blood urea nitrogen, and glomerular filtration rate.

Determination of P-glycoprotein expression

The concentration of P-glycoprotein in the crude membrane fraction of the liver, kidney and brain was determined by Western immunoblotting after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously reported (Huang et al 2000). Briefly, SDS-PAGE was carried out according to the reported method (Laemmli 1970). Freshly isolated kidney, liver and brain were homogenized, and crude membrane fractions were prepared by centrifugation. The crude membrane (80 μg protein) was loaded onto 7.5 % acrylamide-bisacrylamide gels without prior heating. The proteins were transferred electrophoretically onto a polyvinylidene difluoride membrane (0.45-μm pore size; Bio-Rad Laboratories, CA), and were incubated with a monoclonal antibody C219 (10 μg mL^{-1}). Peroxidase-labelled affinity purified antibody to mouse IgG was used as the secondary antibody. Detection was made according to an enhanced chemiluminescence technique (ECL Western Blotting Detection System; Amersham Pharmacia Biotech, UK). The blots were exposed to Hyperfilm ECL (Amersham Pharmacia Biotech). The optical density of immunoblots was estimated by a computer-aided densitometer with NIH-Image (the public domain program developed at the US National Institutes of Health).

In-vivo clearance study of rhodamine 123 under steady state

In-vivo clearance studies of rhodamine 123 were performed as previously reported (Kunihara et al 1998; Yumoto et al 1999, Huang et al 2000). Briefly, a solution of rhodamine 123 (100 μM) containing mannitol (50 mg mL^{-1}) and inulin (10 mg mL^{-1}) was injected intravenously as a bolus (4.36 mL kg^{-1}), followed by a constant-rate infusion (2 mL h^{-1}) into a femoral vein to give a steady-state plasma concentration of approximately 0.25 μM. Three consecutive 20-min clearance studies were performed by collecting bile and urine samples. Blood was taken from a femoral artery at a midpoint above biological sample collection.

Unbound plasma fraction and tissue distribution of rhodamine 123 under steady state

Blood was collected by heart puncture to obtain plasma immediately after the clearance study. The unbound fraction of rhodamine 123 in plasma was measured by ultrafiltration using semipermeable cellulose membrane with a cut-off at MW 12 000–14 000. The liver, kidney and brain were also isolated to measure rhodamine 123 concentrations in these tissues. The tissue distribution of rhodamine 123 was expressed as the tissue-to-plasma partition coefficient of unbound fraction.

Pharmacokinetic analysis

Pharmacokinetic parameters of rhodamine 123, such as total plasma, biliary and renal excretion clearances, were estimated as reported previously (Kunihara et al 1998; Yumoto et al 1999; Huang et al 2000). The normalized biliary clearance of rhodamine 123 was estimated by dividing the biliary excretion rate using hepatic rhodamine 123 concentration. The renal excretion clearance of the unbound plasma fraction of rhodamine 123 was determined by dividing the renal clearance by the unbound plasma fraction. Glomerular filtration rate was assumed to be equal to the renal clearance of inulin. The net renal secretory clearance of unbound plasma fraction of rhodamine 123 was calculated by subtracting the glomerular filtration rate from the renal excretion clearance of unbound plasma fraction.

Tissue binding of rhodamine 123 in-vitro

The liver was freshly isolated and homogenized in pH 7.4 phosphate-buffered saline (PBS) at 4°C. Binding of rhodamine 123 to the 10% liver homogenate was determined by ultrafiltration at 4°C. The concentration of rhodamine 123 in the liver homogenates was adjusted to 1 μM. In a preliminary experiment, rhodamine 123 was not degraded in liver homogenate at 4°C.

Effect of plasma on transepithelial transport of rhodamine 123 across Caco-2 cell monolayers

The effect of plasma collected from control rats and rats with acute hepatic failure on P-glycoprotein-mediated transport of rhodamine 123 was measured in Caco-2 cells as reported previously (Takano et al 1998; Yumoto et al 1999; Huang et al 2000). Plasma filtrate collected by ultrafiltration was also used in this study. Briefly, Caco-2 cells (passages 63–70) were cultured in a 6-well Transwell chamber (Costar, Cambridge, MA) for 19 or 20 days after seeding. Rhodamine 123 solution (5 μM in Dulbecco's PBS containing 25 mM HEPES and 25 mM glucose, pH 7.4) was placed on the basolateral side. Plasma or plasma filtrate was added to both sides of the chamber to give a designated final volume (10, 25 or 50 μL plasma sample (mL transport medium)$^{-1}$). The transepithelial transport of rhodamine 123 was measured at 37°C for 120 min.

Determination of plasma corticosterone concentration

Plasma concentrations of corticosterone in control rats and in rats with acute hepatic failure were determined. All blood samples were obtained by heart puncture at 15.45–16.15 h under light anaesthesia with ethylether, and were centrifuged to obtain plasma samples. Plasma samples were stored at −30°C until analysis.

Analytical methods

The ATP concentration in the liver and kidney (renal cortex) of control rats and rats with acute hepatic failure was determined as described by Maeda et al (1993). Briefly, the liver and kidney were freshly excised. The renal cortex was prepared using a Stadie-Riggs microtome. These tissues were weighed and homogenized with 4 vols ice-cold 3% perchloric acid. After centrifugation at 1200 *g* for 15 min, the supernatant was neutralized and used to determine ATP concentrations enzymatically with hexokinase and glucose-6-phosphate dehydrogenase (Williamson & Corkey 1969).

Concentrations of rhodamine 123 in various biological samples were determined by HPLC using a

reverse-phase column of TSKgel ODS-80TM (Tosoh, Tokyo, Japan) (Yumoto et al 1999). The mobile phase was a mixture of acetonitrile and 1 % acetic acid (40 : 60, v/v%) at a flow rate of 1 mL min^{-1}. Detection was made at excitation and emission wavelengths of 485 nm and 546 nm, respectively. The concentration of inulin in plasma and urine was determined spectrophotometrically (Dische & Borenfreund 1951). Blood urea nitrogen and plasma activity of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase were measured with BUN B-Test Wako, GOT-UV Test Wako and GPT-UV Test Wako (Wako Pure Chemicals, Osaka, Japan), respectively.

Concentrations of corticosterone in plasma samples were determined as described elsewhere (Thomas & Wofford 1984; Woodward & Emery 1987). Briefly, 20 μL of a methanol solution of 4-androsten-11 β-ol-3, 17-dione (10 μg mL^{-1}) was added to 1 mL plasma sample as an internal standard. The mixture was extracted with 6 mL ethyl acetate by shaking and centrifugation. The organic phase was washed with 0.5 mL 0.1 M NaOH followed by 0.5 mL distilled water, and dehydrated with excess amounts of anhydrous Na_2SO_4. The Na_2SO_4 was then eliminated by filtration and the organic solvent was evaporated to dryness under reduced pressure. The residue was re-dissolved in 100 μL 80 % methanol. The HPLC column used was a reverse-phase TSKgel ODS-80TM (Tosoh, Tokyo, Japan) and the mobile phase was a mixture of methanol, acetonitrile and 1 % phosphoric acid (40 : 5 : 55, v/v%) at a flow rate of 1 mL min^{-1}. Detection was made at 240 nm. The corticosterone peak was assigned by spiking standard corticosterone to plasma samples.

Statistical analysis was performed using the Student's *t*-test. A difference of $P < 0.05$ was considered statistically significant.

Results

Tissue ATP and P-glycoprotein expression in acute hepatic failure

Induction of acute hepatic failure after treatment with CCl_4 was confirmed by biochemical parameters (Table 1). Rats treated with CCl_4 showed significantly higher plasma activity of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase compared with control rats. The indicators of renal function, such as blood urea nitrogen and glomerular filtration rate, remained unchanged. In the rats with acute hepatic failure, bile flow rate and ATP concentration in the liver, but not ATP concentration in the kidney, also decreased significantly. Representative immunoblots for P-glycoprotein in the liver, kidney and brain crude membrane preparations are shown in Figure 1. A greater staining intensity of immunoreactive protein with approximately 150 kDa was observed in the liver of diseased rats. Densitometric analysis indicated a significant increase in P-glycoprotein expression in the liver, but not in the kidney of diseased rats. The concentration of P-glycoprotein in the brain was slightly, but not significantly, higher than that of control rats. These results indicate that the expression of P-glycoprotein is increased rather than suppressed in rats with CCl_4-induced acute hepatic failure.

Table 1 Biochemical parameters for control rats and rats with acute hepatic failure.

	Control	Acute hepatic failure
Glutamate oxaloacetate transaminase (int. units L^{-1})	32.1 ± 1.2	815.5 ± 62.6*
Glutamate pyruvate transaminase (int. units L^{-1})	10.7 ± 1.2	583.5 ± 97.2*
Blood urea nitrogen (mg dL^{-1})	21.0 ± 1.4	18.7 ± 0.7
Glomerular filtration rate (mL min^{-1})[a]	1.31 ± 0.14	1.42 ± 0.14
Bile flow rate (μL min^{-1})	21.9 ± 1.1	14.9 ± 1.8*
ATP concn in liver (μmol g^{-1})	0.81 ± 0.03	0.29 ± 0.04*
ATP concn in renal cortex (μmol g^{-1})	0.67 ± 0.03	0.57 ± 0.03
P-glycoprotein concn in liver (ratio)	1	1.49 ± 0.18*
P-glycoprotein concn in kidney (ratio)	1	0.99 ± 0.12
P-glycoprotein concn in brain (ratio)	1	1.25 ± 0.12

Rats were injected intraperitoneally with 50 % (v/v) CCl_4 (acute hepatic failure) or olive oil (control) at a volume of 5 mL kg^{-1}, and the biochemical parameters were measured 24 h later. [a]The glomerular filtration rate was estimated from the renal excretion clearance of inulin. Data are mean ± s.e.m. of results from four to six trials. *$P < 0.05$, significantly different compared with the value for control rats.

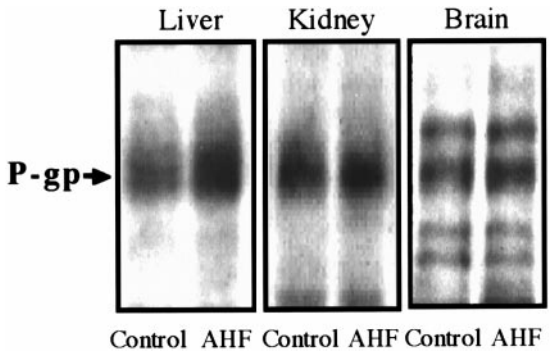

Figure 1 Western blot analysis with a monoclonal antibody for P-glycoprotein (C219) of the liver, kidney and brain crude membranes obtained from control rats and rats with acute hepatic failure (AHF). Rats were injected intraperitoneally with 50% (v/v) carbon tetrachloride (AHF) or olive oil (control) at a volume of 5 mL kg^{-1}, and the concentration of P-glycoprotein (P-gp) was measured 24 h later.

In-vivo clearance of rhodamine 123 under steady state

A steady-state plasma concentration of rhodamine 123 was attained within 50 min after the initiation of constant-rate infusion in both control rats and rats with acute hepatic failure. The in-vivo function of P-glycoprotein in the liver (bile canalicular membrane of hepatocytes), kidney (brush-border membrane of renal proximal tubules) and brain (capillary endothelial cells) was simultaneously evaluated by measuring biliary and renal excretion clearances and brain distribution of rhodamine 123 (Table 2). No difference in total plasma clearance of rhodamine 123 between control rats and rats with acute hepatic failure was observed. In the diseased rats, however, all of the biliary, renal, and net renal secretory clearances of rhodamine 123 decreased significantly compared with control rats. The brain distribution of rhodamine 123 increased significantly. Because the distribution of rhodamine 123 to the liver, but not to the kidney, increased approximately 1.5-fold in diseased rats, a parameter of normalized biliary clearance, which was calculated by dividing the biliary excretion rate by the hepatic rhodamine 123 concentration, was also used. The normalized biliary clearance of rhodamine 123 decreased significantly (44%) and more markedly than the evaluation by biliary clearance (22%) in disease rats. These findings indicate that P-glycoprotein function is systemically suppressed in rats with CCl_4-induced acute hepatic failure, not only in the target organ (liver), but also in other organs (kidney and brain). Acute hepatic failure did not affect other para-

Table 2 Pharmacokinetic parameters of rhodamine 123 under steady-state conditions of plasma concentration in control rats and rats with acute hepatic failure.

	Control	Acute hepatic failure
Steady-state plasma concn (μmol L^{-1})	0.26 ± 0.01	0.30 ± 0.02
Unbound fraction in plasma	0.30 ± 0.01	0.31 ± 0.02
Unbound fraction in 10% liver homogenate[a]	13.3 ± 1.5	16.7 ± 1.2
Unbound fraction in 10% kidney homogenate[a]	19.3 ± 2.2	15.3 ± 0.9
Total plasma clearance (mL min^{-1})	12.9 ± 0.5	11.4 ± 0.8
Biliary clearance (mL min^{-1})	0.81 ± 0.03	0.63 ± 0.08*
Normalized biliary clearance (mg liver min^{-1})[b]	0.09 ± 0.01	0.05 ± 0.01*
Renal clearance (mL min^{-1})	1.36 ± 0.15	0.62 ± 0.02*
Renal clearance of unbound fraction (mL min^{-1})	4.56 ± 0.63	2.02 ± 0.06*
Net renal secretory clearance (mL min^{-1})	3.25 ± 0.72	0.67 ± 0.24*
Brain distribution of plasma unbound fraction[c]	1.08 ± 0.12	1.62 ± 0.13*
Liver distribution of plasma unbound fraction[c]	27.6 ± 3.2	41.4 ± 3.9*
Kidney distribution of plasma unbound fraction[c]	413 ± 20	377 ± 21

Rats were injected intraperitoneally with 50% (v/v) carbon tetrachloride (acute hepatic failure) or olive oil (control) at a volume of 5 mL kg^{-1}, and the clearance study was performed 24 h later. Rhodamine 123 was administered intravenously as a bolus (436 nmol kg^{-1}), followed by constant-rate infusion (200 nmol h^{-1}). [a]The unbound fraction of rhodamine 123 in 10% tissue homogenate was estimated at a concentration of 1 μM by ultrafiltration. [b]The normalized biliary clearance was calculated by dividing the biliary excretion rate by the concentration of rhodamine 123 in the liver. [c]Tissue distribution of rhodamine 123 was estimated by dividing the tissue concentration by the plasma unbound concentration. Data are mean ± s.e.m. of results from four to six trials. *$P < 0.05$, significantly different compared with the value for control rats.

meters, such as unbound fraction of rhodamine 123 in plasma and tissue homogenates.

Effect of plasma on P-glycoprotein-mediated transport of rhodamine 123 in Caco-2 cells

The possible mechanism underlying the systemic suppression of P-glycoprotein function in rats with acute hepatic failure was studied from the viewpoint of involvement of putative endogenous P-glycoprotein-related compounds in the plasma of diseased rats. As reported previously, the transport of rhodamine 123 in the basolateral-to-apical direction across Caco-2 cell monolayers was much higher than that in the opposite direction (Takano et al 1998; Yumoto et al 1999). The functional expression of P-glycoprotein in Caco-2 cells was confirmed by measuring the inhibitory effect of verapamil, a potent P-glycoprotein inhibitor, on the efflux transport of rhodamine 123. The expression of P-glycoprotein in Caco-2 cells was also confirmed by Western blot analysis (data not shown). The effect of plasma on the basolateral-to-apical transport of rhodamine 123 across Caco-2 cell monolayers is summarized in Table 3. Plasma collected from rats with acute hepatic failure inhibited the rhodamine 123 transport in a concentration-dependent manner, and the inhibitory potency of diseased rat plasma was significantly greater than that of control rats. In contrast, plasma filtrate from control rats and rats with acute hepatic failure did not show any inhibitory effect even at a volume of 50 μL (mL transport medium)$^{-1}$. These findings indicate the presence of P-glycoprotein substrates/inhibitors with high protein binding in plasma, and suggest that they accumulate in plasma in the acute hepatic failure state.

Determination of plasma corticosterone concentration

Concentrations of corticosterone, one of the endogenous P-glycoprotein substrates/inhibitors (Rao et al 1994; Orlowski et al 1996), in the plasma of control rats and rats with acute hepatic failure were measured. To avoid the circadian variation in corticosteroid plasma concentrations and the possible effect of handling stress, all blood samples were obtained at 1600 h (1545–1615 h) under light anaesthesia with ethylether (Thomas & Wofford 1984; Barbason et al 1995). Plasma corticosterone concentrations in rats with acute hepatic failure were 2-fold higher than in control rats (0.111 ± 0.021 μg mL^{-1} in control rats and 0.208 ± 0.013 μg mL^{-1} in rats with acute hepatic failure; $P < 0.05$).

Discussion

We studied the effect of CCl_4-induced acute hepatic failure on the expression and function of P-glycoprotein in rats. In this widely used disease model, acute hepatic failure is induced by free radicals produced by bioactivation of CCl_4 by the liver metabolizing cytochrome P450 enzyme system (Comporti 1989; Nelson 1995). As shown in Table 1, bile flow rate, hepatic P-glycoprotein and ATP concentrations, in addition to plasma glutamate oxaloacetate transaminase and glutamate pyruvate

Table 3 Inhibitory percentage of plasma or plasma filtrate collected from control rats and rats with acute hepatic failure on transepithelial transport of rhodamine 123 in the basolateral-to-apical direction across Caco-2 cell monolayers.

	Dose (μL (mL medium)$^{-1}$)	Control (%)	Acute hepatic failure (%)
Plasma	10	-0.22 ± 2.97	-1.30 ± 3.32
	25	1.66 ± 3.49	16.8 ± 4.40†*
	50	18.7 ± 2.46†	26.1 ± 1.26†*
Plasma filtrate	50	-1.90 ± 3.95	-4.04 ± 4.67

Rats were injected intraperitoneally with 50% (v/v) carbon tetrachloride (acute hepatic failure) or olive oil (control) at a volume of 5 mL kg^{-1}, and blood was collected 24 h later. Inhibitory percentage of plasma samples was estimated by comparing the transported amount of rhodamine 123 across Caco-2 cell monolayers for 120 min in the presence and absence of plasma sample. Plasma samples were mixed with the medium of apical and basolateral sides of Caco-2 cells at an indicated volume. Data are mean ± s.e.m. of results from three to four trials. †$P < 0.05$, significantly different compared with the value in the absence of plasma sample. *$P < 0.05$, significantly different compared with the value for control rats.

transaminase activity, were all modulated in rats with acute hepatic failure. In contrast, values for renal function, such as blood urea nitrogen, glomerular filtration rate, and renal P-glycoprotein and ATP concentrations remained unchanged. Acute hepatic failure did not affect the binding of rhodamine 123 to plasma protein and tissue homogenate (Table 2). It has been reported that CCl_4-treatment increases the plasma concentration of α_1-acid glycoprotein, an acute-phase protein and a main binding constituent for weakly basic drugs, in rats (Sugihara et al 1991, 1992). No significant effect of CCl_4-treatment on plasma protein binding of rhodamine 123 would therefore suggest that α_1-acid glycoprotein is not a main binding constituent for rhodamine 123 in plasma. The decreases in bile flow rate and hepatic ATP concentration under CCl_4-intoxication have also been reported (Sandhu et al 1991; Sugihara et al 1992; Murakami et al 1998). With respect to the effect of CCl_4 on P-glycoprotein concentrations, it has been reported that levels of mdr1a and mdr1b mRNA, genes encoding mammalian P-glycoprotein proteins, were increased in rat liver 3 h after administration of CCl_4 and remained increased for the following five days (Nakatsukasa et al 1993; Yamazaki et al 1996), whereas mdr2 mRNA did not increase until 48 h (Brown et al 1993). Similarly, extrahepatic biliary obstruction caused by bile duct ligation reportedly increased both mdr1a and mdr1b mRNA and P-glycoprotein in rats over a period of seven days after ligation (Kagawa et al 1998). We also observed an approximately 1.5-fold increase in P-glycoprotein concentration in the liver of rats with CCl_4-induced acute hepatic failure (Table 1).

An in-vivo study under steady-state conditions of plasma concentration of rhodamine 123 is required for precise evaluation of in-vivo P-glycoprotein function because the hepatic blood flow rate is altered by intoxication with CCl_4 (Sugihara et al 1992). Acute hepatic failure did not affect the steady-state plasma concentration or total plasma clearance of rhodamine 123 in the in-vivo clearance study (Table 2). Rhodamine 123, an ester compound, is extensively metabolized to rhodamine 110 (a deacylated metabolite) and its glucuronide conjugate (Sweatman et al 1990), indicating that the metabolic clearance plays an important role in the total plasma clearance of rhodamine 123. Although the contribution of each clearance to the total plasma clearance is relatively small, biliary and renal tubular secretion clearances and brain distribution of rhodamine 123 are mostly mediated or restricted by P-glycoprotein, as in cases of intestinal exsorption and aqueous humor distribution of rhodamine 123 (Wang et al 1995; Kunihara et al 1998; Takano et al 1998; Kajikawa et al 1999, 2000; Yumoto et al 1999; Huang et al 2000). The biliary clearance, normalized biliary clearance, renal clearance and renal secretory clearance of rhodamine 123 all decreased significantly, and the distribution to the brain increased in rats with acute hepatic failure (Table 2). The increase in hepatic concentration of rhodamine 123 was also observed in rats with acute hepatic failure.

The intracellular unbound concentration of the xenobiotic is actually responsible for the transport from the intracellular compartment to the bile (Yamazaki et al 1996). Thus, a parameter of normalized biliary clearance was used to normalize the change in hepatic concentration of rhodamine 123 under different conditions. This parameter showed a greater suppression of hepatic P-glycoprotein function than that evaluated by conventional biliary clearance (Table 2).

Our data demonstrated that in-vivo P-glycoprotein function is systemically suppressed, not only in the target organ (liver), but also in other organs (kidney and brain) under acute hepatic failure, even though P-glycoprotein concentrations are not decreased in these tissues and are increased in the liver (Table 1). The suppression of hepatic P-glycoprotein function in rats with acute hepatic failure may be explained by the decreased hepatic ATP concentration. P-glycoprotein needs ATP hydrolysis as a driving force to pump out the substrate, with one rhodamine 123 molecule being transported per ATP molecule hydrolysed (Shapiro & Ling 1998). The K_m value for ATP hydrolysis by P-glycoprotein is reportedly over the range 0.4–0.8 mM (Shapiro & Ling 1994; Sharom et al 1995; Liu & Sharom 1997). Taken together, the decrease in hepatic P-glycoprotein function in CCl_4-treated rats observed in the present study may, at least in part, be ascribed to the decrease in hepatic ATP concentration (Table 1 and 2). In contrast, none of the biological parameters examined could account for the suppression of P-glycoprotein function in the kidney and brain of rats with acute hepatic failure. Accordingly, other factors such as endogenous P-glycoprotein substrates/modulators should be considered in the systemic suppression of P-glycoprotein function in rats with acute hepatic failure. Recently, we analysed the expression and function of P-glycoprotein in rats with glycerol-induced acute renal failure, in which the function of P-glycoprotein in the kidney and liver was greatly suppressed, although the P-glycoprotein concentration increased 2.5-fold in the kidney (Huang et al 2000). Interestingly, both renal and the biliary clearances of rhodamine 123 were well correlated with the decreased glomerular filtration rate in rats with acute renal failure. These results suggest that endogenous P-glycoprotein

substrates/inhibitors play an important role in the modulation of P-glycoprotein function, especially in diseased states.

Steroid hormones, such as cortisol, progesterone, aldosterone, and their metabolites (including unidentified compounds), exist in biological fluids as endogenous P-glycoprotein substrate/modulators (Becker et al 1992; Charuk & Reithmeier 1992; Rao et al 1994). In the present study, plasma from rats with acute hepatic failure showed greater inhibition on P-glycoprotein-mediated transport of rhodamine 123 across Caco-2 cells than that from control rats (Table 3). This indicates the presence of endogenous P-glycoprotein substrates/inhibitors in the plasma even under normal conditions and suggests that concentrations of these compounds are increased in the disease state. In the present study, we found that the concentration of corticosterone, a P-glycoprotein substrate (Wolf & Horwitz 1992; Rao et al 1994; Orlowski et al 1996), in acute hepatic failure rats increased approximately 2-fold compared with that in control rats. Also, it was suggested that most of these compounds are bound to plasma protein, at least under normal conditions, because plasma filtrate (50 μL (mL transport medium)$^{-1}$) showed no inhibitory effect. With the accumulation in plasma of endogenous P-glycoprotein substrates/inhibitors in the diseased state, however, the unbound concentrations of these compounds may also increase and cause the systemic suppression of P-glycoprotein function. Further study will be required to mimic the in-vivo condition in an in-vitro transport study and to isolate and identify the endogenous compounds mainly responsible for the modulation of P-glycoprotein function in rats with acute hepatic failure. Our findings also suggest that the in-vivo P-glycoprotein function in the diseased state can not be predicted merely from the expression of protein and mRNA of P-glycoprotein. Many other factors, such as the alteration of the concentration or composition of endogenous P-glycoprotein-related compounds in the plasma, would be involved in the regulation of in-vivo P-glycoprotein function.

In conclusion, it was demonstrated that P-glycoprotein function is systemically suppressed in rats with CCl_4-induced acute hepatic failure, not only in the target organ (liver), but also in other organs (kidney and brain), whereas the P-glycoprotein concentration remained unchanged in the kidney and brain, and increased in the liver. In the systemic suppression of the P-glycoprotein function, the alteration of concentrations or components of endogenous P-glycoprotein-related compounds such as corticosterone would likely be involved in the diseased state.

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