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Expression and function of P-glycoprotein in rats with glycerol-induced acute renal failure

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Abstract

The effect of glycerol-induced acute renal failure on P-glycoprotein expression and function was evaluated in rats. The in vivo function of P-glycoprotein was evaluated by measuring renal secretory and biliary clearance and brain distribution of rhodamine 123 (Rho-123), a P-glycoprotein substrate, under a steady-state plasma concentration. In acute renal failure rats, the P-glycoprotein level increased 2.5-fold in the kidney, but not in the liver and brain. In contrast, P-glycoprotein function in these tissues was suppressed. Interestingly, not only the renal but also the biliary clearance of Rho-123 was correlated with the glomerular filtration rate. In Caco-2 cells, plasma from renal failure rats exhibited a greater inhibitory effect on P-glycoprotein-mediated transport of Rho-123 than did plasma from control rats. In conclusion, P-glycoprotein function was systemically suppressed in acute renal failure, even though the level of P-glycoprotein remained unchanged or rather increased. This may be due to the accumulation of some endogenous P-glycoprotein substrates/modulators in the plasma in disease states.

Keywords: Renal failure, acute; P-glycoprotein level; P-glycoprotein function, in vivo; P-glycoprotein-related compound endogenous; Rhodamine 123; Pharmacokinetics

1. Introduction

The kidney and liver are important organs in the detoxification of xenobiotics, and dysfunction of these organs exerts a large influence on the pharmacokinetics of drugs not only at the target injury organ, but also at other organs. For example, acute renal failure induced by glycerol or uranyl nitrate reduces the hepato-biliary transport of some drugs, modulates the distribution of drugs into the central nervous system and affects the activity of various hepatic microsomal enzymes (Bowmer and Yates, 1984; Yeung, 1991; Zurovsky, 1993; Naora et al., 1999), besides suppressing kidney function including the glomerular filtration and tubular secretion of organic anions and cations (Hori et al., 1985; Lin and Lin, 1988; Inui et al., 1989; Zurovsky, 1993). The mechanism of this systemic alteration of the host defense system in acute renal failure, however, is not yet fully understood.

P-glycoprotein, an ATP-dependent efflux pump, is expressed widely in normal tissues, including brush border membrane of renal proximal tubules, biliary canalicular membranes of hepatocytes and capillary endothelial cells of the brain (Thiebaut et al., 1987; Cordon-Cardo et al., 1989). This protein transports a variety of structurally and pharmacologically unrelated hydrophobic compounds and plays an important role in the excretion of exogenous and endogenous P-glycoprotein substrates to prevent the accumulation of these compounds in tissues (Thiebaut et al., 1987; Lee et al., 1994; Scala et al., 1997; Kajikawa et al., 1999, 2000). Recently, we reported a marked reduction of the renal secretory clearance of rhodamine 123 (Rho-123), a P-glycoprotein substrate, in glycerol-induced acute renal failure rats, indicating the suppression of P-glycoprotein-mediated tubular secretion (Kunihara et al., 1998). The pathogenic mechanisms underlying glycerol-induced acute renal failure include ischemic injury, tubular nephrotoxic-

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ity caused by myoglobin, and the renal action of cytokines released after rhabdomyolysis (Wolfert and Oken, 1989; Vexler et al., 1996). Rho-123 has been used as a probe to assess P-glycoprotein function in various cell lines and to examine whether a test compound is a P-glycoprotein inhibitor or not (Lee et al., 1994; Scala et al., 1997).

In the present study, the effect of glycerol-induced acute renal failure on P-glycoprotein expression and its in vivo function in the liver, kidney and brain was evaluated over 7 days after glycerol injection. In addition, the possible mechanism underlying the systemic modulation of P-glycoprotein function in acute renal failure was examined from the point of view of the involvement of endogenous P-glycoprotein substrates/modulators.

2. Materials and methods

2.1. Materials

Rho-123 was obtained from Kanto Chemical (Tokyo, Japan). Cyclosporin A was kindly supplied by Novartis (Tokyo, Japan). A monoclonal antibody for P-glycoprotein, C219, was from Signet Laboratories (MA, USA) and a secondary antibody, peroxidase-labelled affinity-purified antibody to mouse immunoglobulin G (H + L), was from Kirkegaard & Perry Laboratories (MD, USA). All other chemicals used were of the highest purity available.

2.2. Animal treatment

Experiments with animals 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. Experimental acute renal failure was induced in male Wistar rats (230–300 g) by an injection of glycerol dissolved in saline (50% v/v, 10 ml/kg) into the leg muscle after a 24-h period of water deprivation (Kunihara et al., 1998). Control rats received the same volume of saline. These animals were used for experiments 1, 3, 5 or 7 days after the injection.

2.3. Determination of P-glycoprotein expression level

The amount of P-glycoprotein in the crude membrane fraction of the kidney, liver and brain was determined by Western immunoblotting after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). SDS-PAGE was carried out according to the reported method (Laemmli, 1970). Briefly, freshly isolated kidney, liver and brain were homogenized, and crude membrane fractions were prepared by centrifuging the 3000-g supernatant of tissue homogenates at 24,000 × g for 30 min. The pellet resuspended in loading buffer (4 mg protein/ml in 62.5 mM Tris–HCl, 2% SDS, 7% glycerol, 0.005% bromophenol blue, pH 6.8) was loaded in a volume of 20 μl on 7.5% acrylamide–bisacrylamide gels without prior heating. The proteins were transferred electrophoretically onto a 0.45-μm pore size polyvinylidene difluoride membrane (Bio-Rad Laboratories, CA, USA). Blots were blocked overnight at 4°C with solution A (0.05% Tween 20, 0.15 M NaCl, and 20 mM Tris–HCl, pH 7.5) containing 5 mM sodium azide and 5% (w/v) non-fat powdered milk. The polyvinylidene difluoride membranes were washed three times with solution A, followed once with a solution containing 0.15 M NaCl and 20 mM Tris–HCl (pH 7.5), and were incubated with a monoclonal antibody C219 (10 μg/ml) for 2 h at 37°C. Peroxidase-labelled affinity-purified antibody to mouse immunoglobulin G was used as the secondary antibody. Detection was made according to an enhanced chemiluminescence technique (ECL Western Blotting Detection System, Amersham Pharmacia Biotech, England). The blots were exposed to Hyperfilm ECL™ (Amersham Pharmacia Biotech). The optical densities of immunoblots were estimated by a computer-aided densitometer with NIH Image (the public domain program developed at the US National Institutes of Health). Molecular mass determination was performed over a broad range of protein markers (New England Biolabs, USA).

2.4. In vivo clearance study of Rho-123

In vivo clearance studies of Rho-123 were performed in the same manner as reported previously (Kunihara et al., 1998; Yumoto et al., 1999). Briefly, a solution of Rho-123 (100 μM) containing mannitol (50 mg/ml) and inulin (10 mg/ml) was injected intravenously as a bolus (4.36 ml/kg), followed by a constant infusion (2 ml/h) into a femoral vein, to attain a steady-state plasma concentration of approximately 0.25 μM. In the inhibition study, cyclosporin A (30 mg/kg) was administered intravenously 10 min before the injection of Rho-123. Three consecutive 20-min clearance studies were performed by collecting bile and urine samples under steady-state conditions. Blood was taken from a femoral artery at a midpoint of the above sample collection protocol.

2.5. Plasma unbound fraction and tissue distribution of Rho-123

Blood was collected by heart puncture to obtain plasma after the clearance study. The unbound fraction of Rho-123 in plasma was measured by ultrafiltration, using a semipermeable cellulose membrane. The liver, brain, and kidney were also isolated to measure Rho-123 concentrations in these tissues. The tissue distribution of Rho-123 is expressed as the tissue-to-plasma partition coefficient of unbound fraction (Kpf).

2.6. Pharmacokinetic analysis

Pharmacokinetic parameters such as total plasma (CL_{total}), biliary (CL_{bile}) and renal (CL_{renal}) clearance were estimated in the same manner as reported previously (Kunihara et al., 1998; Yumoto et al., 1999). The normalized biliary clearance, CL^{*}_{bile}, of Rho-123 was estimated by dividing the biliary excretion rate by the hepatic Rho-123 concentration. The renal clearance of unbound Rho-123 (CLr,f) was determined by dividing CL_{renal} by the plasma-unbound fraction. Glomerular filtration rate was assumed to be equal to the CL_{renal} of inulin. The net renal secretory clearance ($CL_{secretory}$) of unbound Rho-123 was calculated by subtracting glomerular filtration rate from CLr,f.

2.7. Tissue binding of Rho-123 in vitro

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

2.8. Effect of plasma on transepithelial transport of Rho-123 across Caco-2 cell monolayers

The effect of plasma collected from control and acute renal failure rats on the transepithelial transport of Rho-123 across Caco-2 cell monolayers was measured in a similar manner as reported previously (Takano et al., 1998, Yumoto et al., 1999). Briefly, Caco-2 cells (passages 60–66) were cultured in a Transwell chamber® (Costar, Cambridge, MA) for 19 or 20 days after seeding. Rho-123 solution (5 μM in Dulbecco's phosphate-buffered saline containing 25 mM HEPES and 25 mM glucose, pH 7.4) was placed either on the apical or the basolateral side. Plasma collected from control or acute renal failure rats was added to both sides of the chamber to give a designated final concentration (10 or 25 μl plasma/ml transport medium). The transepithelial transport of Rho-123 was measured at 37°C for 120 min.

2.9. Analytical methods

Concentrations of Rho-123 in various biological samples were determined by high-performance liquid chromatography using a reverse-phase column of TSKgel ODS-80 TM (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. Detection was made at wavelengths of 485 nm for excitation and 546 nm for emission. The concentration of inulin in plasma and urine was determined spectrophotometrically (Dische and Borenfreund, 1951). Blood urea nitrogen (BUN) and plasma activities of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were measured with the BUN B-Test Wako, the GOT-UV Test Wako and the GPT-UV Test Wako (Wako, Osaka, Japan), respectively.

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

3. Results

3.1. Biochemical parameters of acute renal failure rats

Induction of acute renal failure in rats after treatment with glycerol was evaluated by measuring some biochemical parameters (Table 1). The glycerol-treated rats showed significantly higher BUN values on days 1 and 3 and lower glomerular filtration rate values over 5 days than did the control rats. The plasma glutamic oxaloacetic transaminase and glutamic pyruvic transaminase levels increased only on the first day of the glycerol-induced acute renal failure. These biochemical parameters indicated the induction of acute renal failure and the gradual restoration of

Table 1
Biochemical parameters of control and acute renal failure rats. Control rats received saline alone. Acute renal failure was induced by intramuscular injection of glycerol dissolved in saline (50% v/v, 10 ml/kg). Glomerular filtration rate was determined by measuring the renal clearance of inulin. Each value presented is a mean ± S.E.M. ($n = 4$). BUN, blood urea nitrogen; GFR, glomerular filtration rate; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase

Days after treatment	BUN (mg/dl)	GFR (ml/min)	GOT (IU/l)	GPT (IU/l)
Control	22.6 ± 0.97	1.28 ± 0.25	29.3 ± 1.44	9.24 ± 0.56
1	105 ± 16.8[a]	0.05 ± 0.02[a]	183 ± 58.1[a]	23.2 ± 1.90[a]
3	99.8 ± 14.0[a]	0.22 ± 0.03[a]	30.2 ± 3.43	10.7 ± 0.95
5	44.2 ± 14.9	0.52 ± 0.10[a]	27.4 ± 1.31	11.0 ± 1.03
7	22.6 ± 5.29	1.06 ± 0.22	28.6 ± 4.78	11.0 ± 1.23

[a] $P < 0.05$, significantly different from the values for control rats.

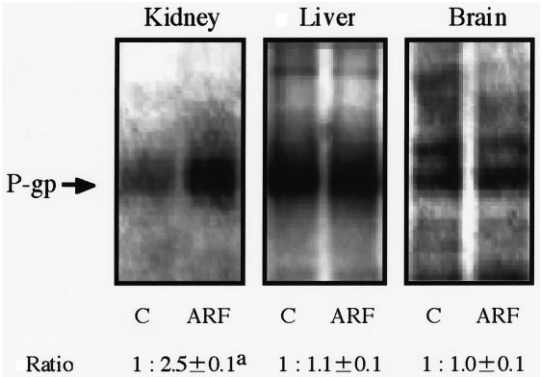

Fig. 1. Western blot analysis with a monoclonal antibody for P-glycoprotein (C219) of crude kidney, liver and brain membranes obtained from control (C) and acute renal failure (ARF) rats. ARF was induced by an intramuscular injection of glycerol dissolved in saline (50% v/v, 10 ml/kg), and the rats were examined 24 h after injection. The ratio represents the relative staining intensity for P-glycoprotein in control and acute renal failure rats. Each value presented is a mean ± S.E.M. ($n = 4$). [a] $P < 0.05$ compared to control rats.

renal function over 7 days, as evaluated by the change in glomerular filtration rate.

3.2. Western blot analysis of P-glycoprotein level in the kidney, liver and brain

Acute renal failure rats showed a stronger staining intensity for P-glycoprotein in the kidney than did control rats (Fig. 1). Densitometric analysis of the P-glycoprotein level indicated a 2.5-fold increase in the kidney on day 1. No change in P-glycoprotein level was found in the liver and brain as compared with control rats. The increased P-glycoprotein level in the kidney was observed for at least 5 days after glycerol injection (2.0 ± 0.6-fold of levels in control rats on day 5, $n = 4$).

Table 2
Pharmacokinetic parameters of Rho-123 in control and cyclosporin A (CsA)-treated rats. Cyclosporin A (30 mg/kg) was administered intravenously 10 min before the initiation of constant infusion of Rho-123. Normalized biliary clearance (CLb^*) was estimated by dividing the biliary excretion rate by the hepatic concentration of Rho-123. Each value presented is a mean ± S.E.M. ($n = 4$–5). GFR, glomerular filtration rate

Parameter	Control	CsA-treated
Cpss (μM)	0.24 ± 0.01	0.25 ± 0.01
Plasma unbound fraction	0.28 ± 0.01	0.27 ± 0.02
GFR (ml/min)	1.28 ± 0.25	0.94 ± 0.15
Bile flow rate (μl/min)	20.2 ± 1.5	19.1 ± 2.4
CL_{total} (ml/min)	14.5 ± 0.56	13.5 ± 0.36
CL_{renal} (ml/min)	1.64 ± 0.18	0.35 ± 0.09[a]
CLr,f (ml/min)	5.71 ± 0.64	1.29 ± 0.34[a]
$CL_{secretory}$ (ml/min)	3.59 ± 0.64	0.44 ± 0.24[a]
CL_{bile} (ml/min)	0.73 ± 0.06	0.07 ± 0.01[a]
CL^*_{bile} (g liver/min)	0.09 ± 0.01	≪ 0.01[a]
Liver Kpf	27.2 ± 4.8	55.3 ± 3.3[a]
Brain Kpf	0.99 ± 0.16	2.73 ± 0.34[a]
Kidney Kpf	410.3 ± 22.5	483.6 ± 50.3

[a] $P < 0.05$, significantly different from the values for control rats.

3.3. Role of P-glycoprotein in Rho-123 pharmacokinetics in normal rats

Cyclosporin A, a P-glycoprotein inhibitor, administered intravenously markedly reduced CL_{renal} of Rho-123 by 75%, $CL_{secretory}$ by 85% and CL_{bile} by 90%, in which glomerular filtration rate was slightly decreased (approximately 30%, no significant difference) and bile flow rate remained unchanged (Table 2). These results indicate that the renal secretion and biliary excretion of Rho-123 are mostly mediated by P-glycoprotein. Cyclosporin A increased the hepatic concentration of Rho-123 to 3.75 ± 0.17 nmol/g from 2.06 ± 0.26 nmol/g in the absence of cyclosporin A. Therefore, to normalize the CL_{bile} with hepatic Rho-123 concentration, a parameter of CL^*_{bile} of

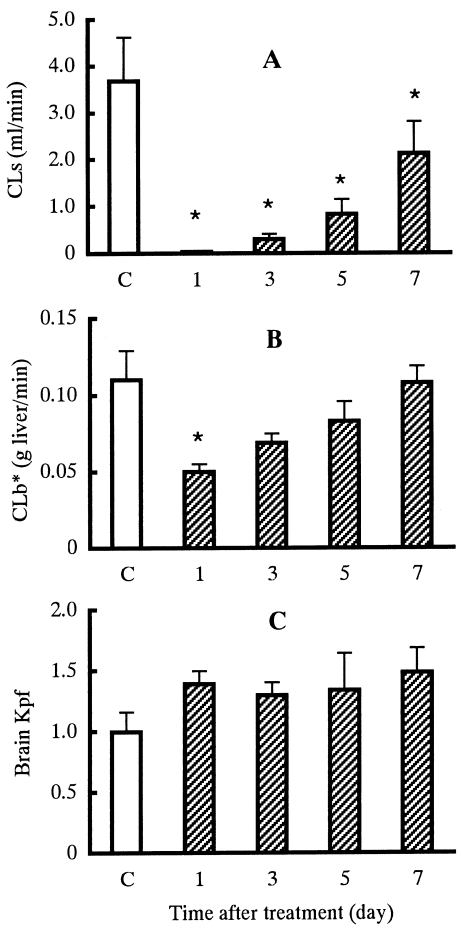

Fig. 2. Changes in renal secretory clearance (CLs, A), normalized biliary clearance (CLb^*, B) and brain distribution of plasma unbound fraction (brain Kpf, C) of Rho-123 under steady-state conditions in control (C, open column) and glycerol-induced acute renal failure rats (hatched column). Acute renal failure was induced by an intramuscular injection of glycerol dissolved in saline (50% v/v, 10 ml/kg). The steady-state plasma concentration (Cpss) of Rho-123 was approximately 0.25 μM. CLb^* was estimated by dividing the steady-state biliary excretion rate by the hepatic concentration of Rho-123. Brain Kpf was estimated by dividing the brain concentration of Rho-123 by a product of Cpss and plasma unbound fraction of Rho-123. Each value presented is a mean ± S.E.M. ($n = 4$). * $P < 0.05$ compared to control rats.

Rho-123 was used. This parameter also indicates that the actual P-glycoprotein function in the liver was potentially suppressed by cyclosporin A. Besides the liver, cyclosporin A increased the brain distribution (brain Kpf) of Rho-123 approximately threefold, while the effect on the kidney distribution of Rho-123 was negligible. The increase in the brain and liver distribution of Rho-123 in cyclosporin A-administered rats was not ascribed to the change in plasma unbound fraction (Table 2).

3.4. Modulation of P-glycoprotein function in acute renal failure rats

$CL_{secretory}$, CL^{*}_{bile} and brain Kpf of Rho-123 under steady-state conditions were determined in acute renal failure rats to evaluate in vivo P-glycoprotein function in the kidney, liver and brain, respectively (Fig. 2). In renal failure rats, $CL_{secretory}$ of Rho-123 was dramatically decreased 1 day after glycerol injection, and it gradually recovered to the control level over 7 days (Fig. 2A). A significant decrease in CL^{*}_{bile} of Rho-123 was also observed 1 day after glycerol injection (Fig. 2B), although no change was detected in the bile flow rate during the 7-day experimental period (Table 3). As with cyclosporin A-treated rats, the liver Kpf of Rho-123 was increased to 55.3 ± 3.3 from 27.5 ± 4.8 in control rats 1 day after glycerol injection. The brain Kpf of Rho-123 was also higher than in control rats over 7 days, although the difference did not reach statistical significance (Fig. 2C). In this study, acute renal failure did not affect the binding of Rho-123 to plasma proteins or to the liver homogenates even 1 day after glycerol injection (Table 3).

In Fig. 3, $CL_{secretory}$ and CL^{*}_{bile} of Rho-123 are plotted against glomerular filtration rate of each groups of rats. Interestingly, not only $CL_{secretory}$ but also CL^{*}_{bile} was positively correlated with glomerular filtration rate. A positive correlation was also observed between $CL_{secretory}$ and CL^{*}_{bile} ($R^2 = 0.8159$, $P < 0.05$). No positive correlation was found between CL^{*}_{bile} and plasma glutamic ox-

Table 3
Bile flow rate and unbound fraction of Rho-123 in plasma and in 10% liver homogenate from control and glycerol-induced acute renal failure rats. Control rats received saline alone. Acute renal failure was induced by an intramuscular injection of glycerol dissolved in saline (50% v/v, 10 ml/kg). The unbound fraction of Rho-123 in plasma and 10% liver homogenate was measured by ultrafiltration at 4°C. The concentration of Rho-123 was approximately 0.25 μM in plasma and 1 μM in liver homogenate. ND: not determined. Each value presented is a mean ± S.E.M. ($n = 4$–6)

Days after treatment	Bile flow rate (μl/min)	Unbound fraction(%)	
		Plasma	Liver homogenate
Control	20.2 ± 1.5	29.8 ± 0.9	13.3 ± 1.5
1	18.2 ± 1.4	33.2 ± 1.9	13.1 ± 1.7
3	16.7 ± 1.5	32.4 ± 1.2	ND
5	17.0 ± 1.3	35.2 ± 3.3	ND
7	16.6 ± 2.1	29.3 ± 2.3	ND

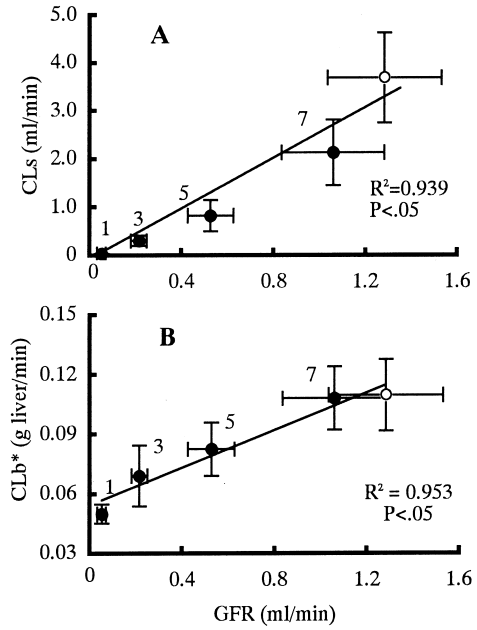

Fig. 3. Relationship between glomerular filtration rate (GFR) and renal secretory clearance (CLs, A) or normalized biliary clearance (CLb^{*}, B) of Rho-123 in control (○) and acute renal failure rats (●, the number for each symbol represents the day after glycerol injection). Glomerular filtration rate was estimated from the steady-state renal clearance of inulin. Each value presented is a mean ± S.E.M. ($n = 4$).

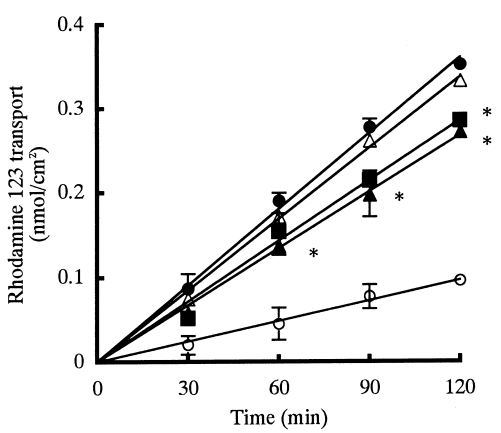

Fig. 4. Effect of plasma obtained from control and acute renal failure rats on transepithelial transport of Rho-123 in the basolateral (b)-to-apical (a) direction across Caco-2 cell monolayers. Acute renal failure was induced by the intramuscular injection of glycerol dissolved in saline (50% v/v, 10 ml/kg), and plasma was collected 24 h after glycerol injection. Key: ○, transport of Rho-123 in the a-to-b direction in the absence of plasma; ●, b-to-a direction in the absence of plasma; △, b-to-a direction in the presence of control plasma (25 μl/ml transport medium); ■, b-to-a direction in the presence of plasma from acute renal failure rats (10 μl/ml); ▲, b-to-a direction in the presence of plasma from acute renal failure rats (25 μl/ml). The initial concentration of Rho-123 in the transport medium was 5 μM. Plasma was added to the transport medium on both apical and basolateral sides. Each value presented is a mean ± S.E.M. ($n = 4$–5). $^{*}P < 0.05$ compared to the transport in the b-to-a direction in the absence of plasma.

aloacetic transaminase or glutamic pyruvic transaminase level (data not shown).

3.5. Inhibitory effect of plasma from acute renal failure rats on transepithelial transport of Rho-123 in Caco-2 cells

As reported previously (Takano et al., 1998), the transport of Rho-123 in the basolateral-to-apical direction across Caco-2 cell monolayers was much greater than that in the apical-to-basolateral direction. Also, the basolateral-to-apical transport of Rho-123 was significantly reduced by verapamil, indicating that P-glycoprotein is functionally expressed in these Caco-2 cells. The expression of P-glycoprotein in Caco-2 cells was also confirmed by Western blot analysis (data not shown). Plasma obtained from acute renal failure rats inhibited the basolateral-to-apical transport of Rho-123, while plasma from control rats showed no effect under these experimental conditions (Fig. 4).

4. Discussion

The present study was carried out to examine the effect of acute renal failure on P-glycoprotein level and in vivo P-glycoprotein function in rats. As to the effect of glycerol-induced acute renal failure on in vivo P-glycoprotein function, we previously demonstrated that the renal tubular secretion of Rho-123 was greatly suppressed in acute renal failure rats. In the present study, we further examined the effect of acute renal failure on in vivo P-glycoprotein function systemically, not only locally at the target injury site, as well as the effect on P-glycoprotein expression. In glycerol-treated rats, there were clear changes in BUN and glomerular filtration rate (Table 1). In particular, it took 7 days for the glomerular filtration rate to recover to the control level after glycerol injection. Also, as reported previously (Coelho et al., 1996), some damage to the liver was observed, as evaluated by plasma glutamic oxaloacetic transaminase and glutamic pyruvic transaminase levels, as an additional complication of glycerol-induced rhabdomyolysis. In acute renal failure rats, the level of P-glycoprotein in the kidney was significantly increased (approximately 2.5-fold of control rats), although the levels in the liver and brain were not altered (Fig. 1).

In normal rats, injection of cyclosporin A dramatically suppressed the CL_{renal}, $CL_{secretory}$, CL_{bile} and CL^{*}_{bile} and increased the brain and liver Kpf values of Rho-123 (Table 2). Cyclosporin A is known to decrease glomerular filtration rate to some extent by suppressing the renal blood flow rate (Bloom et al., 1995; Verbeke et al., 1995). Thus, the effect on glomerular filtration rate would be partly involved in the decreased CL_{renal} of Rho-123 in cyclosporin A-treated rats. However, because $CL_{secretory}$ is independent of glomerular filtration rate, the suppression of $CL_{secretory}$ by cyclosporin A strongly suggests that the renal tubular secretion of Rho-123 is a P-glycoprotein-mediated process, as described previously (Kunihara et al., 1998). For evaluation of the biliary excretion of xenobiotics under steady-state conditions, a parameter of CL_{bile} is generally used. However, the intracellular unbound concentration of the xenobiotic is actually responsible for the transport from the intracellular compartment to the bile (Yamazaki et al., 1996). In the present study, cyclosporin A significantly increased the hepatic concentration of Rho-123. Therefore, a parameter of CL^{*}_{bile} was further used to normalize the change in the hepatic concentration of Rho-123 under different conditions. This parameter indicated that the actual P-glycoprotein function in the liver was potentially suppressed by the presence of cyclosporin A, more than was indicated by the CL_{bile} value. The increase in the brain distribution of Rho-123 in the presence of cyclosporin A observed in the present study was in good agreement with the previously reported value determined by an in vivo microdialysis method (Wang et al., 1995). Taken together, $CL_{secretory}$, CL^{*}_{bile} and brain Kpf of Rho-123 were considered to be suitable for evaluation of in vivo P-glycoprotein function in the kidney, liver and brain.

Acute renal failure was found to cause a systemic suppression of P-glycoprotein function, not only in the target organ (kidney), but also in other organs (liver and brain) (Fig. 2). It has been reported that acute renal failure decreases the transport of organic anions and cations in renal proximal tubular cells, and a decrease in the number and/or availability of transporter has been proposed as a possible mechanism based on membrane vesicle studies (Stein et al., 1978; Hori et al., 1985; Lin and Lin, 1988; Inui et al., 1989). In the present study, the suppression of P-glycoprotein function in acute renal failure rats was not explained by P-glycoprotein level (Fig. 1). P-glycoprotein needs ATP hydrolysis as a driving force to pump out the substrate. However, the ATP content in the renal cortex of acute renal failure rats is not altered as compared with that in control rats (Kunihara et al., 1998). In addition, no significant effect of acute renal failure was observed on other parameters, except for glomerular filtration rate, such as the bile flow rate, plasma unbound fraction of Rho-123, and the binding of Rho-123 to the liver homogenate.

It is generally accepted that a variety of acidic compounds, so-called uremic toxins, such as guanidinosuccinic acid, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid, indole-3-acetic acid, indoxyl sulfate and hippuric acid, accumulate in the central circulation in acute renal failure, and these compounds sometimes modulate the pharmacokinetics of certain acidic compounds, mainly by displacing the drug bound to serum albumin (Sakai et al., 1995; Tanaka et al., 1999; Tsutsumi et al., 1999). In this study, we examined the possible contribution of endogenous P-glycoprotein substrates/modulators in the systemic sup-

pression of P-glycoprotein function, since suppression of hepatic P-glycoprotein function in acute renal failure rats appeared to be related to the decrease in renal function, measured by glomerular filtration rate and $CL_{secretory}$ of Rho-123. In fact, the presence of endogenous P-glycoprotein substrates/modulators in the biological fluid of rats and humans has been reported (Ichikawa et al., 1990; Becker et al., 1992; Charuk and Reithmeier, 1992; Rao et al., 1994). As can be expected from the results of this study, the renal excretion of putative endogenous P-glycoprotein substrates, either by glomerular filtration or tubular secretion or both, should decrease in acute renal failure. If this were the case, the plasma concentration of endogenous P-glycoprotein substrates would increase, which in turn would suppress P-glycoprotein function systemically. To confirm this speculation, the inhibitory potency of plasma obtained from acute renal failure rats on P-glycoprotein-mediated Rho-123 transepithelial transport across Caco-2 cell monolayers was compared with that of plasma from control rats (Fig. 4). A greater inhibitory effect of plasma from acute renal failure rats was observed, as compared with that of plasma from control rats. Thus, the systemic modulation of in vivo P-glycoprotein function in acute renal failure could be, at least in part, due to the accumulation of some endogenous P-glycoprotein-related compounds in the plasma. This accumulation of various endogenous compounds in acute renal failure could also be involved in the systemic modulation of other transporters, including organic anion and cation transporters in the kidney, liver and brain. Further study is necessary to isolate and identify the endogenous compounds responsible for the modulation of P-glycoprotein function in acute renal failure. Our findings also suggest that the actual in vivo function of P-glycoprotein during acute renal failure cannot be predicted merely from the level of expression of protein and/or mRNA of P-glycoprotein, because many other factors, such as alterations in the concentration of the endogenous P-glycoprotein-related compounds in plasma, as described above, participate in the regulation of in vivo P-glycoprotein function.

In conclusion, P-glycoprotein function in acute renal failure rats was found to be suppressed systemically. The systemic suppression of P-glycoprotein function in acute renal failure rats could not be explained by the level of expression of P-glycoprotein, and the accumulation of some endogenous P-glycoprotein-related compounds in plasma is probably involved.

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