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Factors affecting the expression and function of P-glycoprotein in rats: drug treatments and diseased states

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The expression and function of P-glycoprotein (P-gp), an ATP-dependent efflux pump, were examined in rats pretreated with dexamethasone (DEX), an inducer of P-gp, and in rats with glycerol-induced acute renal failure (ARF) and with CCl_4-induced acute hepatic failure (AHF). DEX pretreatment increased the P-gp level and its functional activity in the intestine. In contrast, in ARF and AHF rats, the *in vivo* P-gp function was systemically supressed, even though the level of P-gp remained unchanged or rather increased. In Caco-2 cells, the plasma collected from diseased rats exhibited a greater inhibitory effect on P-gp function than did plasma from control rats. A higher plasma level of corticosterone, an endogenous P-gp substrate/inhibitor, was observed in the desease rats. These findings indicate that the actual *in vivo* function of P-gp cannot be predicted merely from the expression level of P-gp, and suggest that some endogenous P-gp-related compounds such as corticosterone participate in the regulation of *in vivo* P-gp function in diseased states.

1. Introduction

P-Glycoprotein (P-gp), an ATP-dependent multidrug efflux pump, is widely expressed in various normal human and rodent tissues, such as the intestine, kidney, liver, brain, eye, adrenal gland and testis. Substrates transported by P-gp include a variety of structurally and pharmacologically unrelated, hydrophobic compounds such as some anticancer agents, immunosuppressants, steroid hormones, calcium channel blockers, β-blockers, and so on [1–3]. P-gp limits the influx and faciliates the efflux to prevent the accumulation of exogenous and endogenous P-gp substrates in tissues. For example, in the eye (blood-aqueous barrier), quinidine or cyclosporin A (CsA), both are potent P-gp inhibitors, applied topically to the corneal surface markedly increased the aqueous distribution of Rhodamine 123 (Rho123), a P-gp substrate, from blood circulation by 11-fold. Also, topical P-gp inhibitors supressed the ocular clearance (disappearance rate) of Rho123 given intraocularly by approximately 50% [4, 5]. These data indicate that P-gp expressed in the capillary endothelial cells of iris and ciliary muscle, and in epithelia of ciliary non-pigmented cells suppresses the ocular distribution from blood circulation and facilitates the disappearance of a P-gp substrate from the aqueous humor. P-gp is now well recognized as an important host defense mechanism for a living body, along with various metabolic enzymes.

In this paper, the expression and function of P-gp in normal rats, in rats pretreated with dexamethasone (DEX), a P-gp inducer, and in rats with glycerol-induced acute renal failure (ARF) and with carbon tetrachloride (CCl_4)-induced acute hepatic failure (AHF) are summarized. The *in vivo* P-gp function was evaluated by comparing the pharmacokinetics of Rho123 in the presence and absence of a P-gp inhibitor such as CsA. Rho123 has been used as a probe to study the function of P-gp in various multidrug-resistant cells and normal tissues [3, 6]. There are strong overlapping substrate specificities between cytochrome P450 (CYP)3A and P-gp, and many of P-gp substrates are also recognized as CYP3A substrates [7, 8]. However, because Rho123 is not a CYP3A substrate, the change in CYP3A activity possibly occured in DEX-pretreated rats and in diseased rats would not influence on Rho123 pharmacokinetics. Rho123 has an ester moiety in its structure and is metabolized to rhodamine 110 (a deacylated metabolite of Rho123) followed by its glucuronidation *in vivo* [9]. Also, this compound is excreted into bile, urine, and intestinal lumen in an intact form, although the extent of the excretion is not so large. These characteristics of Rho123 are suitable for evaluating *in vivo* P-gp function than P-gp/CYP3A substrates.

2. P-gp-mediated transport of Rho123 in normal rats

As shown in Fig. 1, the total plasma clearance (CLtotal) of Rho123 was not affected by the injection of a potent P-gp inhibitor CsA in normal rats. This would be due to the high metabolic clearance of Rho123. In contrast, the intestinal exsorption clearance (CLexp) of Rho123 was clearly supressed by CsA, indicating the presence of a P-gp-mediated efflux system in the intestine. Also, CsA decreased the biliary clearance (CLbile) of Rho123 to approximately 10% of control. In this study, CsA increased the hepatic concentration of Rho123 approximately 2-fold, although the steady-state plasma concentration of Rho123 was not affected. Therefore, a parameter of CLbile*, a biliary excretion rate divided by hepatic concentration, was also used to normalize the change in the hepatic Rho123 concentration, because the intracellular concentration of the compound is actually responsible for the transport from the intracellular compartment to the bile. This parameter indicated that the actual P-gp function in the liver was potently suppressed by CsA, more markedly than that was indicated by a conventional CLbile. In the kidney, the renal secretory clearance (CLsec) of Rho123 was also supressed by CsA. To estimate the renal excretion of drugs, a parameter of renal clearance (CLrenal) is generally employed. However, CsA may decrease glomerular filtration rate (GFR) to some extent by suppressing the renal blood flow rate [10, 11]. Thus, the effect on GFR would be partly involved in the decrease in CLrenal of Rho123 in CsA-treated rats. Because CLsec is independent of GFR, the suppression of CLsec by CsA strongly suggests that the renal tubular secretion of Rho123 is a P-gp-mediated process. In addition to the liver, the brain Kpf of Rho123 was also increased by CsA by approximately 3-fold, in good agreement with the previously reported value determined by an *in vivo*

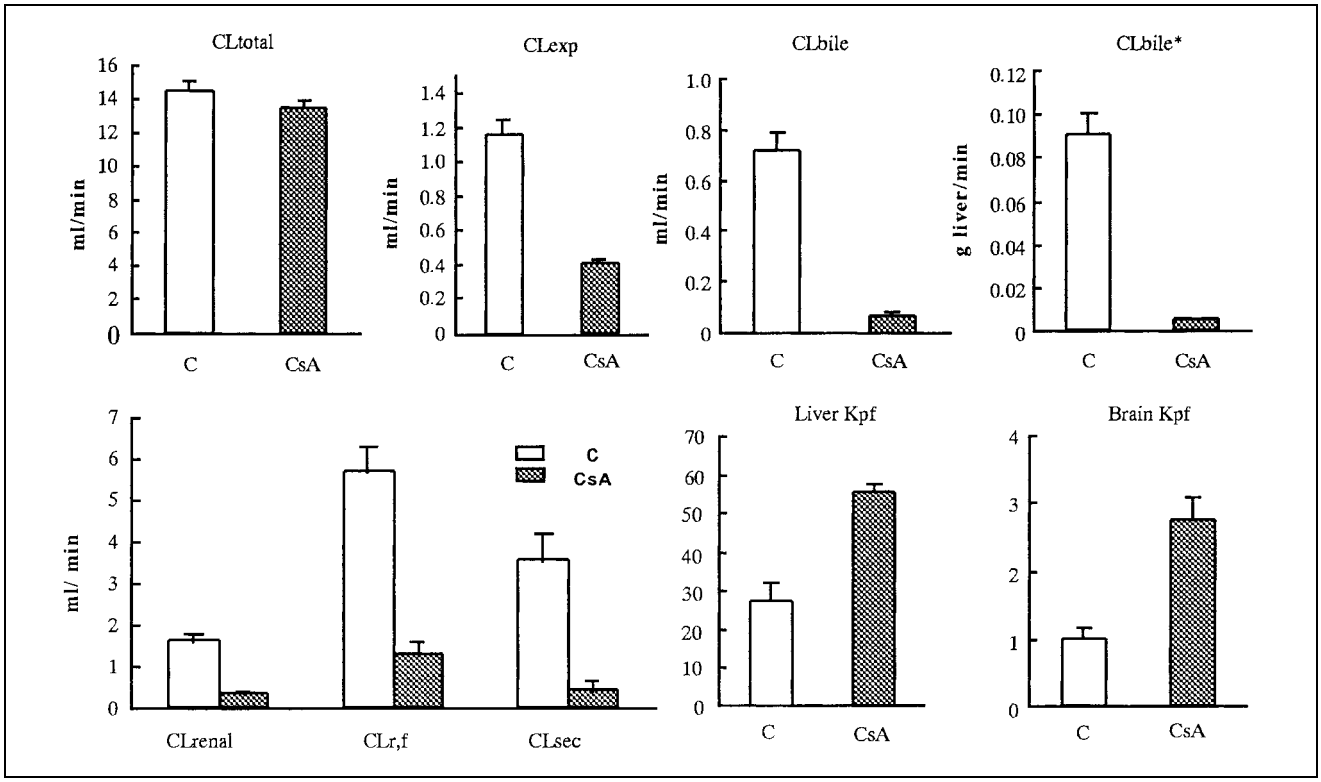

Fig. 1: Pharmacokinetic parameters of rhodamine 123 under a steady-state plasma concentration (0.25 μM) in the absence (C) and presence of cyclosporin A (CsA) in normal rats [34]. CsA was administered intravenously at a dose of 30 mg/kg. Each value represents the mean ± S.E.M. (n = 4–5)

microdialysis method [12]. This result indicates that the brain distribution of a P-gp substrate is physiologically limited by P-gp expressed in capilary endothelial cells of the brain.

3. Expression and function of P-gp in DEX-treated rats

P-gp and/or CYP3A are induced or supressed by various drug treatments. DEX is an inducer of P-gp and CYP3A [7, 8, 13]. In our studies, DEX-treatment increased P-gp level, evaluated by Western blot analysis with a monoclonal antibody for P-gp C219, in the intestine approximately 2-fold of control rats, although the hepatic P-gp level remained unchainged (1.9 ± 0.4 – fold in the intestine and 0.9 ± 0.1 – fold in the liver of control rats). By DEX-treatment, the hepatic CYP3A activity also increased approximately 10-fold, and the plasma AUC of midazolam, a CYP3A substrate, given intrajejunally greatly decreased as reported previously [14, 15]. In such DEX-treated rats, pharmacokinetics of P-gp and/or CYP3A-related compounds, and their drug-drug interactions were found to be significantly altered [16]. P-gp-mediated CLexp of Rho123 increased approximately 2-fold in DEX-treated rats, in good agreement with the results of Western blot analysis (Fig. 2). The CLbile of Rho123 was significantly lower in DEX-treated rats. However, the hepatic concentration of Rho123 also decreased, probably due to the induction of hepatic esterase by DEX-treatment. In fact, it is reported that DEX-pretreatment increased the total hepatic esterase activity more than 10-fold [17]. As a result, CLbile* of Rho123 was almost the same with that in control rats, indicating that P-gp function for Rho123 biliary excretion is not altered by DEX-pretreatment. This result is also in good agreement with the result of Western blot analysis of the hepatic P-gp level. Taken together, the *in vivo* P-gp function in DEX-treated rats was well correlated with the expression level of P-gp.

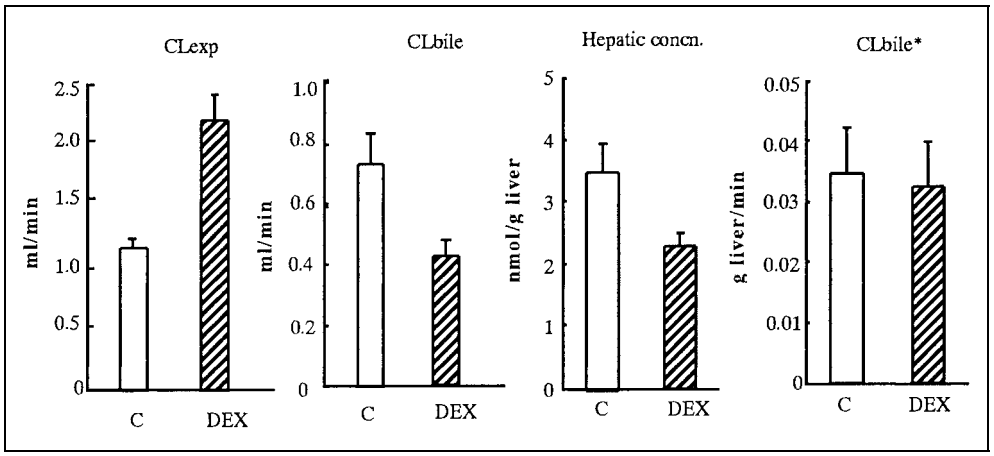

Fig. 2:
Pharmacokinetic parameters of rhodamine 123 under a steady-state plasma concentration (0.25 μM) in control (C) and dexamethasone-treated rats (DEX) [16]. Control rats received DMSO alone (0.5 ml/kg) and DEX-treated rats received 100 mg DEX dissolved in DMSO/kg for 2 days. These rats were used for experiments 24 h after the last dose of DEX. Each value represents the mean ± S.E.M. (n = 3)

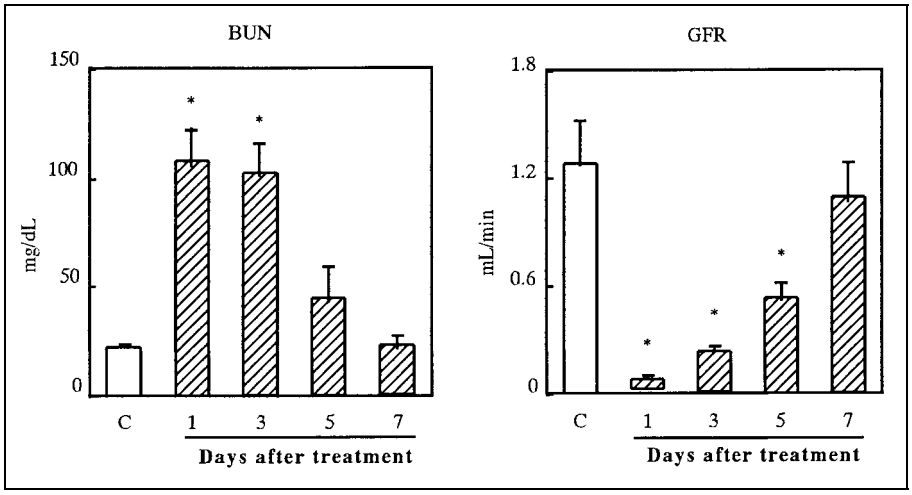

Fig. 3:
Biochemical parameters of control (C, open column) and acute renal failure rats (hatched column) [34]. Control rats received saline alone. Acute renal failure (ARF) was induced by intramuscular injection of glycerol dissolved in saline (5 ml/kg). Glomerular filtration rate was determined by measuring the renal clearance of inulin. Each value represents the mean ± S.E.M. (n = 4)

4. Expression and function of P-gp in ARF rats

In ARF rats, blood urea nitrogen (BUN) was higher on days 1 and 3, and GFR was significantly lower over 5 days, as compared with those in control rats (Fig. 3). On day 1, P-gp level in the kidney increased 2.5-fold, whereas the P-gp levels in the liver and brain remained unchanged. The increase in P-gp level in the kidney remained at least for 5 days (2.0-fold at on day 5). In ARF rats, the concentration of ATP, a driving force for P-gp, in the kidney and liver remained unchanged as compared with control rats as reported previously [18]. As shown in Fig. 4, CLsec of Rho123 decreased markedly on day 1, and it recovered gradually to the control level over 7 days. CLbile* also decreased during 5 days after induction of ARF, and a significant difference was detected on day 1. The brain Kpf of Rho123 was relatively higher than that in control rats, suggesting the inhibition of P-gp function in the brain capillary. These results would indicate that P-gp function is systemically suppressed in ARF rats, not only in the target organ (kidney), but also in other organs (liver and brain). However, the systemic suppression of P-gp in ARF rats was not explained by physiological parameters such as P-gp level, ATP contents, bile flow rate, and plasma and tissue bindings of Rho123.

As shown in Fig. 5, a good linear relationship was observed between the GFR and CLsec or CLbile* of Rho123 in ARF rats. Based on these findings, the possible mechanism underlying the systematic suppression of P-gp function in ARF rats was studied from the viewpoint of involvement of putative, endogenous P-gp-related compounds in the plasma. As reported previously [19], the transport of Rho123 in the basolateral (b)-to-apical (a) direction across Caco-2 cell monolayers was much higher than that in the opposite direction, and the functional expression of P-gp in Caco-2 cells was confirmed by measuring the inhibitory effect of verapamil, a potent P-gp inhibitor, on the efflux transport (b-to-a) of Rho123. As shown in Fig. 6, the plasma collected from ARF rats showed a greater inhibitory effect on P-gp-mediated efflux transport of Rho123 across Caco-2 cell monolayers, while plasma from control rats showed minimal effect. These results would suggest that some endogenous P-gp-related compounds are accumulated in the blood circulation in ARF rats.

5. Expression and function of P-gp in AHF rats

Marked increase in plasma GOT and GPT activities confirmed the induction of AHF. In AHF rats, the P-gp level in the liver increased by 50% as compared with control

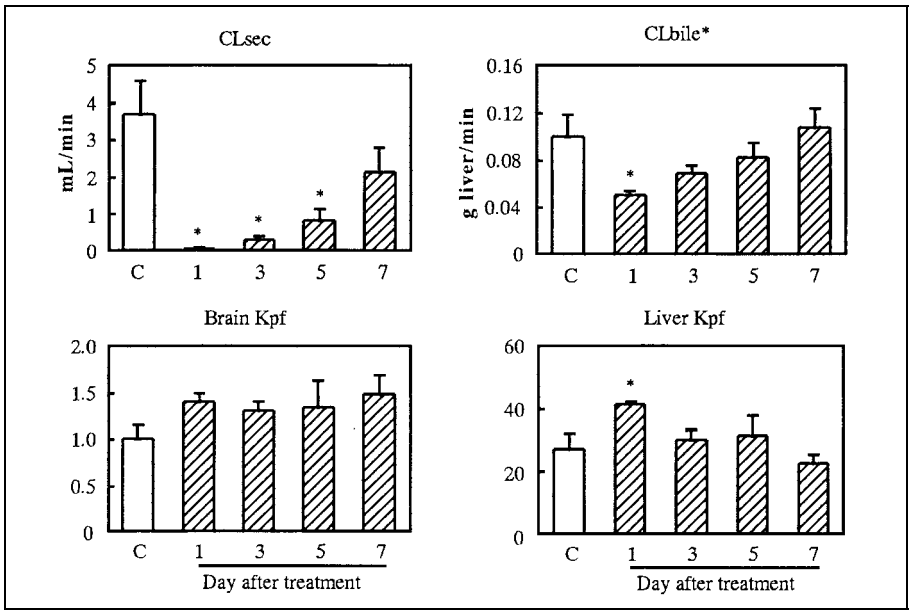

Fig. 4:
Pharmacokinetic parameters of rhodamine 123 under a steady-state plasma concentration (0.25 μM) in control (C) and acute renal failure (ARF, hatched column) rats [34]. Control rats received saline alone. ARF was induced by intramuscular injection of glycerol dissolved in saline (5 ml/kg). *: significantly different from control rats ($p < 0.05$). Each value represents the mean ± S.E.M. (n = 4)

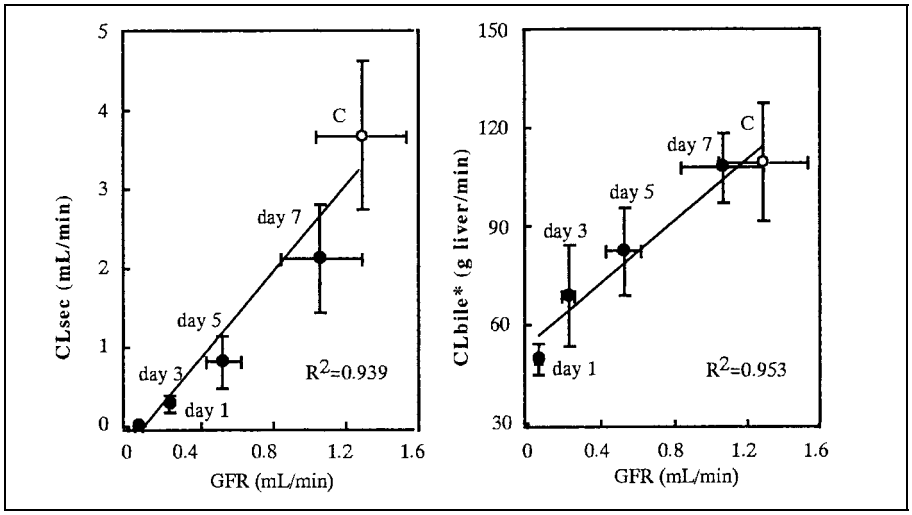

Fig. 5:
Relationship between glomerular filtration rate (GFR) and renal secretory clearance (CLsec) or normalized biliary clearance (CLbile*) of rhodamine 123 in control (○) and acute renal failure rats (●) [34]. Each value represents the mean ± S.E.M. (n = 4)

rats (1.5 ± 0.2 – fold of control rats). Also, the P-gp level in the brain showed slight increase (1.3 ± 0.1 – fold of control rats), although the level in the kidney remained unchanged (1.0 ± 0.1- fold of control rats). The ATP concentration in the liver decreased by 60% in AHF rats, whereas it was almost the same to that of control rats in the kidney (Fig. 7). With respect to the effect of CCL_4 on P-gp level, it has been reported that levels of mdr1a and mdr1b mRMA (mRNAs for P-gp proteins) were increased in rat liver 3 h after administration of CCl_4 and remained increased for the next 5 days, while mdr2 mRNA did not increase until 48 h [20–22]. In good accordance with these reports, we also observed approximately 1.5-fold increase in P-gp level in the liver of CCl_4-induced AHF rats.

As shown in Fig. 8, P-gp function in the liver, kidney and brain in AHF rats was all supressed significantly, as was in ARF rats. The suppression of P-gp function in the liver may be explained by the decrease in hepatic ATP concentrations, at least partly. However, the suppression of P-gp function in other tissues was not explained by ATP contents or by P-gp levels. As well as the case in ARF rats, the plasma obtained from AHF rats showed a significant inhibitory effect on Rho123 transport in Caco-2 cells (Fig. 9). Corticosterone is known as a potent P-gp inhibitor among endogenous steroid compounds [23, 24]. In both ARF and AHF rats, the level of corticosterone in plasma increased 2-fold of normal rats (Table). Like corticosterone, the concentration and/or composition of endogenous P-gp-related compounds would be modulated in these diseased rats.

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 [25–28], besides suppressing the kidney functions including the glomerular filtration and tubular secretion of organic anions and cations [27, 29–31]. Also, AHF induced experimentally with CCl_4 in animals accompanies the reduction of renal tubular secretion of cefpiramine, impared intestinal sugar transport, and so on [32, 33], in addition to the suppression of various hepatic functions. The mechanisms of this systemic alteration of the host defense system in ARF and AHF, however, has not yet fully understood. In the present study, we proposed the possible contribution of endogenous P-gp-related compounds in the regulation of systemic P-gp function, especially in the diseased states such as ARF and AHF, because steroid hormones such as cortisol, progesterone, aldosterone, and their metabolites including

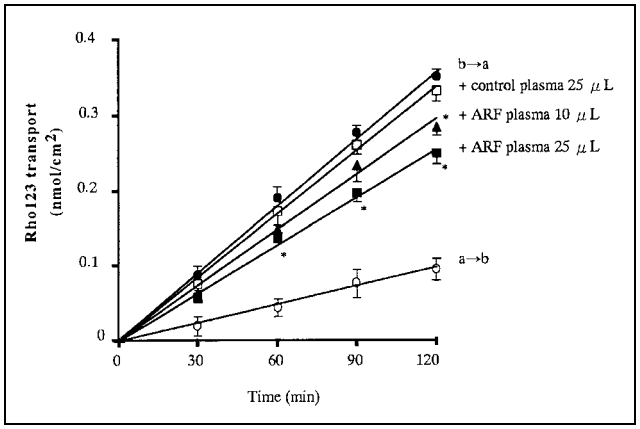

Fig. 6: Effect of plasma obtained from control and acute renal failure (ARF) rats on basal (b)-to-apical (a) transport of rhodamine 123 (Rho123) across Caco-2 cell monolayers [34]. a → b and b → a represent the transport of Rho123 in the absence of plasma. Plasma was added in the transport medium at the indicated volume per ml. Each value represents the mean ± S.E.M. (n = 4)

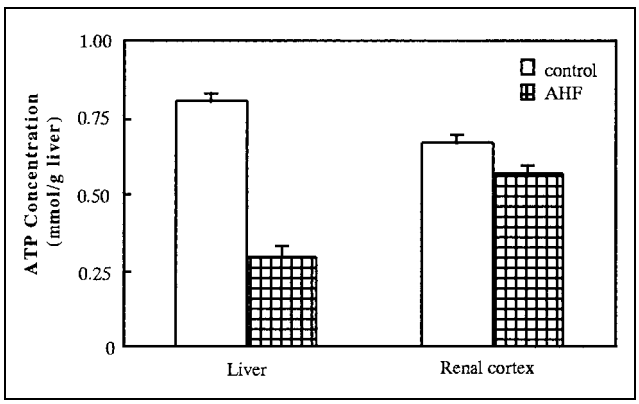

Fig. 7: ATP contents in the renal cortex of control and acute hepatic (AHF) rats [35]. AHF was induced by the injection of 50% CCl_4 intraperitoneally at a volume of 5 ml/kg. These rats were used for experiments 24 h after the CCl_4 injection. Each value represents the mean ± S.E.M. (n = 4–5)

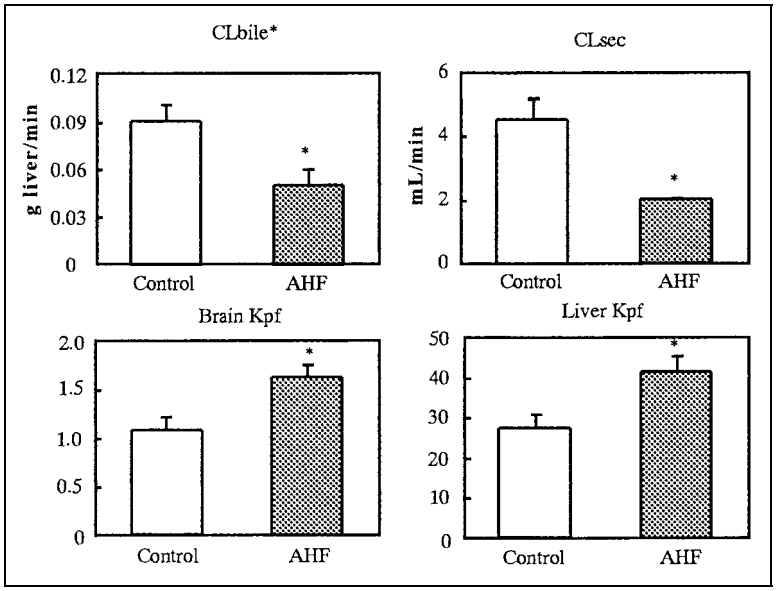

Fig. 8:
Pharmacokinetic parameters of rhodamine 123 under a steady-state plasma concentration (0.25 μM) in control and acute hepatic failure (AHF) rats [35]. *: significantly different from control rats ($p < 0.05$). Each value represents the mean ± S.E.M. (n = 4–5)

unidentified compounds exist in biological fluids as endogenous P-gp substrates/modulators.

In conclusion, we found that 1) DEX-pretreatment increased P-gp levels in the intestine 1.9-fold and P-gp-mediated intestinal exsorption clearance of Rho123 approximately 2-fold, in good agreement with the results of Western blot analysis. 2) In ARF and AHF rats, the P-gp levels in the target injury organs significantly increased. However, P-gp function was systemically suppressed, not only in the target organs, but also in other organs. The *in vivo* P-gp function in the diseased state can not be predicted merely from the expression level of P-gp. 3) The systemic suppression of *in vivo* P-gp function in diseased states may, at least partly, be due to the accumulation of endogenous P-gp-related compounds such as corticosterone in the plasma.

Table: Concentration of corticosterone in plasma of normal, acute renal failure (ARF) and acute hepatic failure (AHF) rats [35]

	Normal (μg/mol)	ARF (μg/mol)	AHF (μg/mol)
Corticosterone	0.11 ± 0.02	0.22 ± 0.01*	0.21 ± 0.01*

* $P < 0.05$, compared to normal rats. Each value represents the mean ± S.E.M. (n = 3–4)

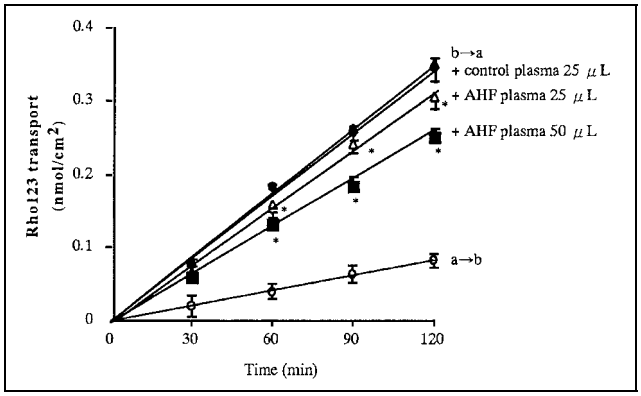

Fig. 9: Effect of plasma obtained from control and acute hepatic failure (AHF) rats to basal (b)-to-apical (a) transport of rhodamine 123 (Rho123) across Caco-2 cell monlayers [35]. a→b and b→a represent the transport of Rho123 in the absence of plasma. Plasma was added in the transport medium at the indicated volume per ml. Each value represents the mean ± S.E.M. (n = 3–4)

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References

1 Thiebaut, F.: Tsuruo, T.; Hamada, H.; Gottesman, M. M.; Pastan, I.; Willingham, M. C.; Proc. Natl. Acad. Sci. USA **84**, 7735 (1987)
2 Lee, J. S.; Paul, K.; Alvarez, M.; Hose, C.; Monks, A.; Grever, M.; Tojo, A. T.; Bates, S. E.: Mol. Pharmacol. **46**, 627 (1994)
3 Scala, S.; Akhmed, N.; Rao, U. S.; Paul, K.; Lan, L. B.; Dickstein, B.; Lee, J. S.; Elgemeic, G. H.; Stein, W. D.; Bates, S. E.: Mol. Pharmacol. **51**, 1024 (1997)
4 Kajikawa, T.; Mishima, H. K.; Murakami, T.; Takano, M.: Curr. Eye Res. **18**, 240 (1999)
5 Kajikawa, T.; Mishima, H. K.; Murakami, T.; Takano, M.: Pharm. Res. **17**, 479 (2000)
6 Hsing, S.; Gatmaintan, Z.; Arias, I. M.: Gastroenterology **102**, 879 (1992)
7 Wacher, V. J.; Wu, C. Y.; Benet, L. Z.: Mol. Carcinog. **13**, 129 (1995)
8 Schuetz, E. G.; Beck, W. T.; Schuetz, J. D.: Mol. Pharmacol. **49**, 311 (1996)
9 Sweatman, A. B.; Seshadri, R.; Israel, M.: Cancer Chemother. Pharmacol. **27**, 205 (1990)
10 Bloom, I. T. M.; Bentley, F. R.; Spain, D. A.; Garrison, R. N.: Br. J. J. Surg. **82**, 195 (1995)
11 Verveke, M.; Van de Voorde, J.; de Ridder, L.; Lameire, N.: Can. J. Physiol. Pharmacol. **73**, 1661 (1995)
12 Wang, Q.; Yang, H.; Miller, D. W.; Elmquist, W. F.: Biochem. Biophys. Res. Commun. **211**, 719 (1995)
13 Salphati, L.; Benet, L. S.: Biochem. Pharmacol. **55**, 387 (1998)
14 Higashikawa, F.; Murakami, T.; Kaneda, T.; Kato, A.; Takano, M.: J. Pharm. Pharmacol. **51**, 67 (1999)
15 Higashikawa, F.; Murakami, T.; Kaneda, T.; Takano, M.: J. Pharm. Pharmacol. **51**, 405 (1999)
16 Yumoto, R.; Murakami, T.; Sanemasa, M.; Nasu, R.; Nagai, J.; Takano, M.: Drug. Metab. Disp. **29**, 145 (2001)
17 Bornheim, L. M.: Toxicol. Appl. Pharmacol. **150**, 158 (1998)
18 Kunihara, M.; Nagai, J.; Murakami, T.; Takano, M.: J. Pharm. Pharmacol. **50**, 1161 (1998)
19 Takano, M.; Hasegawa, R.; Fukuda, T.; Yumoto, R.; Nagai, J.; Murakami, T.; Eur. J. Pharmacol. **358**, 289 (1998)
20 Nakatsukasa, T.; Silverman, J. A.; Gant, T. W.; Evarts, R. P.; Hepatotology **18**, 1202 (1993)
21 Brown, P. C.; Thorgeirsson, S. S.; Silverman, J. A.: Nucleic Acids Res. **21**, 3885 (1993)
22 Yamazaki, M.; Suzuki, H.; Sugiyama, Y.: Pharm. Res. **13**, 497 (1996)
23 Rao, U. S.; Fine, R. L.; Scarborough, G. A.: Biochem. Pharamacol. **48**, 287 (1994)
24 Orlowski, S.; Mir, L. M.; Belehradek, J.; Garrigos, M.: Biochem. J. **317**, 515 (1996)
25 Bowmer, C. J.; Yates, M. S.: Br. J. Pharmacol. **83**, 773 (1984)
26 Yeung, J. H. K.: Methods Find. Exp. Clin. Pharmacol. **13**, 23 (1991)

27 Zurovski, Y.: J. Basic Clin. Physiol. Pharmacol. **4**, 213 (1993)
28 Naora, K.; Ichikawa, N.; Hirano, H.; Iwamoto, K.: J. Pharm. Pharmacol. **51**, 609 (1999)
29 Hori, R.; Takano, M.; Okano, T.; Inui, K.: J. Pharmacol. Exp. Ther. **223** 776 (1985)
30 Lin, J. H.; Lin, T.-H.: J. Pharmacol. Exp. Ther. **246**, 896 (1988)
31 Inui, K.; Takano, M.; Maegawa, H.; Kato, M.; Hori, R.: Pharm. Res. **6**, 954 (1989)
32 Li, C.; Nakayama, S.; Kurosaki, Y.; Nakayama, T.; Kimura, T.: J. Pharmacobiodyn. **13**, 186 (1990)
33 Castilla-Cortazar, I.; Prieto, J.; Urdaneta, E.; Pascual, M.; Nunez, M.; Zudaire, E.; Garcia, M.; Quiroga, J.; Santidrian, S.: Gastroenterology **113**, 1180 (1997)
34 Huang, Z. H.; Murakami, T.; Okochi, A.; Yumoto, R.; Nagai, J.; Takano, M.: Eur. J. Pharmacol. **406**, 453 (2000)
35 Huang, Z. H.; Murakami, T.; Okochi, A.; Yumoto, R.; Nagai, J.; Takano, M.: J. Pharm. Pharmacol. **53**, 873 (2001)
36 Yumoto, R.; Murakami, T.; Nakamoto, Y.; Hasegawa, R.; Nagai, J.; Takano, M.: J. Pharmacol. Exp. Ther. **289**, 149 (1999)

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