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Renal Excretion of Rhodamine 123, a P-Glycoprotein Substrate, in Rats with Glycerol-induced Acute Renal Failure

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Abstract

To clarify renal handling of rhodamine 123, a substrate for P-glycoprotein, in normal and diseased states, in-vivo clearance studies were performed with normal rats and rats with glycerol-induced acute renal failure.

For normal rats the excretion ratio of unbound rhodamine 123-to-inulin was 3·25, indicating the presence of the renal tubular secretion of rhodamine 123. Co-administration of cyclosporin, a P-glycoprotein inhibitor, significantly reduced tubular secretion of rhodamine 123. Administration of glycerol induced both an increase in blood urea nitrogen and a reduction in the glomerular filtration rate, confirming the induction of acute renal failure. Total plasma, renal, and tubular secretory clearances of rhodamine 123 were significantly lower for rats with acute renal failure than for control rats. There was no difference between the ATP content of the renal cortex in control rats and those with acute renal failure. In addition to the decrease in renal clearance, a decrease in the biliary clearance of rhodamine 123 was also observed in rats with acute renal failure.

These results imply that rhodamine 123 is secreted via P-glycoprotein in renal tubules and that the renal secretory clearance of rhodamine 123 was reduced after acute renal failure, probably because of impairment of P-glycoprotein.

Renal dysfunction has a large influence on the pharmacokinetics of drugs and is especially critical if the major elimination pathway of the drug from the body is renal excretion involving glomerular filtration and tubular secretion. It has been reported that renal failure results in altered transport of organic anions and cations in renal tubules (Stein et al 1978; Hori et al 1985; Inui et al 1989).

P-Glycoprotein, an ATP-dependent efflux pump responsible for conferring multidrug resistance on tumour cells (Gottesman & Pastan 1993), transports a variety of structurally and pharmacologically unrelated, hydrophobic drugs. P-Glycoprotein is found not only in tumour cells but also in normal tissues such as the brush-border membranes of renal proximal tubules, the bile canalicular membrane of hepatocytes, the apical membrane of mucosal cells in the intestine, the capillary endothelial cells of the brain and testis, the adrenal gland and placental trophoblasts (Fojo et al 1987; Thiebaut et al 1987; Schinkel et al 1994). The distribution of P-glycoprotein in normal tissues and its function as multidrug efflux pump suggest that it is important in the excretion of xenobiotics and endogenous compounds into urine, bile and the intestinal lumen, and in the prevention of their accumulation in the tissues.

Several reports have shown the important function of P-glycoprotein in normal tissues such as the brain, liver, intestine and kidney (Kamimoto et al 1989; Chianale et al 1995; Schinkel et al 1995). It has been reported that in the kidney P-glycoprotein is involved in the renal tubular secretion of some drugs. Digoxin is eliminated primarily by renal excretion, including renal secretion via P-glycoprotein located on the brush-border membrane of renal proximal tubular cells (Tanigawara et al 1992). The increase in the plasma concentration of digoxin on co-administration with quinidine might, at least in part, be explained by competition for secretory transport via P-glycoprotein. Because daunomycin, cyclosporin analogue, and rapamycin derivative are also secreted via P-glycoprotein in the renal tubules (Miller 1995; Schramm et al 1995; Miller et al 1997), altered renal function might cause a significant change both in the pharmaco-

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kinetics and in the pharmacodynamics of these drugs, especially if the major elimination pathway from the body is renal excretion into urine. Although most P-glycoprotein substrates have a low therapeutic index, and so the change in the plasma concentration in diseased patients could be critical, there is little information about the pharmacokinetics of P-glycoprotein substrate in the diseased state.

In this study the pharmacokinetics, particularly renal handling, of rhodamine 123, a P-glycoprotein substrate, were investigated in normal rats and rats with glycerol-induced acute renal failure.

Materials and Methods

Materials

Rhodamine 123 (methyl *o*-(6-amino-3′-imino-3*H*-xanthen-9-yl)benzoate monohydrochloride) and glycerol were purchased from Kanto (Tokyo, Japan). Hexokinase and glucose-6-phosphate dehydrogenase were purchased from Merck (Darmstadt, Germany). Cyclosporin was kindly supplied by Sandoz (Tokyo, Japan). Other chemicals used were of the highest purity available.

Animal treatment

Acute renal failure was induced in male Sprague-Dawley rats, 210–270 g, by subcutaneous injection of saline containing 50% (v/v) glycerol ($10\,mL\,kg^{-1}$). Rats injected with the same volume of saline were used as controls. Animals were used for the experiment 24 h after injection. All animal experiments were performed in accordance with the Guide for Animal Experimentation, Hiroshima University and the Committee of Research Facilities for Laboratory Animal Sciences, Hiroshima University School of Medicine.

In-vivo clearance studies

The control rats and rats with acute renal failure were anaesthetized by intraperitoneal injection of pentobarbital ($30\,mg\,kg^{-1}$) and the femoral artery and vein were cannulated with polyethylene tubing for blood sampling and drug administration, respectively. The urinary bladder and bile duct were also cannulated for collection of urine and bile. So that a plateau level might be attained quickly, loading was introduced by intravenous bolus injection of rhodamine 123 (64 μg) with mannitol (40 mg) and inulin (10 mg), and plasma rhodamine 123 levels were maintained by constant-rate intravenous infusion of rhodamine 123 ($174\,\mu g\,h^{-1}$), mannitol ($100\,mg\,h^{-1}$) and inulin ($20\,mg\,h^{-1}$) at a rate of $2\,mL\,h^{-1}$. This dosage was determined on the basis of pharmacokinetic parameters obtained in a preliminary bolus-injection study (data not shown). Mannitol was used to maintain sufficient and constant urine flow rate. After 20 min equilibration three consecutive 20-min clearance studies were performed. Cyclosporin ($10\,mg\,kg^{-1}$) was given via the femoral vein before initiation of rhodamine 123 infusion. Blood samples were obtained at the midpoint of urine and bile collection. The plasma unbound fraction (f_u) of rhodamine 123 was determined by ultrafiltration with Ultrafree filter units (Millipore, Bedford, MA).

Determination of the ATP content of the renal cortex

The ATP content of the renal cortex of normal rats and those with acute renal failure was determined as described elsewhere (Maeda et al 1993). Briefly, after the clearance study the kidney was quickly excised and the renal cortex was prepared by use of a Stadie–Riggs microtome. The renal cortex was 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 content enzymatically with hexokinase and glucose-6-phosphate dehydrogenase (Williamson & Corkey 1969).

Analytical methods

The concentrations of rhodamine 123 in plasma, bile and urine were determined fluorimetrically (excitation, 485 nm; emission, 546 nm). Inulin concentrations in plasma and urine were determined spectrophotometrically by a modification of the method of Dische & Borenfreund (1951). The plasma activity of blood urea nitrogen, glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase was measured with commercial kits from Wako Pure Chemicals (Osaka, Japan).

Pharmacokinetic analyses

Pharmacokinetic parameters were calculated by use of standard procedures for each experimental period. The total plasma clearance (CL_t) was calculated by dividing the infusion rate by the steady state plasma concentration (C_{pss}) at the midpoint of urine and bile collection. Renal (CL_r) and biliary (CL_b) clearance were obtained by dividing the urinary and biliary excretion rates, respectively, by C_{pss}. The renal clearance of unbound rhodamine 123 ($CL_{r,f}$) was determined by dividing CL_r by f_u. Glomerular filtration rate (GFR) was assumed equal to the CL_r of inulin. The excretion ratio of unbound rhodamine (ER) was estimated as $CL_{r,f}$ divided by GFR. The net renal secretory clearance of unbound rhodamine 123 (CL_s) was calculated by

subtracting GFR from $CL_{r,f}$. Statistical analysis was performed by means of Student's *t*-test. Differences were considered to be statistically significant when *P* values were < 0.05.

Results

The pharmacokinetic parameters of rhodamine 123 for normal rats were estimated under conditions of the steady state plasma concentration (Table 1). ER for normal rats was 3·25, indicative of the tubular secretion of rhodamine 123. The effect of cyclosporin, a P-glycoprotein inhibitor, on the pharmacokinetics of rhodamine 123 was examined to determine whether active secretion of rhodamine 123 was mediated by P-glycoprotein. Cyclosporin ($10\,mg\,kg^{-1}$) administered intravenously significantly reduced the renal secretory clearance of rhodamine 123 (62·9 ± 10·3% of control, mean ± s.e.m. of results from six trials, $P < 0.05$). These results suggest that rhodamine 123 is, at least in part, secreted via P-glycoprotein in renal proximal tubular cells.

Changes in blood urea nitrogen and GFR were examined to monitor the induction of acute renal failure by administration of glycerol. As shown in Table 2, subcutaneous injection of glycerol significantly increased blood urea nitrogen and reduced GFR. These results indicate the development of acute renal failure as a result of glycerol administration.

The pharmacokinetic parameters of rhodamine 123 in rats with acute renal failure were examined (Table 1). A small but significant decrease in the free fraction of rhodamine 123 was observed for such rats. The C_{pss} in rats with acute renal failure was significantly higher (133·3%) than in normal rats and CL_t, CL_r, $CL_{r,f}$, ER and CL_s were significantly reduced, CL_s being particularly dramatically reduced to 2·4% of that in normal rats. To examine whether the decrease in the CL_s of rhodamine 123 was a result of the change in ATP concentration in renal tubules, we measured the ATP content of the renal cortex; this was unchanged in rats with acute renal failure (Table 2), suggesting that the reduction in the CL_s of rhodamine 123 in such rats could be a result of impairment of P-glycoprotein but not a consequence of any reduction in ATP content.

In normal rats a significant decrease in CL_b (30·9 ± 5·4% of control, mean ± s.e.m. of results from six trials, $P < 0.05$) was induced by treatment with cyclosporin, suggesting that biliary excretion of rhodamine 123 could, at least in part, be mediated by P-glycoprotein. CL_b was also reduced significantly in rats with acute renal failure compared with normal rats (Table 1). Because significant changes in the activity of glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase were not observed in the plasma of rats with acute renal failure (Table 2), the decrease in CL_b in rats with acute renal failure could not have been a result of hepatic failure.

Discussion

Anticancer drugs, immunosuppressive agents and digoxin, which are known substrates of P-glycoprotein, are important drugs often used clinically. Although, because most of these drugs have a low therapeutic index, it is important to monitor changes in the pharmacokinetics of P-glycoprotein substrates in diseased patients, there is little information on the subject. This report describes the renal excretion of a P-glycoprotein substrate in normal rats and the altered pharmacokinetics in rats with acute renal failure.

Rhodamine 123 has been used as a P-glycoprotein substrate in many studies. Previous reports employing different multidrug-resistant cells showed that rhodamine 123 is effectively pumped

Table 1. Pharmacokinetic parameters of rhodamine 123 under conditions of steady state plasma concentration.

	Normal	Acute renal failure
Steady state plasma concentration ($\mu g\,mL^{-1}$)	0·48 ± 0·03	0·64 ± 0·04*
Plasma unbound fraction	0·38 ± 0·01	0·31 ± 0·01*
Total plasma clearance ($mL\,min^{-1}$)	6·14 ± 0·26	4·04 ± 0·26*
Renal clearance ($mL\,min^{-1}$)	2·13 ± 0·23	0·11 ± 0·05*
Renal clearance of unbound fraction ($mL\,min^{-1}$)	5·91 ± 0·46	0·36 ± 0·17*
Excretion ratio	3·25 ± 0·26	1·37 ± 0·48*
Net renal secretory clearance of unbound fraction ($mL\,min^{-1}$)	4·09 ± 0·46	0·10 ± 0·14*
Biliary clearance ($mL\,min^{-1}$)	1·58 ± 0·11	1·01 ± 0·12*

Rats were injected subcutaneously with 50% (v/v) glycerol (acute renal failure) or saline (normal) ($10\,mL\,kg^{-1}$) and the clearance study was performed 24 h later. Data are means ± s.e.m. of results from four trials. * $P < 0.05$, significantly different from the value for normal rats.

Table 2. Biochemical parameters for normal rats and those with acute renal failure.

	Normal	Acute renal failure
Blood urea nitrogen ($mg\,dL^{-1}$)	22·4 ± 1·2	165·9 ± 18·9*
Glutamate-oxaloacetate transaminase ($int.units\,L^{-1}$)	26·5 ± 3·3	14·6 ± 4·9
Glutamate-pyruvate transaminase ($int.units\,L^{-1}$)	13·5 ± 1·2	20·1 ± 7·5
Glomerular filtration rate ($mL\,min^{-1}$)	1·82 ± 0·01	0·26 ± 0·06*
ATP content of renal cortex ($\mu mol\,(g\,cortex)^{-1}$)	1·10 ± 0·11	1·02 ± 0·01

Rats were injected subcutaneously with 50% (v/v) glycerol (acute renal failure) or saline (normal) ($10\,mL\,kg^{-1}$). Data are means ± s.e.m. of results from three to seven trials. * $P < 0·05$, significantly different from the value for normal rats.

out of these cells via P-glycoprotein (Neyfakh et al 1989; Lee et al 1994; Siegsmund et al 1994). Rhodamine 123 has also been used as a marker to examine the function of P-glycoprotein in normal tissues such as brain, intestine, liver and kidney (Hsing et al 1992; Chianale et al 1995; Hirsch-Ernst et al 1995; Wang et al 1995; Ernest & Bello-Reuss 1996).

In this study we monitored the decrease in the renal secretion and biliary excretion of rhodamine 123 as a result of co-administration of cyclosporin, indicating the involvement of P-glycoprotein in the in-vivo transport of rhodamine 123 in the kidney and liver. Recently, Masereeuw et al (1997) reported that rhodamine 123 is secreted by the isolated perfused rat kidney. They suggested that active renal secretion is mediated mainly by the organic-cation-transport system, because they did not observe any inhibitory effect of cyclosporin. The discrepancy between their results and ours remains to be elucidated, although it might be a result of the different methods (isolated perfused kidney experiment and in-vivo clearance experiment). Our results might be supported by reports that P-glycoprotein is expressed in the brush-border of renal proximal tubules (Thiebaut et al 1987) and that renal P-glycoprotein is involved in the tubular secretion of other substrates, such as daunomycin, cyclosporin analogue, and rapamycin derivative (Miller 1995; Schramm et al 1995; Miller et al 1997).

It is well known that acute renal failure causes alteration of organic anion and cation transport in renal proximal tubular cells (Stein et al 1978; Hori et al 1985; Inui et al 1989). We also observed a significant reduction in the renal secretion of phenolsulphonaphthalein, an organic anion, in rats with acute renal failure (data not shown). The renal secretion of rhodamine 123, probably via P-glycoprotein, by rats with acute renal failure was significantly lower than by normal rats. We also investigated whether the reduction in the tubular secretion of rhodamine 123 was a result of the change in the ATP content of the renal cortex, because P-glycoprotein needs ATP hydrolysis as a driving force to pump out the substrate. Because the ATP content of the renal cortex of rats with acute renal failure was no lower than that of normal rats, the reduction in the tubular secretion of rhodamine 123 might be because of a drop in the number or availability of P-glycoprotein molecules, but not because of a decrease in ATP level. Such an assumption is similar to those reported after studies of the transport of organic anions and cations using renal plasma membrane vesicles isolated from rats with acute renal failure (Hori et al 1985; Inui et al 1989).

P-Glycoprotein is also found in canalicular membranes of hepatocytes, and is involved in the biliary excretion of P-glycoprotein substrates (Thiebaut et al 1987; Kamimoto et al 1989; Watanabe et al 1992). We observed a decrease in the biliary excretion of rhodamine 123 in cyclosporin-treated rats and the biliary clearance of rhodamine 123 was also lower in rats with acute renal failure than in normal rats. The decrease in the biliary clearance of rhodamine 123 in rats with acute renal failure would not be a result of hepatic dysfunction because there was little change in the activity of glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase in the plasma of rats with acute renal failure. The reason for reduced biliary excretion in rats with acute renal failure is not clear at present. Several reports suggest the presence of endogenous P-glycoprotein substrate(s) in rat urine and in plasma from man (Ichikawa et al 1990; Charuk & Reithmeier 1992; Charuk et al 1994) and this increase in putative endogenous P-glycoprotein substrate(s) in the plasma of rats with acute renal failure might be involved in the modulation of rhodamine 123 handling in the liver.

In conclusion, this study indicates that rhodamine 123 is secreted via P-glycoprotein in the renal tubules and that the renal secretory clearance of rhodamine 123 was reduced in rats with acute renal failure, probably because of impairment of

P-glycoprotein. These findings could facilitate understanding of the altered pharmacokinetics of P-glycoprotein substrates in acute renal failure.

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