Transport of Furosemide into the Intestinal Lumen and the Lack of Effect of Gastrointestinal Dialysis by Charcoal in Rats with Acute Renal Failure

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The characteristics of exsorption and/or excretion of furosemide into the small intestinal lumen in rats with acute renal failure (ARF rat) were investigated by an *in situ* single-pass perfusion technique. The amount of furosemide, which was exsorbed into the intestinal lumen after an intravenous administration of the drug to rats was only very slight. The exsorption rate of the drug was significantly increased in ARF rats as compared with normal rats. The average amount of the drug exsorbed into the perfusate in normal rats was 0.83% of dose, whereas that in ARF rats was 1.83% of dose. The amounts of furosemide excreted into the bile in normal rats and ARF rats were 1.53% and 2.64%, respectively. The increased exsorption of furosemide in ARF rats appeared to be due primarily to the decreased binding of the drug to the serum protein, because only the unbound drug permeates through the intestinal membrane into the gastrointestinal (g.i.) tract, and, to some extent, to the increased nonrenal excretion caused by poor renal excretion. Oral activated charcoal had little effect on the serum furosemide levels after intravenous administration of the drug at a dose of 10 mg/kg in ARF rats. The lack of effect of activated charcoal on the elimination of the drug may be due to the small amount of the drug excreted into the g.i. tract.

Keywords — furosemide; exsorption rate; intravenous administration; renal failure; biliary excretion; activated charcoal; intestinal dialysis; gastrointestinal tract; nonrenal clearance; serum concentration

Introduction

We have previously shown in studies on the transport (exsorption) of drugs from blood to the gastrointestinal (g.i.) tract that clearance of intravenously administered theophylline,¹⁾ phenobarbital¹⁾ and phenytoin²⁾ was accelerated by orally administered charcoal in rats. The mechanism was confirmed as the transport of the drug and its metabolites into the g.i. tract and their adsorption by charcoal. Accordingly, when the removal of an excess drug from systemic circulation by g.i. dialysis³⁾ was necessary, the process of the exsorption across the intestinal membrane and the excretion *via* the bile duct of the drug plays an important role.

Several reports have shown that subjects with lower clearance of the drug have a larger increase in clearance when treated with activated charcoal.^{4–6)} For example, Park *et al.*⁴⁾ have reported that activated charcoal had a relatively small effect in increasing digoxin elimination in normal subjects who had short digoxin half-lives, but a significant effect in increasing digoxin elimination in the renal failure subject.

Radomski *et al.*⁵⁾ have also reported that the effect of activated charcoal in decreasing the serum theophylline half-life was much greater in subjects with a long serum theophylline half-life in patients such as those suffering from hepatic cirrhosis. As a possible explanation, the above investigators suggested that the total clearance of a drug, during treatment with activated charcoal, is the sum of normal endogenous clearance and clearance through the g.i. tract by adsorption with charcoal and that the percentage change in total body drug clearance caused by the charcoal increases as the endogenous drug clearance (by metabolism or renal elimination) decreases, provided that the activated charcoal produces a constant intestinal drug clearance. In addition, we expected that the increase in the amount eliminated through nonrenal routes (*i.e.*, excretion into the bile and exsorption from blood into the g.i. lumen) in subjects with renal failure exhibiting lower clearance of the drug may contribute to the effect of charcoal.

There has been little information on the transport of the drug from blood into the g.i.

tract under conditions of disease. The present study, therefore, was designed to further investigate the characteristics of exsorption and/or excretion of furosemide into the g.i. tract in rats with acute renal failure (ARF rat) and to evaluate whether the exsorbed drug can be removed by adsorption to orally administered activated charcoal. Furosemide was used as a representative drug because the drug is eliminated almost equally by renal and nonrenal routes and the vast majority of the drug excreted in the urine is in the form of unchanged furosemide.^{7,8)}

Materials and Methods

Materials—Furosemide was kindly supplied by Hoechst Japan Ltd., Tokyo. Lasix® injection (Hoechst Japan Ltd., Tokyo) was used for intravenous injection of furosemide. β -Glucuronidase was a product of Sigma Chemical Co., USA. Activated charcoal was a product of Inuhinode Seiyaku Co., Osaka and the particle size used in this study was less than 62 μ m (250 mesh). All other chemicals used were of analytical grade.

Animals—Male Wistar rats were used for *in situ* (360–400 g) and *in vivo* (260–300 g) studies. Acute renal failure (ARF) in rats was induced by a subcutaneous injection of glycerol (5 ml/kg) and the experiments were performed 48 h after the treatment. Total protein, albumin, blood urea nitrogen (BUN) and serum creatinine concentration were determined after the termination of the study by RABA super kit® (Chugai Co., Ltd., Tokyo). The biochemical data obtained in normal and ARF rats are shown in Table I.

Exsorption Study—Intestinal exsorption

experiments were performed by an *in situ* single-pass perfusion technique reported previously.⁹⁾ The rats were anesthetized by an intraperitoneal injection of ethyl carbamate (1.2 g/kg). The small intestine was exposed by a midline abdominal incision. The upper duodenum and the ileocecal junction were cannulated with polyethylene tubing and the entire small intestine was washed with saline maintained at 37 °C. Isotonic 0.1 M phosphate buffer solution, pH 6.0, was maintained at 37 °C and perfused at the rate of approximately 1.3 ml/min from the duodenum through the small intestine to the ileocecal junction using a perfusion pump (GM-24, Tokyo Rikakikai). Furosemide (10 mg/kg) was injected for a duration of about 1 min into the right femoral vein. After injection, perfusates were collected every 15 min from the ileal outflow into a 25 ml volumetric flask and the perfusates diluted with buffer solution to 25 ml before assay. The exsorption rate was calculated as the amount exsorbed into the intestinal lumen per min. The bile was collected during the experimental period from cannula introduced into the common bile duct. Blood samples to determine serum drug concentrations were taken from the cannula introduced into the left femoral vein at the middle of the perfusate collection period (7.5, 22.5, 37.5, 52.5, 67.5, 82.5, 97.5, and 112.5 min).

In Vivo Study—The rats were fasted overnight with free access to water. Under light anesthesia with ether, furosemide at a dose of 10 mg/kg was administered intravenously *via* the caudal vein. In the case of the treatment with activated charcoal, the activated charcoal suspension in water (150 mg/ml) was administered orally to ARF rats with an initial dose of

TABLE I. Biochemical Data in Normal and ARF Rats

		Normal rats	ARF rats
Total protein	(g/dl)	7.93 $\pm$ 0.55	5.65 $\pm$ 0.35 ^{a)}
Albumin	(g/dl)	4.13 $\pm$ 0.30	2.87 $\pm$ 0.15 ^{a)}
Serum Cr.	(mg/dl)	1.08 $\pm$ 0.026	4.30 $\pm$ 0.91 ^{b)}
BUN	(mg/dl)	26.2 $\pm$ 1.86	40.2 $\pm$ 4.75 ^{b)}

Each value represents the mean $\pm$ S.E.M. of 4 rats. ^{a)} $p < 0.01$. ^{b)} $p < 0.05$.

300 mg at time zero and additional doses of 150 mg each at, 1, 2, 3, and 4 h after the intravenous (i. v.) administration of furosemide. In the case of the control experiment, only 2 ml water at time zero was administered to rats. Blood samples (200 μ l) were collected periodically from the tail. The blood was centrifuged and the serum obtained was stored in the dark at -20°C until assay.

Protein Binding Study—Binding of furosemide to serum protein was determined by an ultrafiltration technique using Centrifree MPS-3 (Amicon Co., Lexington, Mass.) as reported previously.²⁾ Blood samples were collected by cardiocentesis after the termination of the *in situ* exsorption study.

Analytical Method—Furosemide in the serum was determined by high pressure liquid chromatography (HPLC) as previously described with minor modifications.¹⁰⁾ Briefly, to 50 μ l of serum were added 200 μ l methanol containing salicylic acid as an internal standard. The mixture was shaken on a Vortex mixer for 30 s and then centrifuged for 10 min. After filtration by a Millipore filter (0.45 μ m), a 20 μ l portion of the supernatant fluid was injected into the HPLC. Bile juice was incubated overnight in acetate buffer solution, pH 5.0, containing 2000 units of β -glucuronidase at 37°C . After hydrolysis, 0.2 ml portions of the bile samples were extracted with 5 ml of ether containing an internal standard after addition of 1 ml of 4 N HCl solution. The mixture was shaken for 15 min and then centrifuged for 10 min. The organic layer was transferred to a clean tube and evaporated under reduced pressure at room temperature. After evaporation, 100 μ l of methanol were added to the residue and mixed on a Vortex mixer. Twenty microliters of the portion were injected into the HPLC. Furosemide in the perfusate was analyzed after extraction with ether in the same manner as that with bile. Separation was performed with a TSK-Gel 80 column (5 μ m in 4.6 i.d. $\times$ 250 mm, Toyosoda). The mobile phase consisted of methanol, 0.02 M phosphoric acid and acetic acid (50 : 48 : 2). At a flow rate of 1.0 ml/min, the eluate was monitored with an excitation wavelength of 268 nm and an emission wavelength of 410 nm. The

overall assay procedure was performed under dark condition to minimize photochemical degradation of furosemide.¹¹⁾

Pharmacokinetic Analysis—Moment analysis was used as a model-independent manner of estimating the time course of serum levels of furosemide.¹²⁾ The terminal elimination rate constant (β) was determined by least-squares linear regression of the logarithm of the serum concentration-time profiles. The elimination half-life ($t_{1/2\beta}$) was determined from the relationship: $t_{1/2\beta} = 0.693/\beta$. The area under the serum concentration-time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity. Total body clearance (Cl_{tot}) was determined by: $Cl_{\text{tot}} = \text{Dose}/AUC$. The mean residence time (MRT) in the systemic circulation was calculated by: $MRT = AUMC/AUC$, where $AUMC$ is the area under the first moment curve. The steady-state volume of distribution (V_{dss}) was calculated by: $V_{\text{dss}} = \text{Dose} \cdot MRT/AUC$. The unpaired t test was used to assess the effect of charcoal treatment on pharmacokinetic parameters. The intestinal and biliary clearance values of furosemide were calculated by dividing the amount of the drug excreted into the perfusate or into the bile in 2 h by AUC from 0 to 2 h, respectively.

Results

Transport of Furosemide into the Intestinal Lumen

Figure 1 shows the exsorption patterns of furosemide from blood into the perfusate across the small intestinal mucosa following i.v. administration of furosemide to ARF and normal rats at a dose of 10 mg/kg. It was observed that furosemide was exsorbed from blood into the small intestinal lumen and its exsorption rate was decreased as the concentration of the drug in the serum was decreased. Moreover, the exsorption rate of the drug into the perfusate in ARF rats was significantly larger than that in normal rats.

The amounts of furosemide transported into the perfusate and the bile juice in 120 min are shown in Fig. 2. It was shown that only a small amount of furosemide (average of 0.83% of the dose) was exsorbed into the intestinal lumen

Fig. 1. The Concentrations of Furosemide in the Serum and the Exsorption Rate of the Drug into the Perfusate after i.v. Administration of Furosemide at the Dose of 10 mg/kg to Rats during *in Situ* Single-Pass Perfusion

Perfusate was composed of isotonic phosphate buffer at pH 6.0. Each point represents the mean $\pm$ S.E.M. of 4 rats a) $p < 0.05$.

Key: ○, □, normal rats; ●, ■, ARF rats

Fig. 2. Amounts of Furosemide Exsorbed and Excreted into the Perfusate and Bile Juice in Rats in 120 min during *in Situ* Single-Pass Perfusion

Each bar represents the mean $\pm$ S.E.M. of 4 rats. a) $p < 0.05$.

after i.v. administration of the drug to normal rats. On the other hand, the exsorption of the drug (average of 1.83% of the dose) into the intestinal lumen in ARF rats was greater than that in normal rats. The amount of furosemide excreted into the bile was larger than that exsorbed into the perfusate. Moreover, the amount of the drug excreted into the bile in ARF rats tended to be greater than that in normal rats. The average amounts of the drug excreted into the bile in normal rats and ARF rats were 1.53% and 2.64%, respectively.

Effect of Activated Charcoal on Furosemide Clearance

The elimination of furosemide following i.v. administration to ARF rats was apparently prolonged as compared with normal rats. The serum half-life of the drug in normal rats was 0.76 h,

Fig. 3. Serum Furosemide Levels after i.v. Administration of the Drug at the Dose of 10 mg/kg to Rats with or without Treatment with Activated Charcoal

Each point represents the mean $\pm$ S.E.M. of 5 rats.

Key: $\circ$, normal rats without charcoal; $\bullet$, ARF rats without charcoal; $\bullet$, ARF rats with charcoal.

whereas that in ARF rats was 1.27 h in the present study. Thus, the effect of activated charcoal on pharmacokinetic behavior of furosemide was investigated in ARF rats which exhibited lower

clearance of the drug. Figure 3 shows the time course of furosemide levels after i.v. administration of the drug (10 mg/kg) to ARF rats with or without oral activated charcoal treatment. As shown in Fig. 3, the treatment with oral activated charcoal had little effect on the serum furosemide levels. Pharmacokinetic parameters of furosemide (10 mg/kg) after i.v. administration of the drug to ARF rats with or without oral activated charcoal treatment are shown in Table II. There was no significant difference in AUC , MRT , Cl , $t_{1/2\beta}$, and V_{dss} between both treatments in ARF rats.

Discussion

In ARF rats, the exsorption of furosemide from blood into the intestinal lumen was apparently increased and the excretion of the drug into the bile juice also tended to increase as compared with normal rats. These results suggested that the intestinal lumen exhibited an enhanced exsorption/excretion function for the drug in the case of renal failure. Since the total body clearance of furosemide is the sum of the clearance through the renal and nonrenal routes, the decreased clearance through the renal route resulted in the decreased total body clearance in ARF rats. Although renal clearance of the drug was not measured, the intestinal and biliary clearance in normal rats were 1.72 and 3.05 ml/h/kg, respectively, while those in ARF rats were 2.93 and 4.35 ml/h/kg, respectively. The ratio of nonrenal clearance/total body clearance was greater in ARF rats compared to that in normal rats. Thus, an appreciable fraction of furosemide may be eliminated by nonrenal

TABLE II. Pharmacokinetic Parameters of Furosemide after an i.v. Administration to Rats with or without Treatment with Activated Charcoal

	$AUC_{0-\infty}$ (h $\cdot$ μ g/ml)	MRT (h)	Cl (l/h/kg)	$t_{1/2\beta}$ (h)	V_{dss} (l/kg)
Normal rats without charcoal	37.5 ± 7.97	1.11 ± 0.065	0.306 ± 0.048	0.762 ± 0.080	0.333 ± 0.049
Renal failure rats with charcoal	97.8 ± 10.38	2.03 ± 0.19	0.107 ± 0.011	1.29 ± 0.15	0.210 ± 0.014
Renal failure rats without charcoal	121.6 ± 47.6	1.86 ± 0.18	0.119 ± 0.026	1.27 ± 0.14	0.221 ± 0.045

Each value represents the mean $\pm$ S.E.M. of 5 rats.

routes which includes exsorption into the g.i. lumen and excretion into the bile juice due to the lower endogenous clearance produced by impaired renal function. A similar observation which supports our results has been reported for digoxin by Aronson¹³⁾ who showed that fecal excretion of digoxin increased in patients with renal failure. The mechanism involved for increased nonrenal excretion of digoxin has not been established.

In general, permeability of drugs through the biomembrane varies enormously dependant on factors such as the extent of binding to serum proteins, distribution volume, lipophilicity and molecular size of the drug. The extent of binding of the drug to serum proteins is an important factor in the permeation since it is well known that only the unbound or free drug can permeate through capillary walls to the g.i. tract. It has been previously reported that binding of furosemide to serum protein was decreased in renal failure.^{14,15)} The factors for the decrease in drug protein binding in renal failure have been proposed to be due to hypoalbuminemia, structural change of the binding protein and accumulation of endogeneous inhibitors of drug binding.^{15,16)} In fact, the percentage of furosemide bound was significantly decreased from 99.2% in normal rats to 98.1% in ARF rats in the current study ($p < 0.02$, unpaired t test). Accordingly, the decreased binding of furosemide to serum proteins in ARF rats may contribute to the increased exsorption of the drug into the g.i. lumen. It is especially noteworthy that the free fraction of the drug was increased more than two-fold in ARF rats as compared with that in normal rats. An increase in the free fraction of the diffusible drug should lead to an increase in the amount of the drug in the distribution organs including the g.i. lumen.

It is known that most drugs are exsorbed into the gut lumen due to concentration gradients between blood (a region of higher drug concentration) and the g.i. lumen (a region of lower drug concentration) by passive diffusion. Therefore, the higher the serum concentrations of furosemide, the greater exsorption from blood into the g.i. lumen can be expected. The delayed elimination of serum furosemide observed in ARF rats

may further promote the exsorption into the g.i. lumen due to the poor renal excretion.

Furosemide is partly eliminated by renal clearance (mainly *via* tubular secretion) of the unchanged drug and partly by nonrenal routes.^{7,8)} Since renal excretion of furosemide can be inhibited by pretreatment with probenecid, furosemide has been shown to exhibit active secretion in the renal pathway of elimination.¹⁷⁾ Hence, it may be considered that active secretion into the g.i. lumen, in which nonrenal clearance may constitute drug secretion, may take place. Branch¹⁸⁾ has reported that pretreatment of normal subjects with probenecid reduced both renal clearance and nonrenal clearance and that nonrenal clearance can be active transport into the gut since renal tubule can have a transport mechanism in common with the intestinal mucosa. On the contrary, since Valentine *et al.*¹⁹⁾ have reported that there was no change in the intestinal clearance of furosemide after administration of probenecid, which is a competitive inhibitor of organic acid transport across the renal proximal tubule, choroid plexus and biliary tract, active secretion into the intestinal lumen is not likely to occur. In the present study, the smaller amount of furosemide was exsorbed into the intestinal lumen in spite of its higher serum levels. Since it was observed that furosemide was highly bound to serum protein, the drug appears to be transported into the intestinal lumen by passive diffusion with the concentration gradient of an unbound drug.

Thus, the increase of furosemide transported into the intestinal lumen induced by renal failure may enhance the intestinal clearance of the drug by oral administration of activated charcoal. It was expected that orally administered activated charcoal would prevent reabsorption of the drug exsorbed into the g.i. tract by adsorption to the charcoal and promote exsorption due to a greater concentration gradient between blood and the fluid in the g.i. lumen. However, there were no significant differences in serum furosemide levels between the activated charcoal treatment and control in ARF rats.

The lack of the effect of activated charcoal on elimination of the drug may be explained by the small amount of the drug excreted into the g.i.

tract. We have previously shown that the exsorption of theophylline,⁹⁾ phenobarbital²⁰⁾ and phenytoin²⁾ into the perfusate was about 12%, 6.5% and 1.1% of 10 mg/kg doses, respectively. The excretion of these drugs into the bile juice was less than 1.0% of 10 mg/kg dose in 120 min and the total body clearance values of theophylline and phenobarbital were increased by oral activated charcoal. However, that of phenytoin was little affected because of its poor exsorption.

Since furosemide is highly bound to serum protein, there is only a small amount of unbound fraction of the drug available for removal. The exsorption of the drug into the perfusate was slight, 1.83% of dose in ARF rats. It is suggested that exsorption of furosemide from blood into the g.i. tract plays a small role in the transport of the drug. However, the amount (2.6% in ARF rats) of furosemide excreted into the bile was more than those of theophylline (0.17%),⁹⁾ phenobarbital (0.45%)¹⁹⁾ and phenytoin (0.10%).²⁾ Furosemide is excreted mainly as unchanged furosemide and as a glucuronide conjugate in urine and bile.²¹⁾ It has been shown that glucuronide conjugates are not well absorbed from the intestine but are hydrolyzed by bacterial β -glucuronidase to yield their more readily absorbable forms.²²⁾ The deconjugated furosemide produced by the enteric flora may be reabsorbed into the systemic circulation from the g.i. tract. Thus, biliary excretion may play a major role in the transport of the drug into the g.i. lumen and the entero-hepatic circulation of the drug.

In conclusion, exsorption of furosemide from blood into the g.i. tract was confirmed to be increased in ARF rats, but oral administration of activated charcoal had little effect on the pharmacokinetic behaviors of the drug. However, it may be considered that g.i. dialysis may play an important role for removal of toxic drugs from blood because of the very large surface area of the g.i. lumen, provided that the drug is transported in considerably large amounts into the g.i. lumen (e.g. excretion into the g.i. lumen, a nonrenal route, would be increased in patients with a long endogenous serum half-life of drug caused by renal and hepatic failures).

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