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Liver and Gastrointestinal First-pass Effects of Azosemide in Rats

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Abstract

Since considerable first-pass effects of azosemide have been reported after oral administration of the drug to rats and man, first-pass effects of azosemide were evaluated after intravenous, intraportal and oral administration, and intraduodenal instillation of the drug, to rats.

The total body clearances of azosemide after intravenous (5 mg kg^{-1}) and intraportal (5 and 10 mg kg^{-1}) administration of the drug to rats were considerably smaller than the cardiac output of rats suggesting that the lung or heart first-pass effect (or both) of azosemide after oral administration of the drug to rats was negligible. The total area under the plasma concentration-time curve from time zero to time infinity (AUC) after intraportal administration (5 mg kg^{-1}) of the drug was significantly lower than that after intravenous administration (5 mg kg^{-1}) of the drug (1000 vs 1270 μg min mL^{-1}) suggesting that the liver first-pass effect of azosemide was approximately 20% in rats. The AUC from time 0 to 8 h ($AUC_{0-8\ h}$) after oral administration (5 mg kg^{-1}) of the drug was considerably smaller than that after intraportal administration (5 mg kg^{-1}) of the drug (27·1 vs 1580 μg min mL^{-1}) suggesting that there are considerable gastrointestinal first-pass effects of azosemide after oral administration of azosemide to rats. Although the $AUC_{0-8\ h}$ after oral administration (5 mg kg^{-1}) of azosemide was approximately 15% lower than that after intraduodenal instillation (5 mg kg^{-1}) of the drug (27·1 vs 32·0 μg min mL^{-1}), the difference was not significant, suggesting that the gastric first-pass effect of azosemide was not considerable in rats. Azosemide was stable in human gastric juices and pH solutions ranging from 2 to 13. Almost complete absorption of azosemide from whole gastrointestinal tract was observed after oral administration of the drug to rats.

The above data indicated that most of the orally administered azosemide disappeared (mainly due to metabolism) following intestinal first-pass in rats.

Azosemide (5-(4-chloro-5-sulphamoyl-2-thenylaminophenyl)-tetrazole) is a loop diuretic (Fig. 1) closely resembling furosemide in its diuretic action (Krück et al 1978). On a molecular-weight basis, its diuretic potency in man was reported to be higher than that of furosemide after intravenous administration (Brater et al 1983), but equipotent after oral administration (Brater et al 1979). This could be due to high first-pass effects of azosemide after oral administration (Krück et al 1978). Thus, the extent of absolute oral bioavailability (F) was low in man, the values ranging from 10 to 19% (Brater et al 1983). However, the exact metabolizing organ(s) for azosemide was not thoroughly studied. It has been reported (Lee & Lee 1995) from our laboratory that all rat tissues had metabolic activity for azosemide with considerable metabolic activity in liver, lung, heart, kidney, and stomach based on in-vitro tissue homogenate studies. Eleven metabolites including M1, (5-(2-amino-4-chloro-5-sulphamoylphenyl)-tetrazole, Fig. 1), a main metabolite of azosemide, and glucuronide conjugates of both azosemide and M1, were identified after intravenous administration of [^{14}C]azosemide to rats (Asano et al 1984).

Azosemide was found to be absorbed from various gastrointestinal segments of rats, such as stomach, duodenum, jejunum, ileum and large intestine, based on in-situ rat gastrointestinal tract closed-loop study (Lee & Lee 1996). In rat studies (Lee & Lee 1996), percentages of oral dose of azosemide, 5 (n = 7), 10 (n = 9), 20 (n = 11) and 30 (n = 11) mg kg^{-1}, recovered from whole gastrointestinal tract at 24 h as unchanged drug were 4·65 ± 2·61, 8·91 ± 7·09, 11·4 ± 8·83 and 21·4 ± 3·84%, respectively, and the the corresponding values at 8 h after intravenous administration of the drug (mainly due to biliary or gastrointestinal excretion, or both) were 5·97 ± 1·34 (n = 12), 4·78 ± 0·914 (n = 8), 4·68 ± 2·53, (n = 12) and 13·4 ± 4·3% (n = 9). Azosemide was reported (Lee & Lee 1995) to be stable in solutions with pH ranging from 2 to 13 for up to 48 h incubation and in human gastric juices (pH ranging from 1·5 to 8) for up to 4 h incubation. The above data indicated that azosemide could be absorbed essentially completely (especially at low oral doses) from gastrointestinal tract after oral administration of the drug to rats. The areas under the plasma concentration-time curve from time zero to time infinity (AUC) of azosemide after intravenous administration of the drug, 5 (n = 12), 10 (n = 8), 20 (n = 12) and 30 (n = 9) mg kg^{-1}, to rats were 718 ± 172, 2520 ± 378, 6270 ± 1420 and 13700 ± 3080 μg min mL^{-1}, respectively, and the corresponding values up to the last measured time (AUC_{0-t}) after oral administration were 36·2 ± 47·6 (n = 7), 215 ± 90·2 (n = 9), 546 ± 249 (n = 11) and 1160 ± 391 (n = 11) μg min mL^{-1} (Lee & Lee 1996). The above data indicated that there was a considerable first-pass effect of azosemide after oral administration of the drug to rats. Therefore, in the present study, rats were used as an animal model to find out the first-pass organs or tissues involved with azosemide.

The purpose of this study was to find out the first-pass organs or tissues for azosemide after intraduodenal instillation and intravenous, intraportal and oral administration of the drug, 5 mg kg^{-1}, to rats.

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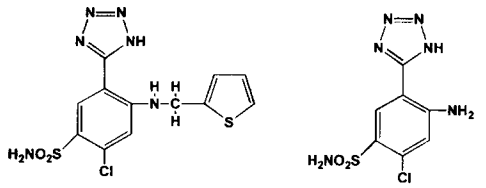

FIG. 1. Chemical structures of azosemide and its metabolite, M1.

Materials and Methods

Chemicals

Azosemide and M1 were donated by Sam Jin Pharmaceutical Company (Seoul, Korea) and Boehringer Manheim GmbH (Manheim, Germany), respectively. β-Glucuronidase was from Sigma (St. Louis, MO). Heparin was a gift from Choong Wae Pharmaceutical Company (Seoul, Korea). Other chemicals were of reagent grade or HPLC grade and used without further purification.

Animals

Male Sprague–Dawley rats, weighing 260–340 g, were purchased from Charles River Company (Atsugi, Japan). The animals were housed in a clean room (College of Pharmacy, Seoul National University, Seoul, Korea) and had free access to food (Samyang Company, Seoul, Korea) and water.

Measurement of liver first-pass effect

The jugular vein and the carotid artery of each rat were catheterized with polyethylene tubing (Clay Adams, Parsippany, NJ) under light ether anaesthesia. Both cannulae were exteriorized to the dorsal side of the neck and terminated with a long Silastic tubing (Dow Corning, Midland, MI). At the same time, the portal vein was also cannulated by the modified Suzuki method (Xu et al 1992). After midline abdominal incision, the middle portion of the portal vein was isolated, and the tapered end of a 23-g needle, bent at 60° angle, was inserted into the pyloric vein, the tributary flowing directly into the hepatic portal vein (to minimize the impairment of blood flow in the portal vein). Bleeding was prevented by applying epoxy glue (Krazy Glue, Krazy Glue Inc., Itasca, IL). A 5-cm piece of the Silastic tubing was attached to the other end of the needle which linked with the dorsal side cannula of the neck. All of the three Silastic tubings were covered with a wire to allow free movement of the rat. The exposed areas, the neck and abdomen, were closed using surgical suture. Each animal was kept individually in a metabolic cage (Daejong Scientific Company, Seoul, Korea) for 2–3 h to recover from anaesthesia.

Azosemide powder (15 mg) was dissolved in NaOH solution (0·1 M; 1 mL), filtered through a 0·45-μm filter, and diluted with 0·9% NaCl injectable solution before use; the final pH was approximately 10. By means of this solution, azosemide was infused in 60 min with the assistance of an infusion pump (Harvard Instrument, Model 2400-006, Southnatick, MA) at a dose of 5 mg kg^{-1} (n = 6) for intravenous administration and 5 (n = 5) and 10 (n = 4) mg kg^{-1} for intraportal administration. The total infusion volume was approximately 1·0 mL. At the same time, the same volume (1·0 mL) of 0·9% NaCl injectable solution was also infused in 60 min via the portal vein for intravenous study and via the jugular vein for intraportal study. Blood samples (0·12 mL) were collected via the carotid artery at appropriate time intervals and centrifuged immediately. A 50-mL sample of each of the plasma samples was stored in the freezer until HPLC analysis for azosemide (Lee & Lee 1994). Heparinized 0·9% NaCl injectable solution (0·3 mL; 20 units mL^{-1}), was used to flush the cannula immediately after each blood sampling to prevent blood clotting. It has been reported (Park et al in press) from our laboratory that the pharmacodynamic effect of intravenous azosemide was dependent on the rate and composition of fluid replacement. Therefore, in the present rat studies, loss of fluid and electrolytes in urine induced by azosemide was replaced immediately volume-for-volume by intravenous infusion of lactated Ringer's solution (Dai-Han Pharmaceutical Company, Seoul, Korea) via the carotid artery for up to 8 h after the dosing. At the end of 8 h, each rat was exsanguinated and killed by cervical dislocation. At the same time, the metabolic cage was rinsed with 10 mL of distilled water. The washings were combined with 8-h urine and the urinary bladder was cut and washed into the combined urine. After measuring the exact volume of each urine output and the combined urine, an aliquot (0·5 mL) of the combined urine sample was stored in the freezer until analysis for azosemide and M1 (Lee & Lee 1994), and also for Na^+, K^+ and Cl^-. At the same time a sample (0·5 mL) of the combined 8-h urine was incubated for 24 h with Sørensen phosphate buffer (pH 7·4; 1 mL) containing 10 000 units of β-glucuronidase in a water-bath shaker (50 oscillations min^{-1} at 37°C for measurement of glucuronide formation of both azosemide and M1). At the same time, the entire gastrointestinal tract (including its contents and faeces) was removed, transferred into a beaker containing NaOH (0·01 M; 50 mL; to facilitate the extraction of azosemide), and cut into small pieces with a pair of scissors. After shaking manually and stirring with a glass rod for 10 min, a sample (100 μL) of the supernatant were collected from each beaker and stored in the freezer until HPLC analysis for azosemide (Lee & Lee 1994).

Measurement of gastrointestinal-tract first-pass effect

Rats were fasted overnight with free access to water. The carotid artery was catheterized with polyethylene tubing (Clay Adams) under light ether anaesthesia. The cannula was exteriorized to the dorsal side of the neck and terminated with a long Silastic tubing (Dow Corning). At the same time, the portal vein was similarly cannulated by the modified Suzuki method (Xu et al 1992). For intraportal administration, 0·9% NaCl injectable solution (1·0 mL) was administered orally using a feeding tubing and the same volume of the solution was also instilled into the duodenum using a 23-g needle. Thereafter, azosemide (the same solution as described for the measurement of liver first-pass effect), 5 mg mL^{-1} (1·0 mL), was infused in 60 min via the portal vein with the assistance of Harvard infusion pump (Harvard Instrument) to rats (n = 4). For intraduodenal instillation, azosemide, 5 mg mL^{-1} (1·0 mL), was instilled into the duodenum of rats (n = 6), followed by oral administration of 0·9% NaCl injectable solution (1·0 mL) using a feeding tubing. At the same

time, 0·9% NaCl injectable solution (1·0 mL) was similarly infused in 60 min via the portal vein. For oral administration, after instillation of 0·9% NaCl injectable solution (1·0 mL) into the duodenum, azosemide, 5 mg kg^{-1} (1·0 mL), was administered orally using a feeding tubing (n = 6). At the same time, the same volume of 0·9% NaCl solution (1·0 mL) was similarly infused in 60 min via the portal vein. Blood samples (0·12 mL) were collected at appropriate time intervals. Other procedures were similar to those described for the measurement of liver first-pass effect.

Analytical procedure

The concentrations of azosemide and M1 were analysed by a published sensitive HPLC method (Lee & Lee 1994). Concentrations of chloride in urine were determined by chemical analyser (Gilford SBA 300, Coming Laboratory Science Company, Oberlin, OH), and sodium and potassium in urine by flame photometry (Model IL 943, Instrumentation Laboratory SpA via 41-20128, Milan, Italy).

Pharmacokinetic analysis

The total area under the plasma concentration-time curve from time zero to time infinity (AUC) or to the last measured time, up to 8 h, ($AUC_{0-8\ h}$) was calculated by the trapezoidal rule-extrapolation method (Lee et al 1994) employing the logarithmic trapezoidal rule (Chiou 1978) for the calculation of the area during the declining plasma-level phase and the linear trapezoidal rule for the rising plasma-level phase. The area from the last data point to time infinity for the calculation of AUC was estimated by dividing the last measured concentration by the terminal rate constant.

A standard method (Gibaldi & Perrier 1982) was used to calculate the time-averaged total body clearance (CL), the area under the first moment of plasma concentration-time curve (AUMC), the mean residence time (MRT), the apparent volume of distribution at steady-state (V_{ss}), and the time-averaged renal and nonrenal clearances (CL_R and CL_{NR}, respectively; Lee et al 1994).

The mean values of the terminal half-life (Eatman et al 1977), V_{ss} (Chiou 1979) and each clearance (Chiou 1980) were determined by the harmonic mean method.

Pharmacodynamic analysis

The diuretic, natriuretic, kaluretic and chloruretic efficiencies, respectively, were calculated by dividing the total urine output (mL) and total amount (mmol) of sodium, potassium and chloride excreted in 8-h urine by the total amount (mg) of azosemide excreted in 8-h urine.

Statistical analysis

A P value of less than 0·05 was considered to be statistically significant using unpaired t-test or Duncan's multiple range test of posteriori analysis of variance using unpaired data of the mean. All results are expressed as mean ± s.d.

Results and Discussion

Pharmacokinetics and pharmacodynamics of azosemide after intravenous and intraportal administration of the drug to rats

The mean arterial plasma concentration-time curves after intravenous, 5 mg kg^{-1} (n = 6), and intraportal, 5 (n = 5) and 10 (n = 4) mg kg^{-1}, administration of azosemide to rats are shown in Fig. 2; some relevant pharmacokinetic parameters are listed in Table 1. The plasma concentrations of azosemide increased during infusion and declined polyexponentially post-infusion with mean terminal half-lives of 73·7, 53·2 and 71·3 min after intravenous, 5 mg kg^{-1}, and intraportal, 5 and 10 mg kg^{-1}, administration, respectively. The CL of azosemide based on plasma data after intravenous and intraportal administration of the drug (3·52–4·99 mL min^{-1} kg^{-1}; Table 1) was significantly lower than the cardiac output of rats based on blood data (74·0 mL min^{-1} per 250 g body weight; Davies & Morris 1993), suggesting that lung or heart first-pass effect (or both) of azosemide could be negligible in rats. Similar results were also obtained after intravenous administration of azosemide, 5–30 mg kg^{-1}, to rats (Lee & Lee 1996). The AUC of azosemide after intraportal administration of the drug, 5 mg kg^{-1}, was significantly lower (approximately 20%) than the value after intravenous infusion of 5 mg kg^{-1} of the drug (Table 1). These data suggested that approximately 20% of azosemide disappeared (due to both liver metabolism and biliary excretion) following first-pass through the liver after intraportal administration of the drug to rats.

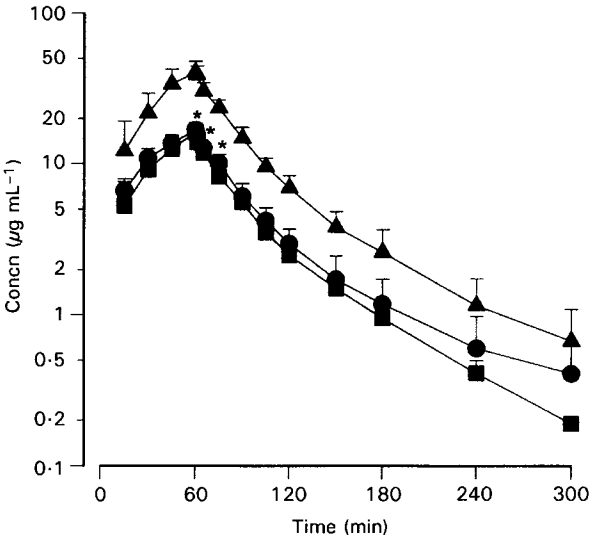

FIG. 2. Mean arterial plasma concentration-time curves of azosemide after 60-min intravenous infusion, 5 mg kg^{-1} (●, n = 6), and intraportal infusion, 5 mg kg^{-1} (■, n = 5) and 10 mg kg^{-1} (▲, n = 4) to rats. Vertical bars represent standard deviation. *$P < 0.05$ compared with intraportal administration of 5 mg kg^{-1} azosemide.

After intraportal administration of azosemide, 5 mg kg^{-1}, to rats, the terminal half-life decreased significantly (by approximately 28%). However, CL (approximately 27%) and CL_{NR} (approximately 47%) increased significantly compared with those after intravenous administration of 5 mg kg^{-1} azosemide to rats (Table 1). This could be due to the liver first-pass effect of azosemide (approximately 20%) after intraportal administration to rats. After intraportal administration of azosemide, 10 mg kg^{-1}, to rats, the AUC (approximately 184%) and terminal half-life (approximately 34%) increased significantly, however, the CL (approximately 29%) and CL_{NR} (approximately 38%) decreased significantly compared with

Table 1. Mean (±s.d.) pharmacokinetic and pharmacodynamic parameters of azosemide and its metabolites after 60-min intravenous and intraportal infusion of azosemide to rats.

Parameters	Intravenous (5 mg kg^{-1}, n=6)	Intraportal (5 mg kg^{-1}, n=5)	Intraportal (10 mg kg^{-1}, n=4)
Body weight (g)	306·0±28·4	324·0±37·8	303·0±5·0
Terminal half-life (min)[a,b]	73·7±14·1	53·20±5·36	71·3±15·0
Area under the plasma concentration-time curve from time zero to time infinity (μg min mL^{-1})[a,b]	1270±118	1000±86	2840±703
Mean residence time (min)	58·5±18·1	49·90±5·08	55·50±7·47
Time-averaged total body clearance (mL min^{-1} kg^{-1})[a,b]	3·940±0·376	4·990±0·447	3·520±0·798
Time-averaged renal clearance (mL min^{-1} kg^{-1})	1·310±0·563	1·120±0·554	1·170±0·303
Time-averaged nonrenal clearance (mL min^{-1} kg^{-1})[a,b]	2·42±0·50	3·56±0·88	2·190±0·812
Apparent volume of distribution at steady state (mL kg^{-1})[b]	341·0±45·5	396·0±57·7	302·0±54·8
Amount of azosemide recovered from 8-h gastrointestinal tract (% of dose)[a]	8·08±4·74	9·21±5·15	2·130±0·932
Amount of azosemide-glucuronide recovered from 8-h gastrointestinal tract (% of dose)[c]	2·32±1·39	0·452±0·306	1·36±0·43
Amount of M1 recovered from 8-h gastrointestinal tract (% of dose)[c]	1·650±0·875	1·77±1·08	0·546±0·245
Amount of M1-glucuronide recovered from 8-h gastrointestinal tract (% of dose)[c]	1·64±2·05	0·508±0·083	0·4674±0·098
Amount of 3 azosemide metabolites recovered from 8-h gastrointestinal tract (M1 and glucuronides of both M1 and azosemide) (% of dose)[c]	4·17±2·99	2·13±1·04	2·38±0·20
Amount of azosemide excreted in 8-h urine (% of dose)	36·7±12·0	27·4±11·5	34·5±11·0
Amount of azosemide-glucuronide excreted in 8-h urine (% of dose)[b,c]	4·40±2·38	2·48±1·36	6·91±0·60
Amount of M1 excreted in 8-h urine (% of dose)[c]	6·06±1·42	4·82±1·97	4·850±0·877
Amount of M1-glucuronide excreted in 8-h urine (% of dose)[c]	5·88±2·89	2·99±0·94	3·19±1·61
Amount of 3 azosemide metabolites excreted in 8-h urine (M1 and glucuronides of both M1 and azosemiide) (% of dose)[b,c]	14·20±6·22	9·20±3·83	14·9±2·3
Eight-hour urine output (mL per 100 g body weight)[a]	12·90±4·34	7·71±3·04	19·8±8·3
Eight-hour urinary excretion of sodium (mmol per 100 g body weight)[b]	1·53±0·63	0·818±0·463	2·47±1·14
Eight-hour urinary excretion of potassium (mmol per 100 g body weight)	0·354±0·120	0·2910±0·0888	0·472±0·155
Eight-hour urinary excretion of chloride (mmol per 100 g body weight)[b]	1·64±0·60	0·927±0·433	2·44±1·03
Eight-hour diuretic efficiency (mL mg^{-1})	70·00±5·17	59·4±21·9	63·5±31·7
Eight-hour natriuretic efficiency (mmol mg^{-1})	8·09±1·22	6·20±3·31	7·93±4·17
Eight-hour kaluretic efficiency (mmol mg^{-1})	1·980±0·432	2·330±0·939	1·510±0·644
Eight-hour chloruretic efficiency (mmol mg^{-1})	8·820±0·875	7·10±3·14	7·81±3·89

[a]Significant difference ($P < 0·05$) between intravenous, 5 mg kg^{-1} and intraportal, 5 mg kg^{-1} administration. [b]Significant difference ($P < 0·05$) between intraportal, 5 mg kg^{-1} and 10 mg kg^{-1} administration. [c]Expressed in terms of azosemide.

those after intraportal administration of 5 mg kg^{-1} (Table 1). This could be due to saturable metabolism of azosemide in rats, and similar results were also reported (Lee & Lee 1996) after intravenous administration of azosemide, 5–30 mg kg^{-1}, to rats.

A negligible amount of azosemide was excreted as unchanged drug in urine after 8 h and the urine loss was replaced only up to 8 h in the present study, therefore, the following discussion on the pharmacodynamics of azosemide will be confined to this period of time (8 h). The 8-h urine output decreased significantly (by approximately 40%) after intraportal administration of azosemide, 5 mg kg^{-1}, compared with that after intravenous administration of 5 mg kg^{-1}. This could be due to a decreased amount of azosemide (because of liver first-pass effect) being excreted unchanged in 8-h urine after intraportal administration of azosemide, 5 mg kg^{-1} (Table 1).

However, the total amount of sodium, potassium and chloride excreted in 8-h urine was not significantly different between intravenous and intraportal administration of azosemide, 5 mg kg^{-1}, although each value after intravenous administration tended to be higher than that after intraportal administration (Table 1). The diuretic, natriuretic, kaluretic and chloruretic efficiencies were also not significantly different between intravenous and intraportal administration of azosemide, 5 mg kg^{-1} to rats (Table 1).

Pharmacokinetics and pharmacodynamics of azosemide after intraportal infusion, intraduodenal instillation and oral administration of the drug to rats

The mean arterial plasma concentration-time curves after intraportal infusion, intraduodenal instillation, and oral administration of azosemide, 5 mg kg^{-1}, to rats are shown in Fig. 3; some relevant pharmacokinetic parameters are listed in Table 2. After intraportal infusion, the plasma concentration of azosemide increased during the infusion and declined monoexponentially post-infusion with a mean terminal half-life of 64·5 min (Table 2). After both intraduodenal instillation and oral administration, however, the plasma concentrations of azosemide were almost constant up to 8 h due to continuous absorption of the drug from the various rat gastrointestinal segments. The $AUC_{0-8\ h}$ after oral administration of azosemide was significantly lower (only 1·72% of intraportal administration) than that after intraportal infusion suggesting that the gastrointestinal first-pass effect of azosemide was considerable in rats. It has been reported (Lee & Lee 1996) that the pharmacokinetic parameters of azosemide are dependent on intravenous doses of 5–30 μg kg^{-1} of azosemide in rats, and in particular the CL_{NR} of azosemide decreased with increasing intravenous doses of azosemide in rats due to the saturable metabolism. Therefore, the measurement of the extent of absolute bioavailability of azosemide after oral administration (F) could not be possible in rats. However, in the present study,

Table 2. Mean (± s.d) pharmacokinetic and pharmacodynamic parameters of azosemide and its metabolites after intraportal infusion (treatment I), intraduodenal (treatment II) and oral (treatment III) administration of azosemide to rats.

Parameters	Treatment I (5 mg kg^{-1}, n = 4)	Treatment II (5 mg kg^{-1}, n = 6)	Treatment III (5 mg kg^{-1}, n = 6)
Body weight (g)	268·0 ± 14·4	278·0 ± 15·7	274·0 ± 14·3
Terminal half-life (min)	64·5 ± 16·5		
Area under the plasma concentration-time curve from time 0 to 8 h (μg min mL^{-1})[a]	1580 ± 296	32·0 ± 13·5	27·1 ± 11·2
Mean residence time (min)	70·50 ± 7·99		
Time-averaged total body clearance (mL min^{-1} kg^{-1})	3·060 ± 0·622		
Time-averaged renal clearance (mL min^{-1} kg^{-1})[b]	1·040 ± 0·301	1·75 ± 0·41	0·754 ± 0·197
Time-averaged nonrenal clearance (mL min^{-1} kg^{-1})	1·990 ± 0·593		
Apparent volume of distribution at steady state (mL kg^{-1})	309·0 ± 50·1		
Amount of azosemide recovered from 8-h gastrointestinal tract (% of dose)[c]	7·29 ± 3·71	8·30 ± 1·00	13·90 ± 4·89
Amount of azosemide-glucuronide recovered from 8-h gastrointestinal tract (% of dose)[b,d]	U.D.	2·56 ± 1·43	U.D.
Amount of M1 recovered from 8-h gastrointestinal tract (% of dose)[a,d]	0·966 ± 0·119	0·542 ± 0·282	0·672 ± 0·270
Amount of M1-glucuronide recovered from 8-h gastrointestinal tract (% of dose)[d]	2·170 ± 1·581	0·261 ± 0·169	0·594 ± 0·304
Amount of 3 azosemide metabolites recovered from 8-h gastrointesinal tract (M1 and glucuronides of both M1 and azosemide) (% of dose)[d]	1·530 ± 0·503	2·46 ± 1·90	1·27 ± 0·39
Amount of azosemide excreted in 8-h urine (% of dose)[e]	33·20 ± 4·95	1·090 ± 0·307	0·435 ± 0·212
Amount of azosemide-glucuronide excreted in 8-h urine (% of dose)[a,d]	U.D.	2·23 ± 0·73	2·580 ± 0·288
Amount of M1 excreted in 8-h urine (% of dose)[a,d]	2·300 ± 0·501	1·070 ± 0·488	0·578 ± 0·357
Amount of M1-glucuronide excreted in 8-h urine (% of dose)[d]	2·17 ± 1·581	1·48 ± 0·41	1·710 ± 0·667
Amount of 3 azosemide metabolites excreted in 8-h urine (M1 and glucuronides of both M1 and azosemide) (% of dose)[d]	4·47 ± 1·90	3·79 ± 0·25	4·30 ± 1·11
Eight-hour urine output (mL per 100 g body weight)[a]	10·90 ± 3·31	0·902 ± 0·213	0·913 ± 0·359
Eight-hour urinary excretion of sodium (mmol per 100 g body weight)[a]	1·310 ± 0·506	0·0208 ± 0·0238	0·0595 ± 0·0476
Eight-hour urinary excretion of potassium (mmol per 100 g body weight)[a]	0·1770 ± 0·0132	0·0543 ± 0·0210	0·0522 ± 0·0112
Eight-hour urinary excretion of chloride (mmol per 100 g body weight)[a]	1·320 ± 0·436	0·0366 ± 0·0313	0·066 ± 0·038
Eight-hour diuretic efficiency (mL mg^{-1})[a]	64·6 ± 11·6	175·00 ± 0·41	437·0 ± 289·0
Eight-hour natriuretic efficiency (mmol mg^{-1})[e]	7·74 ± 2·06	3·11 ± 3·17	29·8 ± 17·6
Eight-hour kaluretic efficiency (mmol mg^{-1})[e]	1·090 ± 0·213	9·440 ± 1·960	30·20 ± 17·90
Eight-hour chloruretic efficiency (mmol mg^{-1})[c]	7·83 ± 1·71	5·87 ± 4·10	34·4 ± 16·5

[a]Treatment I was significantly different ($P < 0·05$) from II and III. [b]Treatment II was significantly different ($P < 0·05$) from I and III. [c]Treatment III was significantly different ($P < 0·05$) from I and II. [d]Expressed in terms of azosemide. [e]Each treatment was significantly different ($P < 0·05$). U.D., under detection limit.

F was estimated for comparison; the value after oral administration was approximately 2·13% by comparing the AUC (Table 1) and $AUC_{0-8\ h}$ (Table 2) values after intravenous and oral administration. The considerably low F value was not due to incomplete absorption of azosemide after oral administration to rats; the percentages of orally administered azosemide recovered at 8 h from whole gastrointestinal tract as unchanged drug were 7·29% (due to biliary or GI excretion, or both) and 13·9% (due to unabsorbed and gastrointestinal or biliary excretion, or both) for intraportal and oral administration, respectively (Table 2). Moreover, azosemide was stable after incubation in human gastric juices and solutions with pH ranging from 2 to 13 (Lee & Lee 1995). These data suggested that there are considerable gastrointestinal first-pass effects of azosemide after oral administration of the drug to rats. Although the $AUC_{0-8\ h}$ of azosemide after intraduodenal instillation was approximately 15% less than that after oral administration (Table 1), they were not significantly different, suggesting that gastric first-pass effect of azosemide was not considerable in rats. After oral administration of azosemide, 5 mg kg^{-1}, to rats, F value was approximately 2·13%, liver first-pass effect was approximately 20%, and gastric first-pass effect was not considerable. Therefore, it could be concluded that the intestinal first-pass effect of azosemide was considerable after oral administration of the drug to rats. It was not unexpected because rat intestine has considerable metabolic activity for azosemide based on in-vitro tissue metabolism studies (Lee & Lee 1995) and has a large surface area. It should be noted, however, that the values of liver and gastrointestinal first-pass effects of azosemide could alter with various doses of azosemide because azosemide metabolism was saturated in rats (Lee & Lee 1996).

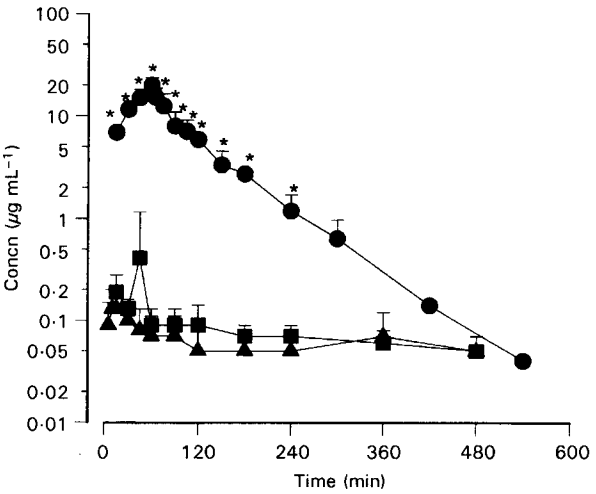

FIG. 3. Mean arterial plasma concentration-time curves of azosemide after 60-min intravenous infusion, 5 mg kg^{-1} (●, n = 4), and intraduodenal instillation, 5 mg kg^{-1} (■, n = 6), and oral administration, 5 mg kg^{-1} (▲, n = 6) to rats. Vertical bars represent standard deviation. *$P < 0·05$ compared with intraduodenal and oral administration of 5 mg kg^{-1}.

The 8-h urine output decreased significantly after both introduodenal instillation and oral administration compared with that after intraportal infusion of azosemide, 5 mg kg^{-1} each (Table 2). This was due to a significantly reduced amount of azosemide being excreted in 8-h urine as unchanged drug after both intraduodenal instillation and oral administration compared with that after intraportal infusion (Table 2). The total amount of sodium, potassium and chloride excreted in 8-h urine and diuretic efficiency after intraportal infusion were significantly higher than those after both intraduodenal instillation and oral administration of azosemide to rats (Table 2). The natriuretic, kaluretic and chloruretic efficiencies were significantly different with intraportal infusion, intraduodenal instillation and oral administration of azosemide to rats (Table 2).

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References

Asano, T., Inoue, T., Kurono, M. (1984) Disposition of azosemide. 1. Distribution, metabolism and excretion following intravenous administration to rats. Yakugaku Zasshi 104: 1181–1190

Brater, D. C., Day, B., Anderson, S. A., Stowig, S. (1979) Azosemide, a 'loop' diuretic, and furosemide. Clin. Pharmacol. Ther. 25: 435–439

Brater, D. C., Day, B., Anderson, S. A., Seiwell, R. (1983) Azosemide kinetics and dynamics. Clin. Pharmacol. Ther. 34: 454–458

Chiou, W. L. (1978) Critical evaluation of potential error in pharmacokinetic studies using the linear trapezoidal rule method for the calculation of the area under the plasma level-time curve. J. Pharmacokinet. Biopharm. 6: 539–546

Chiou, W. L. (1979) New calculation method for mean apparent drug volume of distribution and application to rational dosage regimen. J. Pharm. Sci. 68: 1067–1069

Chiou, W. L. (1980) New calculation method of mean total body clearance of drugs and its application to dosage regimens. J. Pharm. Sci. 69: 90–91

Davies, B., Morris, T. (1993) Physiological parameters in laboratory animals and humans. Pharm. Res. 10: 1093–1095

Eatman, F. B., Colburn, W. A., Boxenbaum, H. G., Posmanter, H. N., Weinfeld, R. E., Ronfeld, R., Weissman, L., Moore, J. D., Gibaldi, M., Kaplan, S. A. (1977) Pharmacokinetics of diazepam following multiple dose oral administration to healthy human subjects. J. Pharmacokinet. Biopharm. 5: 481–494

Gibaldi, M., Perrier, G. (1982) Pharmacokinetics. 2nd edn, Dekker, New York

Krück, F., Bablock, W., Besenfelder, E., Betzien, G., Kaufmann, B. (1978) Clinical and pharmacological investigations of the new saluretic azosemide. Eur. J. Clin. Pharmacol. 14: 153–161

Lee, S. H., Lee, M. G.. (1994) Determination of azosemide and its metabolite in plasma, blood, urine and tissue homogenates by high-performance liquid chromatography. J. Chromatogr. 656: 367–372

Lee, S. H., Lee, M. G. (1995) Stability, tissue metabolism, tissue distribution and blood partition of azosemide. Biopharm. Drug Dispos. 16: 547–561

Lee, S. H., Lee, M. G. (1996) Pharmacokinetics and pharmacodynamics of azosemide after intravenous administration and oral administration to rats: absorption from various GI segments. J. Pharmacokinet. Biopharm. 24: 551–568

Lee, S. H., Lee, M. G., Kim, N. D. (1994) Pharmacokinetics and pharmacodynamics of bumetanide after intravenous and oral administration to rats: absorption from various GI segments. J. Pharmacokinet. Biopharm. 22: 1–17

Park, K. J., Yoon, W. H., Shin, W. G., Lee, M. G. Effects of the rate and composition of fluid replacement on the pharmacokinetics and pharmacodynamics of intravenous azosemide. Biopharm. Drug Disposit. (In press)

Xu, Z.-X., Tang, J.-P., Badr, M., Melethil, S. (1992) Kinetics of aluminum in rats: effect of route of administration. J. Pharm. Sci. 81: 160–163