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Circadian Changes in the Pharmacokinetics and Pharmacodynamics of Azosemide in Rats

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Abstract

The circadian changes in the pharmacokinetics and pharmacodynamics of azosemide were investigated after intravenous and oral administration of the drug (10 mg kg^{-1}) to rats at 1000 or 2200 h.

After intravenous administration of azosemide the percentage of the dose excreted in 8-h urine as unchanged azosemide was significantly higher in the 1000 h group than in the 2200 h group (41·7 compared with 28·9%) and this resulted in a significant increase in 8-h urine output (84·7 compared with 36·6 mL/100 g). After intravenous administration the time-averaged renal clearance (CL_R) of azosemide was significantly faster (2·86 compared with 1·76 mL min^{-1} kg^{-1}) and urinary excretion of sodium (46·4 compared with 25·9 mmol/100 g) and chloride (35·6 compared with 18·8 mmol/100 g) increased significantly in the 1000 h group. However, after oral administration, the percentages of oral dose of azosemide excreted in 8-h urine as unchanged azosemide were significantly higher (1·88 compared with 0·67%) and the CL_R of azosemide was significantly faster (3·64 compared with 0·79 mL min^{-1} kg^{-1}) in the 2200 h group. This could be at least partly because of increased absorption of azosemide from the gastrointestinal tract in the 2200 h group; the percentages of oral dose of azosemide recovered from the gastrointestinal tract in 8 h as unchanged azosemide was significantly smaller (5·7 compared with 13·2%) in the 2200 h group. The pharmacodynamic parameters of azosemide were not significantly different after oral administration of the drug to both groups of rats.

If these data could be extrapolated to man, the intravenous dose of azosemide could be modified on the basis of circadian time.

Circadian changes in pharmacokinetics (chronopharmacokinetics) and pharmacodynamics have been reported in animals and in man for over one hundred drugs (Reinberg & Smolensky 1982; Bruguerolle 1993). Circadian changes can be involved at each step of pharmacokinetic processes—temporal variations in drug absorption from the gastrointestinal tract, in plasma protein binding and drug distribution, in drug metabolism (temporal variations in enzyme activity and hepatic blood flow), and in renal drug excretion might be important (Bruguerolle 1993).

Azosemide, 5-(4-chloro-5-sulphamoyl-2-thenylaminophenyl)tetrazole, is a loop diuretic (Figure 1) closely resembling furosemide in its diuretic action (Krück et al 1978). Because azosemide is subject to a high first-pass metabolism its bioavailability is low (10–19%) after oral administration in man (Brater et al 1983). After intravenous and oral administration in man, 17–37% and less than 10% of the dose, respectively, were recovered in urine as unchanged azosemide (Beerman & Grind 1987). Although the pharmacokinetics and pharmacodynamics of azosemide after intravenous and oral administration to man (Brater et al 1979, 1983; Kuzuya 1983; Beerman & Grind 1987) and animals (Asano et al 1984; Inoue et al 1984; Lee & Lee 1994a, 1996, 1997; Ha et al 1996; Park et al 1996, 1997a, b, 1998) have been investigated, it seems that no detailed studies on the time-dependent pharmacokinetic and pharmacodynamic changes of azosemide have even been reported to date. It has been reported (Fujimura & Ebihara 1986) that the diuretic effects of furosemide, another loop

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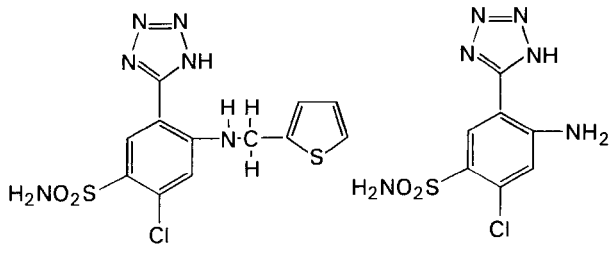

Figure 1. The chemical structures of azosemide and its metabolite 5-(2-amino-4-chloro-5-sulphamoylphenyl)tetrazole (M1).

diuretic, were greater when it was administered at 1000 h, corresponding to the sleeping period, than when it was administered at 2200 h, the awake period.

The purpose of this study was to investigate time-dependent pharmacokinetic and pharmacodynamic changes of azosemide using rats as an animal model after intravenous and oral administration at 1000 or 2200 h.

Materials and Methods

Chemicals

Azosemide and its metabolite M1 [5-(2-amino-4-chloro-5-sulphamoylphenyl)tetrazole], were donated by Sam Jin Pharmaceutical Company (Seoul, Korea) and Boehringer Mannheim (Mannheim, Germany), respectively. β-Glucuronidase was purchased from Sigma (St Louis, MO). Other chemicals were of reagent grade or HPLC grade and used without further purification.

Pretreatment of rats

Male Sprague–Dawley rats, 280–320 g, were purchased from Charles River Company (Atsugi, Japan) and randomly divided into two groups (1000 h and 2200 h). The animals were housed in a clean room under conditions of light from 0700 to 1900 h and dark from 1900 to 0700 h; the animals had free access to food and tap water.

The carotid artery (for blood sampling) and the jugular vein (for drug administration, in the intravenous study only) were cannulated individually with polyethylene tubing (Clay Adams, Parsippany, NJ) under light ether anaesthesia. Both cannulae were exteriorized to the dorsal side of the neck and connected individually with long Silastic tubing (Dow Corning, Midland, MI). Both pieces of Silastic tubing were covered with wire to allow free movement of the rat. The exposed areas were surgically sutured. Each rat was housed individually in a metabolic cage (Daejong Scientific, Seoul, Korea) and left to recover from anaesthesia for 4–5 h before the commencement of the experiment. They were not restrained at any time during the study. Heparinized 0·9% NaCl injectable solution (20 units mL^{-1}, 0·3 mL) was used to flush each cannula to prevent blood clotting.

Intravenous infusion study

Azosemide powder (15 mg) was dissolved in NaOH solution (0·1 M; 1 mL) of final pH 10, filtered through a 0·45 μm filter, and diluted with normal saline injectable solution. Azosemide, 10 mg kg^{-1}, was infused in 1 min via the jugular vein of rats at 1000 h (n = 7) or 2200 h (n = 5). Total injection volume was approximately 1 mL. Blood (approx. 0·12 mL) was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of the infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240 and 300 min after intravenous administration. Heparinized 0·9% NaCl injectable solution (20 units mL^{-1}, approx. 0·3 mL) was used to flush the cannula immediately after each blood sampling. Because it has been reported (Park et al 1997b) that the pharmacodynamic effect of intravenous azosemide was dependent on the rate and composition of fluid replacement, in the current studies azosemide-induced losses of fluid and electrolytes in the urine were replaced immediately by intravenous infusion of lactated Ringer's solution via the carotid artery for up to 8 h after dosing. After 8 h each rat was exsanguinated and killed by cervical dislocation. At the same time, the metabolic cage was rinsed with distilled water (10 mL) and the rinsings were combined with the 8-h urine. After measuring the exact volume of each urine output and the combined urine, samples of the plasma (0·05 mL) and of the combined urine samples were frozen until the HPLC analysis for azosemide and M1 (Lee & Lee 1994b), and sodium, potassium and chloride. A sample (0·5 mL) of the combined 8-h urine was also added to Sørensen's phosphate buffer (pH 7·4; 1 mL) containing 10 000 units of β-glucuronidase, and the mixture was incubated for 24 h in a water-bath shaker kept at 37°C and at a rate of 50 oscillations min^{-1} to measure glucuronide conjugates 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 solution (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, two 100-μL samples of the supernatant were collected from each beaker and stored in the freezer until the HPLC analysis for

azosemide and M1 (Lee & Lee 1994b). A sample (0·5 mL) of the supernatant was also incubated with β-glucuronidase.

Oral study

At 1000 h (n = 8) or 2200 h (n = 8) after fasting of the rats for 12 h with free access to tap water, azosemide (the same solution as used in intravenous study; 10 mg kg^{-1}) was administered orally in 10 s to rats by use of a feeding tube. The total oral volume was approximately 1 mL. The blood sampling times were 0 (control), 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min after oral administration. The other procedures were similar to those used for the intravenous study.

Analytical procedure

The concentrations of azosemide and M1 were analysed by a sensitive HPLC method reported elsewhere (Lee & Lee 1994b); 2·5 vols acetonitrile for analysis of azosemide or 1 vol saturated aqueous $Ba(OH)_2$ and $ZnSO_4$ solution for the analysis of M1 were added to the sample. After vortex-mixing and centrifugation, a 50-μL sample of the supernatant was injected directly on to the HPLC column. The mobile phase, phosphoric acid (0·03 M)–acetonitrile (50 : 40, v/v) for azosemide, or phosphoric acid (0·03 M)–acetic acid (0·2 M) in acetonitrile (83 : 17, v/v) for M1, was delivered at a flow rate of 1·5 mL min^{-1} and the column effluent was monitored by UV detection at 240 nm for azosemide or 236 nm for M1. The detection limits for both azosemide and M1 in both rat plasma and urine were 50 ng mL^{-1}. The concentrations of sodium, potassium and chloride in the urine were determined by means of a chemical analyser (Ciba Corning 644, Ciba Corning Company, Boston, MA).

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to time infinity (AUC for the intravenous study) or to the last measured time, up to 8 h ($AUC_{0-8\ h}$ for the oral study) was calculated by the trapezoidal rule method (Kim et al 1993); this method employed the logarithmic trapezoidal rule recommended by Chiou (1978) for 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 (in the calculation of AUC) was estimated by dividing the last measured plasma concentration by the terminal rate constant.

Standard methods (Gibaldi & Perrier 1982) were used to calculate the time-averaged total body clearance (CL), the area under the first moment of the plasma concentration–time curve (AUMC), the mean residence time (MRT), the apparent volume of distribution at steady state (Vd_{SS}), and the time-averaged renal (CL_R) and non-renal (CL_{NR}) clearances (Kim et al 1993):

$$CL = dose/AUC \quad (1)$$

$$AUMC = \int_0^\infty tC_p dt \quad (2)$$

$$MRT = AUMC/AUC \quad (3)$$

$$Vd_{SS} = CL \times MRT \quad (4)$$

$$CL_R = Xu_{azo}/AUC \quad (5)$$

$$CL_{NR} = CL - CL_R \quad (6)$$

where C_p is the plasma concentration of azosemide at time t and Xu_{azo} is the amount of azosemide excreted into the urine up to time infinity (this was assumed to equal the total amount of azosemide excreted in 8 h, because no azosemide could be detected in urine collected later). In the calculation of CL_R of azosemide after oral study, the 8-h values of AUC and Xu_{azo} were used.

The harmonic mean method was used for the calculation of the mean values of terminal half-life (Eatman et al 1977), total body clearance (Chiou 1980), and Vd_{SS} (Chiou 1979).

Statistical analysis

$P < 0·05$ was considered to be indicative of statistical significance when using the unpaired *t*-test. All the data were expressed as mean ± standard deviation.

Results and Discussion

Intravenous study

The mean arterial plasma concentration–time curves of azosemide after intravenous administration of the drug, 10 mg kg^{-1}, to rats at 1000 h (n = 7) or 2200 h (n = 5) are shown in Figure 2, and the relevant pharmacokinetic and pharmacodynamic parameters are given in Table 1. After intravenous administration, the plasma concentrations of azosemide declined similarly for both groups of rats with mean terminal half-lives of 65·3 and 57·4 min (Table 1) for the 1000 h and 2200 h groups, respectively. The values were not significantly different. The percentages of the intravenous dose of azosemide excreted in 8-h urine as unchanged azosemide were significantly higher in the 1000 h group than in the 2200 h group (41·7

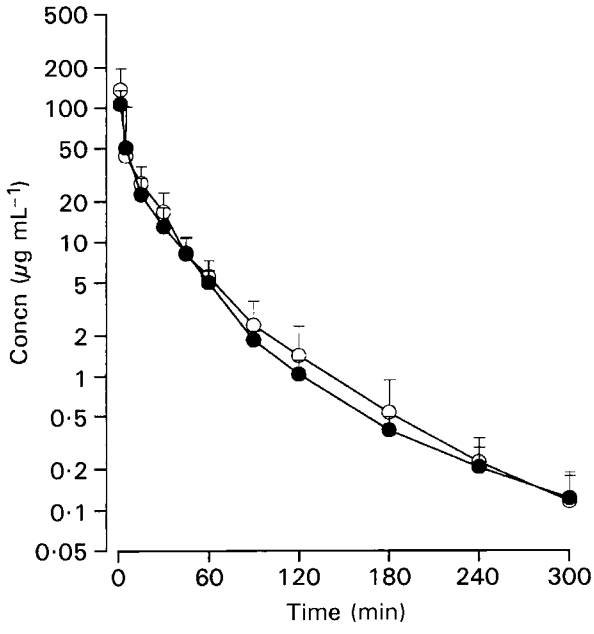

Figure 2. Mean arterial plasma concentration–time profiles of azosemide after 1-min intravenous infusion of 10 mg kg^{-1} to rats at 1000 (●, n = 7) and 2200 h (○, n = 5). Vertical bars represent the standard deviation.

compared with 28·9%) and this resulted in a significantly faster CL_R of azosemide in the 1000 h group (2·86 compared with 1·76 mL min^{-1}) as listed in Table 1. Because it has been reported (Lee & Lee 1996) that CL, CL_R, and CL_{NR} of azosemide were dose-dependent after intravenous administration of various doses of the drug to rats, each clearance of azosemide in the current rat studies (Tables 1 and 2) are given as time-averaged values.

The contribution of biliary or gastrointestinal excretion, or both, of unchanged azosemide to the CL_{NR} of azosemide did not seem to be considerable; the percentages of the intravenous dose of azosemide remaining in the entire gastrointestinal tract as unchanged azosemide 8 h after intravenous dosing were 4·73 and 4·70%, respectively, for the 1000 h and 2200 h groups (Table 1). Similar results have already been reported for rats (Asano et al 1984; Ha et al 1996; Lee & Lee 1996). Therefore, the CL_{NR} of azosemide could represent non-renal metabolism of azosemide in the current rat intravenous study. As mentioned above, the percentages of intravenous dose of azosemide excreted in 8-h urine as unchanged azosemide were significantly higher in the 1000 h group suggesting that metabolism of azosemide was greater in the 2200 h group; this inference was supported by the significantly higher amounts of the intravenous dose of azosemide excreted in 8-h urine as M1-glucuronide (8·27 compared with 4·71%, expressed in terms of azosemide). The amounts of the intravenous dose of azosemide excreted in 8-h urine, as the sum of the amounts of the three metabolites, M1, M1-glucuronide, and azosemide-glucuronide, tended to be higher in the 2200 h group (12·9 compared with 16·4%, expressed in terms of azosemide, $P < 0.252$, Table 1). However, the corresponding values for the three metabolites recovered from the entire gastrointestinal tracts of the two groups of rats were not significantly different (Table 1). Eleven metabolites of azosemide were found in urine and bile after intravenous administration of azosemide to rats (Asano et al 1984). It has been reported (Fujimura & Ebihara 1986) that the urinary excretion of furosemide was also significantly higher in 1000 h rats and reduced metabolism of furosemide in 1000 h rats was also suggested. Many drugs have been reported to be metabolized by rats faster at night-time than during daytime (Radzialowski & Bousquet 1968; Holcslaw et al 1975). It has been reported on the basis of an in-vitro tissue-homogenate study (Lee & Lee 1995) that all rat tissues were metabolically active towards azosemide, with considerable metabolic activity in the liver, lung, heart, kidney and stomach; rhythmic variations in the activities of many enzymes in liver, kidney and brain in rats were also documented (Bruguerolle 1993).

Negligible amounts of azosemide were excreted as unchanged azosemide in 8-h urine and the urine loss was replaced for up to 8 h only in the current study. Therefore, the following discussion on the pharmacodynamics of azosemide will be confined to this period of time (8 h). In 1000 h group the 8-h urine output was significantly higher (84·7 compared with 36·6 mL/100 g); this could be because of a significant increase in the amounts of the intravenous azosemide dose excreted in 8-h urine as unchanged azosemide (Table 1). However, this could not be because of changes in glomerular filtration rate and plasma protein binding of azosemide in the 1000 h group. It has been reported (Lee & Lee 1996) that the fraction of azosemide filtered by the glomerulus is small considering the high plasma protein binding of the drug in rats (94–95%; Lee & Lee (1997)), and active secretion by the proximal tubule is of great importance to the diuretic effect. Similar results were also reported for the other loop diuretics furosemide (Boles Ponto & Schoenwal 1990) and bumetanide (Odlind et al 1983). Furthermore, this could not be because of increased urinary excretion of M1 in the 1000 h group—the 8-h urinary excretion of M1 by the two groups of rats was not significantly different (Table 1), and M1 did not have significant diuretic effect after intravenous administration of 10 mg kg^{-1} to rats (Lee & Lee 1997). The 8-h urinary excretion of

Table 1. Pharmacokinetic and pharmacodynamic parameters of azosemide and its metabolites after 1-min intravenous infusion of azosemide (10 mg kg^{-1}) to rats at 1000 h and 2200 h.

Parameter	1000 h (n = 7)	2200 h (n = 5)
$t^{1}/_{2}$ (min)	65·3 ± 20·2	57·4 ± 10·6
$AUC_{0-\infty}$ (μg min mL^{-1})	1390 ± 256	1600 ± 354
MRT(min)	35·8 ± 5·58	34·4 ± 9·13
CL (mL min^{-1} kg^{-1})	7·22 ± 1·51	6·25 ± 1·66
CL_R (mL min^{-1} kg^{-1})	2·86 ± 0·87*	1·76 ± 0·52
CL_{NR} (mL min^{-1} kg^{-1})	3·79 ± 1·62	4·43 ± 1·27
Vd_{ss} (mL kg^{-1})	248 ± 80·8	206 ± 40·1
Azosemide excreted in 8-h urine (% of dose)	41·7 ± 13·5*	28·9 ± 4·75
Azosemide-glucuronide excreted in 8-h urine (% of dose)	4·24 ± 3·09	3·89 ± 3·51
M1 excreted in 8-h urine (% of dose)	5·15 ± 2·33	4·11 ± 1·88
M1-glucuronide excreted in 8-h urine (% of dose)	4·71 ± 1·91†	8·27 ± 2·01
Azosemide metabolites excreted in 8-h urine (M1 and glucuronides of both M1 and azosemide) (% of dose)	12·9 ± 6·23	16·4 ± 7·23
Azosemide recovered from 8-h gastrointestinal tract (% of dose)	4·73 ± 2·76	4·70 ± 2·47
Azosemide-glucuronide recovered from 8-h gastrointestinal tract (% of dose)	1·93 ± 1·45	2·24 ± 1·89
M1 recovered from 8-h gastrointestinal tract (% of dose)	2·82 ± 2·53	2·45 ± 1·96
M1-glucuronide recovered from 8-h gastrointestinal tract (% of dose)	1·65 ± 1·05	1·58 ± 1·72
Azosemide metabolites recovered from 8-h gastrointestinal tract (% of dose)	5·34 ± 4·95	5·67 ± 4·86
8-h Urinary output (mL/100 g)	84·7 ± 29·7*	36·6 ± 20·2
8-h Urinary excretion of sodium (mmol/100 g)	46·4 ± 9·10‡	25·9 ± 12·1
8-h Urinary excretion of potassium (mmol/100 g)	2·97 ± 1·36	2·05 ± 0·58
8-h Urinary excretion of chloride (mmol/100 g)	35·6 ± 9·38*	18·8 ± 10·9

Values are mean ± s.d. *$P < 0.05$, significant difference between results for 1000 h and 2200 h. †$P < 0.001$, significant difference between results for 1000 h and 2200 h. ‡$P < 0.01$, significant difference between results for 1000 h and 2200 h.

sodium (46·4 compared with 25·9 mmol/100 g) and chloride (35·6 compared with 18·8 mmol/100 g) were also significantly higher in the 1000 h group. However, the corresponding values for potassium (2·97 compared with 2·05 mmol/100 g) by the two groups of rats were not significantly different (Table 1). Similar results have also been obtained for furosemide in man (Branch et al 1977), dogs (Lee et al 1986), and rats (Khan et al 1983; Jang et al 1994), bumetanide in rabbits (Ryoo & Lee 1993), and azosemide in rats (Lee & Lee 1996; Park et al 1996, 1997a, 1998). This might be because of the constant rate of potassium secretion in the distal tubule (Giebisch 1976).

Oral study

The mean arterial plasma concentration–time profiles of azosemide after oral administration of 10 mg kg^{-1} to rats at 1000 h (n = 8) or 2200 h (n = 8) are shown in Figure 3, and the relevant pharmacokinetic and pharmacodynamic parameters are listed in Table 2. After oral administration the plasma concentrations of azosemide were similar for both groups of rats (Figure 3). In the 2200 h group the CL_R was significantly faster (3·64 compared with 0·791 mL min^{-1} kg^{-1}), which could be because of a significant increase in the percentages of oral dose of azosemide excreted in 8-h urine as unchanged azosemide (1·88 compared with 0·672%, Table 2). The significant increase in the percentages of the oral dose of azosemide excreted in 8-h urine as unchanged azosemide in the 2200 h group could be at least partly a result of the increased absorption of azosemide from the gastrointestinal tract and this was supported by the significant reduction in the amounts of the oral dose of azosemide remaining in the gastrointestinal tract as unchanged azosemide after 8 h in the 2200 h group (5·69 compared with 13·2%, Table 2). Note that multiple peaks appeared in the plasma con-

Table 2. Pharmacokinetic and pharmacodynamic parameters of azosemide and its metabolites after oral administration of azosemide (10 mg kg^{-1}) to rats at 1000 h and 2200 h.

Parameter	1000 h (n = 8)	2200 h (n = 8)
$AUC_{0-8\,h}$ (μg min mL^{-1})	68·4 ± 18·5	68·2 ± 12·9
CL_R (mL min^{-1} kg^{-1})	0·791 ± 0·820*	3·64 ± 0·144
Azosemide excreted in 8-h urine (% of dose)	0·672 ± 0·321*	1·88 ± 0·492
Azosemide-glucuronide excreted in 8-h urine (% of dose)	0·223 ± 0·177	0·258 ± 0·188
M1 excreted in 8-h urine (% of dose)	0·851 ± 0·981	0·358 ± 0·286
M1-glucuronide excreted in 8-h urine (% of dose)	0·148 ± 0·121	0·171 ± 0·167
Azosemide metabolites excreted in 8-h urine (M1 and glucuronides of both M1 and azosemide) (% of oral dose)	1·51 ± 1·48	0·723 ± 0·612
Azosemide recovered from 8-h gastrointestinal tract (% of dose)	13·2 ± 3·62*	5·69 ± 1·98
Azosemide-glucuronide recovered from 8-h gastrointestinal tract (% of dose)	N.D.	N.D.
M1 recovered from 8-h gastrointestinal tract (% of dose)	0·151 ± 0·136	0·191 ± 0·151
M1-glucuronide recovered from 8-h gastrointestinal tract (% of dose)	0·291 ± 0·168	0·202 ± 0·181
Azosemide metabolites recovered from 8-h gastrointestinal tract (% of dose)	0·428 ± 0·306	0·411 ± 0·291
8-h Urinary output (mL/100 g)	8·40 ± 5·38	10·4 ± 5·42
8-h Urinary excretion of sodium (mmol/100 g)	23·0 ± 11·0	18·4 ± 4·86
8-h Urinary excretion of potassium (mmol/100 g)	8·15 ± 6·36	6·76 ± 5·83
8-h Urinary excretion of chloride (mmol/100 g)	8·77 ± 1·73	9·63 ± 5·86

†$P < 0·01$, significant difference between results for 1000 h and 2200 h. N.D., not detectable.

centrations of azosemide after oral administration to each rat of both groups. However, the appearance of multiple peaks is not readily apparent in Figure 3, because the concentrations of azosemide in this figure are the mean values of plasma concentrations with different peak times and different peak concentrations from each rat. Similar results were also obtained from other rat studies and the reasons for the multiple peak phenomena of azosemide in rats have been thoroughly studied and found to be mainly a consequence of differences in gastric emptying pattern (Lee & Lee 1996).

It has been reported (Lee & Lee 1996) that the CL_{NR} of azosemide was dose-dependent after intravenous administration of the drug to rats and so the extent of bioavailability of azosemide could not be estimated. However, the extent of bioavailability was estimated for comparison in this study by comparing AUC values after intravenous and oral administration; the values were less than 5% for both groups of rats. The reason for the low bioavailability of azosemide in rats has been extensively studied and is a result of a considerable intestinal first-pass effect of azosemide in rats (Kim

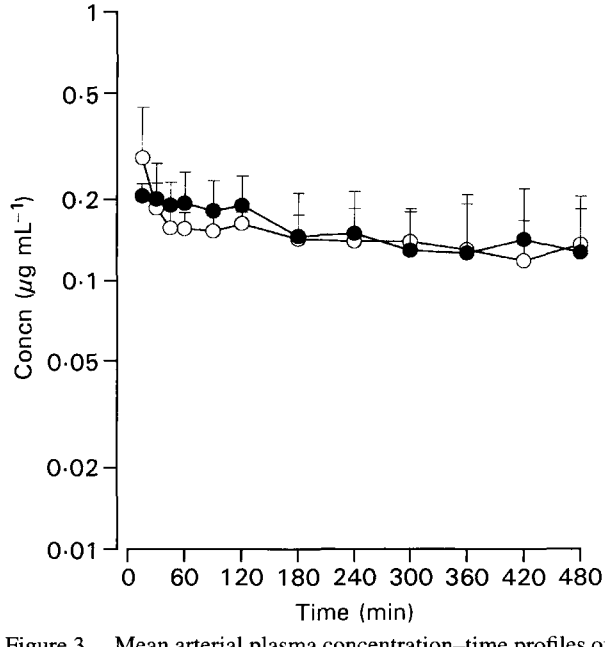

Figure 3. Mean arterial plasma concentration–time profiles of azosemide after oral administration of 10 mg kg^{-1} to rats at 1000 (●, n = 8) and 2200 h (○, n = 8). Vertical bars represent the standard deviation.

et al 1997). It is of interest to note that the pharmacodynamic parameters of azosemide listed in Table 2 were not significantly different after oral administration of the drug to both groups of rats.

In conclusion, urine output and urinary excretion of sodium and chloride were significantly higher after intravenous administration of azosemide to rats at 1000 h, however, the values were not significantly different after oral administration of the drug to both groups of rats. If this rat data could be extrapolated to man, the intravenous dose of azosemide could be modified on the basis of circadian time.

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