BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **19**: 357–364 (1998)

Pharmacokinetic and Pharmacodynamic Changes of Furosemide After Intravenous and Oral Administration to Rats with Alloxan-Induced Diabetes Mellitus

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ABSTRACT: Because some physiological changes occurring in diabetes mellitus patients could alter the pharmacokinetics and pharmacodynamics of the drugs to treat the disease, the pharmacokinetics and pharmacodynamics of furosemide were investigated after intravenous (i.v.) and oral administration of the drug (6 mg per whole body weight) to control rats and alloxan-induced diabetes mellitus rats (AIDRs). After i.v. administration, the total body clearance (5.47 versus 7.05 mL min^{-1} kg^{-1}) was significantly slower in AIDRs and this was due to significantly slower renal clearance (2.35 versus 4.33 mL min^{-1} kg^{-1}) because the nonrenal clearance was comparable between two groups of rats. The 8 h urinary excretion of furosemide after i.v. administration decreased significantly (2280 versus 3760 μg) in AIDRs due to impaired kidney function; the glomerular filtration rate measured by creatinine clearance was significantly slower (2.86 versus 4.33 mL min^{-1} kg^{-1}) and both the plasma urea nitrogen (43.5 versus 17.3 mg dL^{-1}) and kidney weight (0.953 versus 0.749% of body weight) increased significantly in AIDRs. This resulted in a significant decrease in the 8 h urine output per g kidney (17.8 versus 43.6 mL) in AIDRs. However, the 8 h diuretic efficiency was not significantly different between two groups of rats. After oral administration, the area under the plasma concentration–time curve from time 0 to 8 h decreased significantly in AIDRs (1200 versus 1910 μg·min mL^{-1}) due to considerably decreased absorption of furosemide from gastrointestinal tract of AIDRs. After oral administration, the 8 h urine output per g kidney (18.6 versus 36.4 mL) also decreased significantly in the AIDRs due to significantly decreased 8 h urinary excretion of furosemide (405 versus 2210 μg), however, the 8 h diuretic efficiency increased significantly (127 versus 35.2 mL mg^{-1}) in AIDRs. © 1998 John Wiley & Sons, Ltd.

Key words: furosemide; pharmacokinetics; pharmacodynamics; alloxan-induced diabetes mellitus rats

Introduction

Many diabetic patients develop serious complications during the course of the disease, including cardiovascular disorders, nephropathy, neuropathy, and retinopathy [1]. Thiazide diuretics are generally effective in treating early hypertension in diabetic patients [2], but when creatinine clearance (Cl_{cr}) is less than 30–35 mL min^{-1}, loop diuretics should be used [3]. Animal models of insulin-dependent diabetes mellitus by administration of a number of chemicals, principally alloxan, streptozotocin, and zinc chelators, have been reported [4].

Some physiological changes such as gastroparesis, decreased plasma albumin level, elevated plasma fatty acid level, glycosylation of plasma proteins, and changes in cytochrome P450 content were reported to occur in diabetes mellitus [1,5]. The above physiological changes in diabetes mellitus could alter the pharmacokinetics and hence the pharmacodynamics of drugs in such patients. The effects of diabetes mellitus on the pharmacokinetics or pharmacodynamics of some drugs in the patients or alloxan-induced diabetes mellitus rats (AIDRs) have been reviewed [1,5].

The pharmacokinetics and/or pharmacodynamics of furosemide, a loop diuretic, in humans [6–8] and animals [9–14] have been extensively studied, however, the effects of diabetes mellitus on the pharmacokinetics and pharmacodynamics of furosemide seemed not to be thoroughly studied. Pharmacokinetic and pharmacodynamic changes of azosemide, another loop diuretic, after its intravenous (i.v.) and oral administration to AIDRs have been reported [15] from our laboratory. The purpose of this study was to investigate the effect of alloxan-induced diabetes mellitus on the pharmacokinetics and pharmacodynamics of furosemide after its i.v. and oral administration to control rats and to AIDRs.

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CCC 0142–2782/98/060357–08$17.50

Received 12 March 1997
Accepted 17 October 1997

Materials and Methods

Chemicals

Furosemide i.v. solution (Lasix, 20 mg per 2 mL) was kindly supplied by Han Dok Pharmaceutical Company (Seoul, Republic of Korea), and 4-chloro-5-sulphamoyl anthranilic acid (CSA), a possible metabolite of furosemide, was purchased from US Pharmacopoeia (Rockville, MD, USA). Tris base, the reduced form of nicotinamide adenosine dinucleotide phosphate (NADPH), uridine diphosphoglucuronic acid (UDPGA), and alloxan were products of Sigma Chemical Company (St. Louis, MO, USA). Other chemicals were of reagent grade or high-performance liquid chromatographic (HPLC) grade and used without further purification.

Induction of Diabetics Mellitus in Rats by Alloxan Injection

Alloxan dissolved in 0.9% NaCl injectable solution, 40 mg kg^{-1}, was administered intravenously via the tail vein (total injection volume was approximately 0.2 mL) of the overnight-fasted Sprague–Dawley rats [15] (200–250 g, Central-Research Institute, Dong Shin Pharmaceutical Company, Pyungtak, Republic of Korea) for two consecutive days. On the third day, blood glucose level was measured and the rats with blood glucose levels higher than 200 mg dL^{-1} were chosen as AIDRs.

Intravenous Study

In the early morning on the fourth day after the start of alloxan treatment, the carotid artery and the jugular vein were catheterized individually with polyethylene tubing (Clay Adams, Parsippany, NJ, USA) under light ether anaesthesia. Both canulae were exteriorized to the dorsal side of the neck where each canula terminated individually with long Silastic tubing (Dow Corning, Midland, MI, USA). Both Silastic tubings were covered with a wire to allow free movement of the rat. Each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, Republic of Korea) and allowed to recover from anaesthesia for 4–5 h before the study began. They were not restrained at any time during the study. Water and electrolyte losses through urine induced by furosemide were replaced volume-by-volume by i.v. infusion of Ringer's lactate solution (Dai Han Pharmaceutical Company, Seoul, Republic of Korea) via the jugular vein for up to 8 h of the experiment, for it has been reported [16] that the pharmacodynamic effects of i.v. furosemide were dependent on the rate and composition of fluid replacement. Each litre of Ringer's lactate solution contained approximately 130 mmol sodium, 4 mmol potassium, 6 mmol calcium, 109 mmol chloride, and 28 mmol lactate. Food and water were restrained throughout the whole experimental period.

Lasix, 6 mg per whole body weight, was administered by i.v. infusion in 1 min via the jugular vein (total injection volume was 0.6 mL) of control rats ($n = 10$) or AIDRs ($n = 9$). Blood samples (0.12 mL) were 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, 300, 360 and 480 min after i.v. administration of furosemide. Heparinized 0.9% NaCl injectable solution (20 units mL^{-1}, 0.25 mL) was used to flush the cannula after each blood sampling to prevent blood clotting. Blood samples were centrifuged immediately to minimize the 'blood storage effect' (the change in plasma concentration of furosemide due to time elapsed between collection and centrifugation of the blood sample) of the plasma concentrations of furosemide [17], and a 0.05 mL aliquot of plasma was stored at $-20°C$ until the HPLC analysis of furosemide. At the end of 24 h after i.v. administration of furosemide, a large volume of blood was collected through the abdominal artery and each rat was sacrificed by cervical dislocation. The plasma sample was stored in the freezer ($-20°C$) until the analysis for creatinine, urea nitrogen, and glucose. After measuring the exact urine volume, each metabolic cage was rinsed with 10 mL of distilled water. The rinsings were combined with urine, and urinary bladder was cut and washed into the combined 24 h urine. After measuring the exact volume of the combined urine, an aliquot of the combined 24 h urine was collected and frozen ($-20°C$) until the analysis for furosemide, CSA, sodium, potassium, chloride, and creatinine. At the same time, the liver and kidney of each rat were excised and weighed. At the end of the experiment, the whole gastrointestinal (GI) tract (including its contents and faeces) was removed, transferred to a 500 mL beaker filled with 50 mL 0.01 M NaOH (to facilitate the extraction of furosemide [9]) and cut into small pieces with a pair of scissors. After vigorously shaking for 10 min, two 0.1 mL aliquots of the supernatant were collected from each beaker and stored in the freezer ($-20°C$) until the HPLC analysis of furosemide. All biological samples were protected from light [18,19] during collection and HPLC analysis.

Oral Study

Lasix, 6 mg per whole body weight, was administered orally in 10 s (total oral volume was 0.6 mL) to control rats ($n = 9$) and AIDRs ($n = 7$) using a feeding tubing after overnight fasting with water *ad libitum*. Blood samples were collected via the carotid artery at 0 (to serve as a control), 15, 30, 45, 60, 90, 120, 180, 240, 300, and 480 min after oral administration of furosemide. The other procedures were similar to those of the i.v. study.

Plasma Protein Binding Studies

The plasma protein binding of furosemide was determined by the equilibrium dialysis technique using plasma from additional control rats ($n = 4$) and AIDRs ($n = 4$). One millilitre of the plasma was dialyzed against 1 mL of isotonic Sørensen phosphate buffer of pH 7.4 containing 3% dextran (to minimize volume shifts [20,21]) using 1 mL dialysis cells (Fisher Scientific Company, Fair Lawn, NJ, USA). To reduce equilibration time, furosemide was spiked into plasma side [22] with an initial plasma concentration of 10 μg mL^{-1}. The spiked dialysis cells were incubated for 24 h in a water-bath shaker kept at 37°C and at a rate of 50 oscillations min^{-1} (opm). The plasma protein binding of furosemide has been reported [23] to be relatively constant up to furosemide concentrations of 36 μg mL^{-1} using the equilibrium dialysis method.

Metabolism in Homogenate of Rat Stomach, Liver and Kidney

The procedures were similar to the method [9,10] reported by Litterst *et al*. [24]. In the early morning of the fourth day after alloxan treatment, additional control rats ($n = 5$) and AIDRs ($n = 5$) were sacrificed by cervical dislocation. One gram of each stomach, liver, and kidney was excised, rinsed in 50 mmol of Tris–HCl buffer (pH 7.4), blotted dry with tissue paper, and weighed. All subsequent procedures were conducted at 4°C. Each tissue was minced into small pieces with a pair of scissors and then homogenized with 4 volumes of cold 0.25 M sucrose in a tissue homogenizer (Ultra-Turrax T25, Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany). Each homogenate was then centrifuged (Beckman Model J2-21, Palo Alto, CA, USA) at $9000 \times g$ for 20 min. After discarding the floating fat layer the $9000 \times g$ supernatant fraction was collected.

Metabolic activity was initiated by adding 1 mL of the above $9000 \times g$ supernatant to a glass test tube containing 25 μL of Lasix solution (diluted with 0.9% NaCl injectable solution, 50 μg of furosemide), 100 μL of NADPH (1 mM), 1.9 mL of pH 7.4 Tris–HCl buffer (100 mM), and 25 μL of UDPGA (3.3 mM). The mixture was then thoroughly mixed by hand and shaken in a water-bath shaker kept at 37°C and at the rate of 50 opm. After 30 min of incubation, 1 mL 1 M NaOH was added to terminate enzyme activity and stored in the freezer (−20°C) until the HPLC analysis of furosemide.

Analytical Procedure

The concentrations of furosemide and CSA were determined by the reported sensitive HPLC methods [9]. Sample preparation was simple: a 2.5 volume of acetonitrile was added to the biological samples, and for the tissue homogenate, 0.1 mL aliquot of 0.3% HCl was added after addition of the 2.5 volume of acetonitrile [11]. After vortex-mixing and centrifugation, an aliquot of the supernatant was injected directly onto the reversed-phase column. Therefore, the formation of CSA in the present rat urine sample was neither due to photodegradation [18,19] nor an artifact in the process of acid extraction for the sample preparation [25].

Concentrations of creatinine in plasma and urine, and of glucose and urea nitrogen in plasma were determined using the Abbot Spectrum (High Performance Diagnostic System, Irving, TX, USA) and those of sodium, potassium, and chloride in urine were determined using the Lytening System 30 (Na/K/Cl Instant ISE Analyzer, Baxter Lytening System, Danvers, MA, USA).

Pharmacokinetic Analysis

The area under the plasma concentration–time curve from time zero to time infinity (AUC the for the i.v. studies) or to 8 h ($AUC_{0-8\ h}$ for the oral studies) was calculated by the trapezoidal rule-extrapolation method [10]; this method employed the logarithmic trapezoidal rule recommended by Chiou [26] for the calculation of 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 plasma concentration by the terminal rate constant.

Standard method [27] was used to calculate the following pharmacokinetic parameters after i.v. administration [10]; the time-averaged total body clearance (Cl), area under the first moment of the plasma concentration–time curve (AUMC), mean residence time (MRT), apparent volume of distribution at steady state (V_{ss}), and time-averaged renal (Cl_r) and nonrenal (Cl_{nr}) clearances:

$$Cl = dose/AUC, \tag{1}$$

$$AUMC = \int_0^\infty t \cdot C_p \, dt, \tag{2}$$

$$MRT = AUMC/AUC, \tag{3}$$

$$V_{ss} = Cl \cdot MRT, \tag{4}$$

$$Cl_r = X_{u_{fu}}/AUC, \tag{5}$$

$$Cl_{nr} = Cl - Cl_r, \tag{6}$$

where C_p is the plasma concentration of furosemide at time t and $X_{u_{fu}}$ is the amount of furosemide excreted in urine for up to time infinity. In the estimation of Cl_r after i.v. administration, the amount of furosemide excreted in urine for up to

time infinity was assumed to equal the total amount excreted in 24 h, since negligible amounts of furosemide could be found in urine collected later. The total AUC instead of $AUC_{0-24\ h}$ (AUC from time 0 to 24 h) was employed for the calculation of Cl_r after i.v. studies, since the mean contribution of AUC from the last measured time to time infinity to the total AUC was lower than 0.392 and 0.898% for control rats and AIDRs, respectively. The $X_{u_{0-8\ h}}$ and $AUC_{0-8\ h}$ were employed for the estimation of Cl_r after oral administration. The Cl_{cr} was estimated assuming that the kidney function was stable during the study.

The mean values of terminal half-life [28], V_{ss} [29], and each clearance [30] were calculated by the harmonic mean method.

Pharmacodynamic Analysis

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

Statistical Analysis

Levels of statistical significance were assessed using the *t*-test between two means for unpaired data. Significant differences were judged as $p < 0.05$. All results are expressed as mean $\pm$ standard deviation (S.D.).

Results and Discussion

The mean arterial plasma concentration–time curves of furosemide after i.v. administration to control rats and AIDRs are shown in Figure 1, and some relevant pharmacokinetic parameters are listed in Table 1. After i.v. administration, the plasma levels of furosemide declined polyexponentially with significantly higher levels in AIDRs than those in control rats (Figure 1), and this resulted in a significantly higher AUC (4280 versus 3190 μg min mL^{-1}) in AIDRs (Table 1). As expected from the AUC values, Cl was significantly slower (5.47 versus 7.05 mL min^{-1} kg^{-1}) in AIDRs compared with that in control rats, and this was due to significantly slower Cl_r (2.35 versus 4.33 mL min^{-1} kg^{-1}) in AIDRs since Cl_{nr} was not significantly different between two groups of rats (Table 1). Since it has been reported that the Cl of furosemide was dose [31] or concentration [32] dependent in rats, and Cl_r of furosemide was urine flow-dependent in rabbits [33], the values of Cl, Cl_r, and Cl_{nr} listed in Table 1 were time-averaged values. The amount (2280 versus 3760 μg) and percentages of i.v. dose excreted in 8 h urine ($X_{u_{fu,\,0-8\,h}}$) as unchanged furosemide decreased significantly (38.1 versus 62.8%) in AIDRs, and similar results were also obtained from both $X_{u_{fu,\,0-24\,h}}$ (2610 versus 3770 μg and 43.5 versus 62.9% of i.v. dose) and $X_{u_{CSA,\,0-8\,h}}$ (1120 veusus 1870 μg and 18.7 versus 31.1% of i.v. dose expressed in terms of furosemide) as listed in Table 1. This could be due to impaired kidney function brought about by alloxan treatment [34]; 'kidney is perhaps next in importance to the pancreas as a site of lesions in alloxan diabetes and the severest damage occurs in the convoluted tubules and appears to be generally proportional to the size of the dose.' The impaired kidney function in the present AIDRs was also supported by the significantly slower glomerular filtration rate (GFR) as measured by Cl_{cr} (2.86 versus 4.33 mL min^{-1} kg^{-1}) and the significant increase in both plasma urea nitrogen (43.5 versus 17.3 mg dL^{-1}) and kidney weight (0.953 versus 0.749% of body weight) in AIDRs (Table 1). Furosemide resided longer in AIDRs than that in control rats; the MRT increased significantly (32.7 versus 17.8 min) in AIDRs (Table 1). However, the values of V_{ss} and terminal $t_{1/2}$ were not significantly different between two groups of rats (Table 1). The amount of glucuronide formation of furosemide was not measured in the present rat urine since the glucuronide formation of furosemide were reported to be negligible (less than 3% of i.v. dose expressed in terms of furosemide) in rats [9–11], although the glucuronide formation of furosemide was reported in humans [35], dogs [36], and rabbits (unpublished data). The exact reason for the negligible formation of the glucuronide of furosemide in rats is unknown; however, it may be due to inhibition of

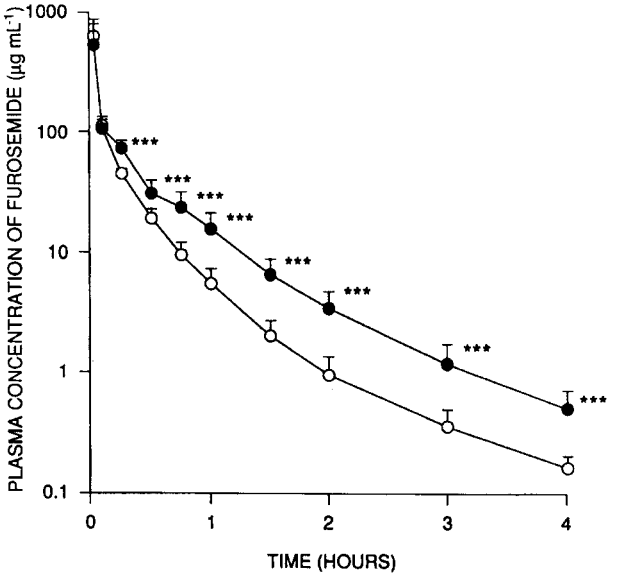

Figure 1. Mean arterial plasma concentration–time profiles of furosemide after 1 min intravenous infusion of furosemide (6 mg per whole body weight) to control rats (○, $n = 10$) and AIDRs (●, $n = 9$). Bars represent standard deviation. *** $p < 0.001$

Table 1. Mean ($\pm$ standard deviation) pharmacokinetic and pharmacodynamic parameters of furosemide after 1 min intravenous (i.v.) infusion and oral administration of furosemide (6 mg per whole body weight) to control rats and alloxan-induced diabetes mellitus rats (AlDRs)

Parameters	Intravenous administration		Oral administration	
	Control rats ($n = 10$)	AIDRs ($n = 9$)	Control rats ($n = 9$)	AIDRs ($n = 7$)
Body weight (BW, g)	271 ± 18.0	257 ± 12.7	243 ± 16.3	221 ± 35.5
AUC or $AUC^{a}_{0-8\ h}$ ($\mu g \cdot min\ mL^{-1}$)	3190 ± 424	4280 ± 1210*	1910 ± 555	1200 ± 570*
TeIminal $t_{1/2}$ (min)	44.8 ± 6.79	44.5 ± 5.20		
MRT (min)	17.8 ± 3.77	32.7 ± 6.48***		
V_{ss} (mL kg^{-1})	119 ± 32.7	168 ± 80.1		
$X_{u_{fu,\ 0-8\ h}}$ (μg)	3760 ± 656	2280 ± 307***	2210 ± 546	405 ± 235***
$X_{u_{fu,\ 0-8\ h}}$ (% of i.v. dose)	62.8 ± 11.6	38.1 ± 5.12***	36.9 ± 9.10	6.74 ± 3.92***
$X_{u_{fu,\ 0-24\ h}}$ (μg)	3770 ± 695	2610 ± 459***	2420 ± 585	495 ± 247***
$X_{u_{fu,\ 0-24\ h}}$ (% of i.v. dose)	62.9 ± 11.6	43.5 ± 8.11***	40.3 ± 9.75	8.25 ± 4.11***
$X_{u_{CSA,\ 0-8\ h}}$[b] (μg)	1870 ± 837	1120 ± 357*	1160 ± 306	732 ± 314*
$X_{u_{CSA,\ 0-8\ h}}$[b] (% of i.v. dose)	31.1 ± 14.0	18.7 ± 5.94*	19.3 ± 5.11	12.2 ± 5.23*
Cl (mL min^{-1} kg^{-1})	7.05 ± 0.868	5.47 ± 1.69*		
Cl_r (mL min^{-1} kg^{-1})	4.33 ± 1.16	2.35 ± 0.632***	4.57 ± 2.20	1.02 ± 1.09**
Cl_{nr} (mL min^{-1} kg^{-1})	2.20 ± 0.516	2.98 ± 1.30		
Cl^{c}_{cr} (mL min^{-1} kg^{-1})	4.33 ± 1.24	2.86 ± 0.656***	3.40 ± 1.93	1.57 ± 4.72
% of dose recovered from gastrointestinal tract as intact furosemide at 24 h	0.412 ± 0.264	0.414 ± 0.0806	8.27 ± 4.70	16.3 ± 9.54*
Plasma glucose (mg dL^{-1})	161 ± 10.6	394 ± 91.2***	158 ± 14.8	520 ± 344**
Plasma urea nitrogen (mg dL^{-1})	17.3 ± 7.93	43.5 ± 10.3***	31.6 ± 13.6	78.1 ± 42.2**
$UO_{0-8\ h}$ (mL g^{-1} kidney)	43.6 ± 13.6	17.8 ± 4.98***	36.4 ± 12.7	18.6 ± 13.0*
$X_{u_{Na+,\ 0-8\ h}}$ (mmol g^{-1} kidney)	5.11 ± 1.49	1.90 ± 0.581***	4.18 ± 1.75	2.29 ± 1.80
$X_{u_{K+,\ 0-8\ h}}$ (mmol g^{-1} kidney)	0.762 ± 0.169	0.311 ± 0.0602***	0.494 ± 0.149	0.387 ± 0.125
$X_{u_{Cl-,\ 0-8\ h}}$ (mmol g^{-1} kidney)	5.19 ± 1.38	2.19 ± 0.574***	4.30 ± 1.65	2.44 ± 1.73*
Diuretic $efficiency_{0-8\ h}$ (mL mg^{-1})	24.1 ± 8.84	18.8 ± 4.30	35.2 ± 21.2	127 ± 120*
Natriuretic $efficiency_{0-8\ h}$ (mmol mg^{-1})	2.85 ± 1.07	2.05 ± 0.616	4.04 ± 2.70	14.8 ± 7.00***
Kaluretic $efficiency_{0-8\ h}$ (mmol mg^{-1})	0.434 ± 0.184	0.339 ± 0.0740	0.475 ± 0.264	3.56 ± 2.94**
Chloruretic $efficiency_{0-8\ h}$ (mmol mg^{-1})	2.89 ± 1.04	2.37 ± 0.630	4.14 ± 2.59	16.1 ± 6.86***
Liver weight (% of BW)	3.27 ± 0.189	3.66 ± 0.404	3.30 ± 0.260	3.36 ± 0.393
Kidney weight (% of BW)	0.749 ± 0.0657	0.953 ± 0.0628***	0.808 ± 0.0509	1.10 ± 0.133***

fu, furosemide: CSA, 4-chloro-5-sulphamoylanthranilic acid.
[a] AUC for the i.v. studies and $AUC_{0-8\ h}$ tor the oral studies.
[b] Expressed in terms of furosemide.
[c] Creatinine clearance.
* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with control.

UDP-glucuronyltransferase activity by furosemide in rats [37]. Note that there is controversy on the formation of furosemide metabolites. CSA was not detected in human urine, but was detected in rat urine, and glucuronide was detected in human urine after oral administration of furosemide [14].

The contribution of biliary and/or GI excretion of furosemide to Cl_{nr} after i.v. administration to rats seemed to be minor, since 0.412 and 0.414% of i.v. dose were recovered as intact furosemide from the GI tract at 24 h after i.v. dose in control rats and AIDRs, respectively (Table 1). Similar results were also reported; less than 5% of i.v. dose was excreted in bile [38] or recovered from GI tract [9,12,14,15] after i.v. administration of furosemide to rats. Therefore, the Cl_{nr} could represent the metabolism of furosemide to rats. The Cl_{nr} was not significantly different between two groups of rats (Table 1), indicating that metabolism of furosemide was not affected by alloxan-induced diabetes mellitus in rats. This could be supported at least in part by the result of the *in vitro* incubation of 50 μg furosemide in the $9000 \times g$ supernatant fraction of rat stomach, kidney, and liver homogenate ($n = 5$); the mean amount of furosemide remaining per gram rat stomach (43.3 ± 2.44 versus 42.7 ± 0.73 μg), kidney (48.2 ± 4.03 versus 49.7 ± 3.58 μg), and liver (44.4 ± 2.21 versus 45.5 ± 1.54 μg) after 30 min of incubation were not significantly different between two groups of rats. The weight of liver per body weight was also not significantly different between two groups of rats (Table 1). Note that the induction of diabetes mellitus was evident by administration of alloxan to rats; the mean plasma glucose level measured at the end of the experiment, 394 mg dL^{-1}, in AIDRs was significantly higher than in control rats, 161 mg dL^{-1} (Table 1).

The mean arterial plasma concentration–time curves of furosemide after oral administration to control rats and AIDRs are shown in Figure 2, and some relevant pharmacokinetic parameters are also listed in Table 1. The plasma concentrations of furosemide in AIDRs seemed to be lower for up to 6 h than in control rats (Figure 2), and this resulted in a significant decrease in $AUC_{0-8\ h}$ (1200 versus 1910 μg min mL^{-1}) in AIDRs (Table 1). Note that after i.v. administration of furosemide to rats, the AUC was significantly higher in AIDRs (Table 1). Therefore, significant decrease in $AUC_{0-8\ h}$ after oral administration of furosemide to AIDRs could be due to decreased absorption of furosemide from GI tract of AIDRs; the percentages of oral dose recovered from GI tract at 24 h as intact furosemide were 8.27 and 16.3% ($p < 0.05$) for control rats and AIDRs, respectively (Table 1). Furosemide was reported to be stable in human gastric juice [9,35] and in duodenal fluids [35]. It has also been reported [1] that 'the rate and extent of absorption of drugs given orally could be expected to be altered in diabetes mellitus patients; disorders of GI tracts, such as diarrhoea, constipation, and delayed gastric emptying occurred due to the gastroparesis in as many as 20% of diabetic patients who have had the disease for several years.' As expected from the significantly slower GFR as measured by Cl_{cr} (1.57 versus 3.40 mL min^{-1} kg^{-1}), the significant increase in both plasma urea nitrogen (78.1 versus 31.6 mg dL^{-1}) and kidney weight (1.10 versus 0.808% of body weight) in AIDRs, the $X_{u_{fu,\,0-8\,h}}$ also decreased significantly (405 versus 2210 μg and 6.74 versus 36.9% of oral dose) in AIDRs and similar results was also obtained from both $X_{u_{fu,\,0-24\,h}}$ (495 versus 2420 μg and 8.25 versus 40.3% of oral dose) and $X_{u_{CSA,\,0-8\,h}}$ (732 versus 1160 μg and 12.2 versus 19.3% of oral dose expressed in terms of furosemide) as listed in Table 1. As shown in the i.v. study, the Cl_r of furosemide was also significantly slower after oral administration (1.02 versus 4.57 mL min^{-1} kg^{-1}) by treatment with alloxan (AIDRs). The mean percentages of oral dose recovered from GI tract at 24 h after oral dose in control rats were 8.27% (Table 1), and a comparable value, 13.3% [12] has also been reported. However, this value was considerably lower than the values, 40.1 [10] and 40.3% [9] which are estimated at 8 h after oral administration of furosemide to Sprague–Dawley rats. The above data suggested that some of the orally administrated furosemide could be absorbed from GI tract between 8 and 24 h after oral administration. The absorption of furosemide from rat stomach, duodenum, jejunum, ileum, and large intestine has been reported [14] from our laboratory. Note that the plasma glucose increased significantly (520 versus 158 mg dL^{-1}) in AIDRs (Table 1).

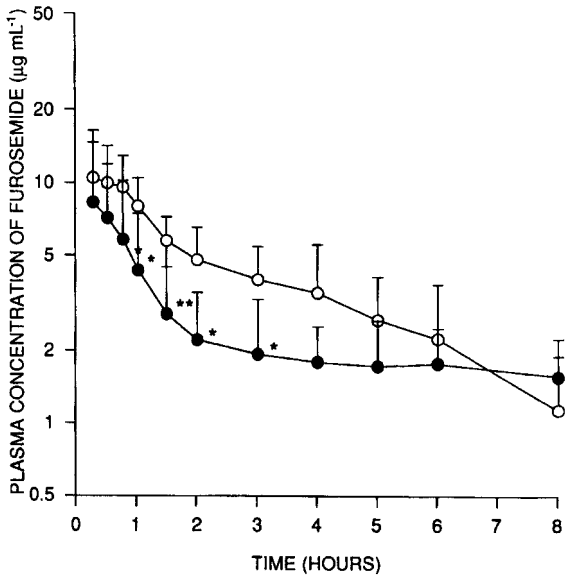

Figure 2. Mean arterial plasma concentration–time profiles of furosemide after oral administration of furosemide (6 mg per whole body weight) to control rats (○, $n = 9$) and AIDRs (●, $n = 7$). Bars represent standard deviation. * $p < 0.05$ and ** $p < 0.01$

The pharmacodynamic parameters of furosemide after i.v. administration to control rats and AIDRs are also listed in Table 1. Since 99.5 and 87.4% of the 24 h urinary excretion of furosemide were excreted in 8 h urine after i.v. administration to control rats and AIDRs, respectively (the corresponding values were 91.3 and 81.8% after oral administration), and the loss of water and electrolytes in urine induced by furosemide was replaced with Ringer's lactate solution for up to 8 h for both i.v. and oral studies, the proceeding diuretic effect will be confined to 8 h data. The 8 h urine output per gram kidney ($UO_{0-8\ h}$) decreased significantly (17.8 versus 43.6 mL) in AIDRs compared with that in control rats (Table 1). Some factors could be proposed to explain the above phenomena. First, the decreased urinary excretion of CSA ($X_{u_{CSA,\,0-8\,h}}$, Table 1) which may have diuretic effect after i.v. administration of furosemide to AIDRs could have been a factor. However, this was ruled out because CSA did not cause considerable diuretic effect in rabbits (unpublished data). Second, the decreased urinary excretion of furosemide glucuronide in AIDRs could have been another factor. But this was also ruled out since glucuronide formation of furosemide was negligible in rats as discussed earlier. However, the possibility of decreased urinary excretion of other metabolite(s) of furosemide having diuretic effect in AIDRs could not be totally ruled out. Third, the changes in plasma protein binding of furosemide in AIDRs could have been one of the factors. Again, this was unlikely because the values of plasma protein binding of furosemide were 89.6 and 89.5% for control rats and AIDRs, respectively. Fourth, the decreased GFR as measured by Cl_{cr} in AIDRs (Table 1) could have been one of the factors. Again, this was also unlikely because it has been reported [39] that the

majority of furosemide excreted in the urine is delivered by active secretion rather than passive filtration when considering the high plasma protein binding of furosemide. In the present i.v. study, the Cl_r of furosemide estimated based on unbound furosemide was approximately 9.62 and 7.85 times higher than that of Cl_{cr} for control rats and AIDRs, respectively. Finally, the above phenomena could be due to significantly reduced amount of furosemide excreted in 8 h urine (2280 versus 3760 μg) in AIDRs. The total amount of sodium ($X_{u_{Na^+,0-8\,h}}$, 1.90 versus 5.11 mmol), chloride ($X_{u_{Cl^-,0-8\,h}}$, 2.19 versus 5.19 mmol), and potassium ($X_{u_{K^+,0-8\,h}}$, 0.311 versus 0.762 mmol) excreted in 8 h urine per gram kidney were also decreased significantly in AIDRs compared with control rats (Table 1). However, the 8 h diuretic, natriuretic, kaluretic, and chloruretic efficiencies were not significantly different between two groups of rats (Table 1).

The pharmacodynamic parameters of furosemide after oral administration are also listed in Table 1. The $UO_{0-8\ h}$ decreased significantly (18.6 versus 36.4 mL g^{-1} kidney) in AIDRs and this could be due to significant decrease in the amount of furosemide excreted in 8 h urine (405 versus 2210 μg, Table 1) in AIDRs as discussed in the i.v. studies. The amount of sodium and potassium excreted in 8 h urine were not significantly different between two groups of rats. However, the 8 h diuretic, natriuretic, kaluretic, and chloruretic efficiencies increased significantly in AIDRs (Table 1).

In conclusion, the 8 h urine output decreased significantly after both i.v. and oral administration of furosemide to AIDRs compared with control rats. The above results might suggest (if they can be extrapolated and applied to humans) that both the i.v. and oral dose of furosemide for diabetes mellitus patients would require some modifications.

Acknowledgements

This work was supported in part by the Korea Science and Engineering Foundation (KOSEF) through the Research Center of New Drug Development (KOSEF-RCNDD) at Seoul National University.

References

1. P.R. Gwilt, R.R. Nahhas and W.G. Tracewell, The effects of diabetes mellitus on pharmacokinetics and pharmacodynamics in humans. *Clin. Pharmacokinet.*, **20**, 477–490 (1991).
2. J.C. Joseph and A.A. Schuna, Management of hypertension in the diabetic patient. *Clin. Pharm.*, **9**, 864–873 (1990).
3. M.C. Houston, Adverse effect of antihypertensive drug therapy on glucose tolerance. *Cardiovas. Clin.*, **4**, 117–135 (1986).
4. J.C. Pickup and G. Williams, *Textbook of Diabetes*, Vol. 1, Blackwell Scientific Publication, Oxford, UK, 1991, pp. 151–155.
5. P. O'Connor and J. Feely, Clinical pharmacokinetics and endocrine disorders, therapeutic implications. *Clin. Pharmacokinet.*, **13**, 345–364 (1987).
6. L.Z. Benet, Pharmacokinetics/pharmacodynamics of furosemide in man: A review. *J. Pharmacokinet. Biopharm.*, **7**, 1–27 (1979).
7. R.E. Cutler and A.D. Blair, Clinical pharmacokinetics of furosemide, *Clin. Pharmacokinet.*, **4**, 279–296 (1979).
8. M. Hammerlund-Udenaes and L.Z. Benet, Furosemide pharmacokinetics and pharmacodynamics in health and disease-An update. *J. Pharmacokinet. Biopharm.*, **17**, 1–46 (1989).
9. M.G. Lee and W.L. Chiou, Evaluation of potential causes for the incomplete bioavailability of furosemide: Gastric first-pass metabolism. *J. Pharmacokinet. Biopharm.*, **11**, 623–640 (1983).
10. S.H. Kim, Y.M. Choi and M.G. Lee, Pharmacokinetics and pharmacodynamics of furosemide in protein-calorie malnutrition. *J. Pharmacokinet. Biopharm.*, **21**, 1–17 (1993).
11. Y.M. Choi, S.H. Kim and M.G. Lee, Effects of phenobarbital and 3-methylcholanthrene pretreatment on the pharmacokinetics and pharmacodynamics of furosemide in rats. *J. Pharm. Sci.*, **80**, 638–642 (1991).
12. S.H. Jang, M.G. Lee and N.D. Kim, Pharmacokinetics and pharmacodynamics of furosemide after intravenous and oral administration to spontaneously hypertensive rats and DOCA-salt induced hypertensive rats. *Biopharm. Drug Dispos.*, **15**, 185–206 (1994)
13. M.J. Kang, W.H. Yoon, O.N. Kim and M.G. Lee, Effects of water deprivation for 48 hours on the pharmacokinetics and pharmacodynamics of furosemide in rats, *J. Clin. Pharm. Ther.*, **20**, 13–21 (1995).
14. W.I. Lee, W.H. Yoon, W.G. Shin, I.S. Song and M.G. Lee, Pharmacokinetics and pharmacodynamics of furosemide after direct administration into the stomach or duodenum, *Biopharm. Drug Dispos.*, **18**, 753–767 (1997).
15. K.J. Park, W.H. Yoon, W.G. Shin and M.G. Lee, Pharmacokinetics and pharmacodynamics of azosemide after intravenous and oral administration to rats with alloxan-induced diabetes mellitus. *J. Pharm. Pharmacol.*, **48**, 1093–1097 (1996).
16. T. Li, M.G. Lee and W.L. Chiou, Effects of the rate and composition of fluid replacement on the pharmacokinetics and pharmacodynamics of intravenous furosemide. *J. Pharmacokinet. Biopharm.*, **14**, 495–509 (1986).
17. M.G. Lee, M.-L. Chen and W.L. Chiou, Pharmacokinetics of drugs in blood II: Unusual distribution and storage effect of furosemide. *Res. Commun. Chem. Pathol. Pharmacol.*, **34**, 17–23 (1981).
18. A.L.M. Kerremans, Y. Yan, C.A.M. van Ginneken and F.W.L. Gribnan, Specimen handling and high-performance liquid chromatographic determination of furosemide. *J. Chromatogr.*, **229**, 129–139 (1982).
19. D.E. Moore and V. Sithipitaks, Photolytic degradation of furosemide. *J. Pharm. Pharmacol.*, **35**, 489–493 (1982).
20. H.J. Shim, M.G. Lee and M.H. Lee, Factors influencing the protein binding of bumetanide using an equilibrium dialysis technique. *J. Clin. Pharm. Ther.*, **16**, 467–476 (1991).
21. W.G. Shin, M.G. Lee, M.H. Lee and N.D. Kim, Factors influencing the protein binding of vancomycin. *Biopharm. Drug Dispos.*, **12**, 637–646 (1991).
22. S. Øie and T.W. Guentert, Comparison of equilibrium time in dialysis experiments using spiked plasma or spiked buffer. *J. Pharm. Sci.*, **71**, 127–128 (1982).
23. J. Prandota and A.W. Pruitt, Furosemide binding to human albumin and plasma of nephrotic children. *Clin. Pharmacol. Ther.*, **17**, 159–165 (1975).
24. C.L. Litterst, E.G. Mimnaugh, R.I. Reagan and T.E. Gram, Comparison of *in vitro* drug metabolism by lung, liver and kidney of several common laboratory species. *Drug Metab. Dispos.*, **3**, 259–265 (1975).

25. D.E. Smith, E.T. Lin and L.Z. Benet, Absorption and disposition of furosemide in healthy volunteers, measured with a metabolic-specific assay. *Drug Metab. Dispos.*, **8**, 337–342 (1980).
26. W.L. Chiou, Critical evalution 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 (1978).
27. M. Gibaldi and D. Perrier, *Pharmacokinetics*, 2nd edn, Dekker, New York, 1982.
28. F.B. Eatman, W.A. Colburn, H.G. Boxenbaum, H.N. Posmanter, R.E. Weinfeld, R. Ronfeld, L. Weissman, J.D. Moore, M. Gibaldi and S.A. Kaplan, Pharmacokinetics of diazepam following multiple dose oral administration to healthy human subjects. *J. Pharmacokinet. Biopharm.*, **5**, 481–494 (1977).
29. W.L. Chiou, New calculation method for mean apparent drug volume of distribution and application to rationale dosage regimens. *J. Pharm. Sci.*, **68**, 1067–1069 (1979).
30. W.L. Chiou, New calculation method of mean total body clearance of drugs and its application to dosage regimens. *J. Pharm. Sci.*, **69**, 90–91 (1980).
31. M.M. Hammarlund and L.K. Paalzow, Dose-dependent pharmacokinetics of furosemide in the rat, *Biopharm. Drug Disposit.*, **3**, 345–359 (1982).
32. D.E. Smith and L.Z. Benet, Relationship between urinary excretion rate, steady-state plasma levels and diuretic response of furosemide in the rat, *Pharmacology*, **19**, 301–306 (1979).
33. W.L. Chiou, A new simple approach to study the effect of changes in urine flow and/or urine pH on renal clearance and its applications. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **24**, 519–529 (1986).
34. S. Warren, P.M. LeCompte and M.A. Legg, *The Pathology of Diabetes Mellitus*, 4th edn, Lea and Febiger, Philadelphia, PA, 1966, pp. 458–467.
35. B. Beerman, E. Dálen, B. Lindström and A. Rosén, On the fate of furosemide in man, *Eur. J. Clin. Pharmacol.*, **9**, 57–61 (1975).
36. G.J. Yakatan, D.D. Maness, J. Scholler, J.T. Johnson, W.J. Noveck, Jr. and J.T. Doluisio, Plasma and tissue levels of furosemide in dogs and monkeys following single and multiple oral doses, *Res. Commun. Chem. Pathol. Pharmacol.*, **24**, 456–482 (1979).
37. F. Sorgel, F.E. Beyhl and E. Mutschler, Inhibition of uridine diphosphate glucuronyltransferase caused by furosemide, *Experimentia*, **36**, 861–863 (1980).
38. S. Inui, H. Yamamoto, H. Nakae and S. Asada, Dose dependency of loop diuretics, furosemide and piretanide in the rat, *Yakugaku Zasshi*, **102**, 1053–1060 (1982).
39. L.L. Boles Ponto and R.D. Schoenwald, A pharmacokinetic/pharmacodynamic review (part I and II). *Clin. Pharmacokinet.*, **18**, 381–408, 460–471 (1990).