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Research Paper

Effects of cytochrome P450 inducers and inhibitors on the pharmacokinetics of intravenous furosemide in rats: involvement of CYP2C11, 2E1, 3A1 and 3A2 in furosemide metabolism

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Abstract

Objectives It has been reported that the non-renal clearance of furosemide was significantly faster in rats pretreated with phenobarbital but was not altered in rats pretreated with 3-methylcholanthrene. However, no studies on other cytochrome P450 (CYP) isozymes have yet been reported in rats.

Method Furosemide 20 mg/kg was administered intravenously to rats pretreated with various CYP inducers – 3-methylcholanthrene, orphenadrine citrate and isoniazid, inducers of CYP1A1/2, 2B1/2 and 2E1, respectively, in rats – and inhibitors – SKF-525A (a non-specific inhibitor of CYP isozymes), sulfaphenazole, cimetidine, quinine hydrochloride and troleandomycin, inhibitors of CYP2C6, 2C11, 2D and 3A1/2, respectively, in rats.

Key findings The non-renal clearance of furosemide was significantly faster (55.9% increase) in rats pretreated with isoniazid, but slower in those pretreated with cimetidine or troleandomycin (38.5% and 22.7% decreases, respectively), than controls. After incubation of furosemide with baculovirus-infected insect cells expressing CYP2C11, 2E1, 3A1 or 3A2, furosemide was metabolized via CYP2C11, 2E1, 3A1 and 3A2.

Conclusions These findings could help explain possible pharmacokinetic changes of furosemide in various rat disease models (where CYP2C11, 2E1, 3A1 and/or CYP3A2 are altered) and drug–drug interactions between furosemide and other drugs (mainly metabolized via CYP2C11, 2E1, 3A1 and/or 3A2).

Keywords enzyme inducers and inhibitors; furosemide; pharmacokinetics; rats

Introduction

Furosemide, a loop diuretic, is widely used for the treatment of ascites and oedema of cardiac, renal or hepatic origin, and also hypertension; approximately 50% of intravenous furosemide is excreted via the kidney.[1,2] Furosemide is metabolized solely to a glucuronide conjugate in humans.[3] Glucuronide formation of furosemide has also been reported in dogs[4] and rabbits.[5] 4-Chloro-5-sulfamoylanthranilic acid was found in rats.[6]

There have been a few reports on the metabolism of furosemide via hepatic microsomal cytochrome P450 (CYP) isozymes in humans and rats. For example, the time-averaged non-renal clearance (CL_{NR}) of furosemide tended to be faster (but not significantly so) among smokers (3-methylcholanthrene (3-MC) type; a main inducer of CYP1A1/2).[7] After intravenous administration of furosemide at a dose of 2 mg to rats pretreated with 3-MC or phenobarbital (inducers of CYP1A1/2 and 2B1/2, respectively, in rats),[8] the CL_{NR} of furosemide was significantly faster (34.9% increase) in phenobarbital-treated rats, but was not altered in 3-MC-treated rats.[9] The oxidation of furosemide by rat hepatic microsomes has been reported.[10] Also, formation of the γ-ketocarboxylic acid metabolite of furosemide was CYP-dependent in rats.[11] However, the CYP isozymes responsible for the metabolism of furosemide in rats *in vivo* does not seem to be have been thoroughly studied. Thus, in the present study, furosemide at a dose of 20 mg/kg was infused for 1 min to rats pretreated with the CYP inducers 3-MC, orphenadrine citrate or isoniazid (inducers of CYP1A1/2, 2B1/2 and 2E1, respectively, in

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rats[8]) or the inhibitors SKF-525A (a non-specific inhibitor of CYP isozymes), sulfaphenazole, cimetidine, quinine hydrochloride or troleandomycin (inhibitors of CYP2C6, 2C11, 2D, 3A1 and 3A2, respectively, in rats[8,12–14]) and untreated control rats. Furosemide was also incubated with microsomes from baculovirus-infected insect cells expressing CYP2C11, 2E1, 3A1 and 3A2.

The purpose of this study was to report the involvement of hepatic CYP2C11, 2E1, 3A1 and 3A2 in the metabolism of furosemide in male Sprague-Dawley rats.

Materials and Methods

Chemicals

Furosemide intravenous solution (Lasix; 2 ml ampoule; 10 mg/ml) and telithromycin (internal standard for the HPLC analysis of furosemide) were from Han Dok Pharmaceutical Company (Seoul, South Korea) and Sanofi-Aventis (Paris, France), respectively. NADPH as a trisodium salt, 3-MC, orphenadrine citrate, isoniazid, cimetidine, SKF-525A, sulfaphenazole, quinine hydrochloride and troleandomycin were purchased from Sigma-Aldrich Corporation (St Louis, MO, US). Microsomes from baculovirus-infected insect cells expressing CYP2C11, 2E1, 3A1 and 3A2 (Supersomes) were obtained from Gentest Corp. (Woburn, MA, US). Other chemicals were of reagent or HPLC grade.

Animals

Protocols for animal studies were approved by the Institute of Laboratory Animal Resources of the Seoul National University, Seoul, South Korea. Male Sprague-Dawley rats (6–8 weeks old, weighing 245–315 g) were purchased from Taconic Farms Inc. (Samtako Bio Korea, O-San, South Korea). They were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at a temperature of 22 ± 2°C with a 12 h light–dark cycle (lights on 07:00–19:00) and relative humidity of 55 ± 5%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered pathogen-free air, with food (Samyang Company, Pyungtaek, South Korea) and water available ad libitum.

Pretreatment of rats with cytochrome P450 inducers and inhibitors

Rats were treated as follows. Sulfaphenazole: single intravenous injection of 80 mg (2 ml)/kg (dissolved in distilled water with a minimum amount of 10 M NaOH to make a pH of approximately 8.0);[13] SKF-525A: a single intraperitoneal injection of 50 mg (3.3 ml)/kg (dissolved in 0.9% NaCl injectable solution);[15] cimetidine: 150 mg (5 ml)/kg (dissolved in 0.9% NaCl injectable solution acidified with HCl to make a pH of 4.0);[12] troleandomycin: 500 mg (5 ml)/kg (dissolved in 0.9% NaCl injectable solution acidified with HCl to make a pH of 4.0);[16] quinine hydrochloride: 20 mg (5 ml)/kg (dissolved in 0.9% NaCl injectable solution;[14] isoniazid: three daily intraperitoneal injections of 150 mg (3 ml)/kg (dissolved in 0.9% NaCl injectable solution;[17] orphenadrine citrate: 60 mg (5 ml)/kg (dissolved in 0.9% NaCl injectable solution;[18] 3-MC: four daily intraperitoneal injections of 20 mg (3.3 ml)/kg (dissolved in corn oil).[9,19] Control groups received an intraperitoneal or intravenous injection of 5 ml kg^{-1} 0.9% NaCl injectable solution, or 3.3 ml kg corn oil for the 3-MC control group. Rats had free access to food and water during the pretreatment.

Intravenous study

Early in the morning, the jugular vein (for drug administration) and the carotid artery (for blood sampling) of each rat were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ, US) under light ether anaesthesia.[20] Both cannulae were exteriorized to the dorsal side of the neck and terminated with a long silastic tube (Dow Corning, Midland, MI, US). Both silastic tubes were inserted into a wire sheath to allow free movement of the rat. Rats were then housed individually in metabolic cages (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from the anaesthesia for 4–5 h before beginning the experiment. Rats were not restrained.

An experiment was performed just after the injection for the sulfaphenzole groups,[13,21] after the first hour for the SKF-525A and quinine groups,[14,15] after 1.5 h for the cimetidine groups,[12] after 2 h for the troleandomycin groups,[16,22,23] on the fourth day for the isoniziad and orphenadrine citrate groups,[16–18,23,24] and on the fifth day for the 3-MC groups.[9,11]

Furosemide was administered intravenously at a dose of 20 mg (2 ml)/kg for 1 min via the jugular vein of all rats (n = 5–8 per group). Blood samples (approximate 0.12 ml) were taken via the carotid artery at 0 (control), 1 (end of the infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after the start of the intravenous infusion of furosemide. A heparinized 0.9% NaCl injectable solution (20 units/ml; 0.25 ml) was used to flush the cannula after each blood sampling to prevent blood clotting. Blood samples were immediately centrifuged (16 000g for 5 min) to minimize the 'blood storage effect' (the change in plasma concentration of furosemide due to time elapsed between collection and centrifugation of the blood samples),[25] and a 50 μl aliquot of each plasma sample was stored at −70°C prior to HPLC analysis of furosemide. At the end of 8 h, each metabolic cage was rinsed with 15 ml distilled water, and the rinsed material was combined with the 8 h urine sample. The exact volume of the combined urine sample was measured, and two 50 μl aliquots of the combined urine sample were stored at −70°C prior to HPLC analysis of furosemide. At the same time (8 h), rats were exsanguinated and killed by cervical dislocation.

Metabolism of furosemide by microsomes from baculovirus-infected insect cells expressing CYP2C11, 2E1, 3A1 and 3A2

The disappearance of furosemide was measured in microsomes from baculovirus-infected insect cells expressing CYP2C11, 2E1, 3A1 and 3A2 (final concentration of each 60 pmol/ml). Microsomes were incubated in 100 mM phosphate buffer (pH 7.4) to which was added a 10 μL aliquot of Sørensen phosphate buffer (pH 7.4) containing 5 mM furosemide (final concentration 100 μM) and a 50 μl aliquot of Sørensen phosphate buffer (pH 7.4) containing 1.2 mM NADPH, in a final volume of 500 μl. Incubations were done

in a thermomixer (Thermomixer 5436; Eppendorf, Hamburg, Germany) at 37°C and 500 oscillations per min. The reaction was terminated by addition of 0.5 ml acetonitrile containing 500 μg/ml telithromycin (internal standard) after 30 min' incubation.

Measurement of rat plasma protein binding of furosemide using equilibrium dialysis

Protein binding of furosemide to fresh plasma from the quinine-treated and control rats was measured using equilibrium dialysis[26] at a furosemide concentration of 10 μg/ml. Plasma (1 ml) was dialysed against 1 ml isotonic Sørensen phosphate buffer (pH 7.4) containing 3% (w/v) dextran ('the buffer') to reduce volume shift[27] in a 1 ml dialysis cell (Spectrum Medical Industries, Los Angeles, CA, US) using a Spectra/Por 4 membrane (molecular weight cut-off 12–14 kDa; Spectrum Medical Industries). To reduce equilibrium time between the plasma and the buffer compartments, furosemide was spiked into the plasma side.[28] After incubation for 24 h at 37°C, a 50 μL aliquot was collected from each of the buffer and plasma compartments, and stored at −70°C prior to HPLC analysis. Binding of furosemide to human albumin was independent of furosemide concentrations ranging from 1.8 to 36 μg/ml; the mean value was 97.2%.[29] In the preliminary study, the protein binding values of furosemide at 1, 10 and 100 μg/ml in rat plasma were constant. Thus, a furosemide concentration of 10 μg/ml was chosen for this plasma protein binding study.

HPLC analysis of furosemide

Concentrations of furosemide were determined using a slight modification of a reported HPLC method.[30] Telithromycin was used as the internal standard. Briefly, a 0.3 ml aliquot of acetonitrile containing 500 μg/ml telithromycin was added to a 50 μl aliquot of sample. After vortex mixing and centrifugation, a 50 μl aliquot of the supernatant was injected directly onto a reverse-phase (C_{18}) HPLC column. The mobile phase was 0.005% (v/v) phosphoric acid in water: acetonitrile at a ratio of 78:22 (v/v), run at a flow rate of 1.5 ml/min at room temperature. The column eluent was monitored using a fluorescence detector at an excitation wavelength of 226 nm and an emission wavelength of 388 nm. The retention times of furosemide and telithromycin were approximately 4.3 and 7.8 min, respectively, in rat plasma and urine samples. The quantitation limits of furosemide in rat plasma and urine samples were 0.05 and 0.5 μg/ml, respectively. The inter- and intra-day coefficients of variation were below 10.9% and 9.50%, respectively, in the concentration ranges 0.05–1000 μg/ml for rat plasma and 0.5–100 μg/ml for rat urine.

Because furosemide is reported to be photodegraded,[31,32] all samples were covered or wrapped with aluminum foil or kept in the dark during the experiment or when they were not in use.

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to time infinity (AUC) was calculated using the trapezoidal rule/extrapolation method.[33] The area from the last datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal-phase rate constant.

Standard methods[34] were used to calculate the following pharmacokinetic parameters using a non-compartment analysis (WinNonLin v 2.1; Pharsight, Mountain View, CA, US): the time-averaged total body and non-renal clearances (CL and CL_{NR}, respectively), mean residence time (MRT) and apparent volume of distribution at a steady state (V_{ss}).[20]

Statistical analysis

Statistical analysis was performed using SPSS software. Data are expressed as mean ± SD. One-way analysis of variance was used to compare differences between the three or four means for the unpaired data and then individual differences among groups were determined using Duncan's multiple range test. The unpaired *t*-test was used to determine differences between the two means for the unpaired data. A P value < 0.05 was deemed significant.

Results

Pharmacokinetics of furosemide in rats pretreated with cytochrome P450 inducers

The mean plasma concentration–time profiles of furosemide following intravenous infusion (20 mg/kg) to rats pretreated with 3-MC, orphenadrine citrate or isoniazid, and control rats are shown in Figure 1; the relevant pharmacokinetic parameters are listed in Table 1. After intravenous infusion of furosemide, the plasma concentrations of furosemide declined in a polyexponential fashion in all groups of rats.

Compared with control rats, the AUC was significantly smaller (24.9% decrease), and CL and CL_{NR} significantly faster (36.3 and 55.9% increase, respectively) in isoniazid-treated rats. However, the pharmacokinetics parameters of furosemide were not significantly different between 3-MC treated and control rats, and between orphenadrine citrate-treated and control rats (Table 1).

Body weight gain was significantly less in the rats treated with orphenadrine citrate or isoniazid than in their respective controls, as reported in other studies.[21,35,36]

Pharmacokinetics of furosemide in rats retreated with cytochrome P450 inhibitors

The mean plasma concentration–time profiles of furosemide infusion (20 mg/kg) to rats pretreated with SKF-525A, sulfaphenazole, cimetidine, quinine or troleandomycin, and their respective controls are shown in Figure 2; relevant pharmacokinetic parameters are listed in Table 2. After intravenous infusion of furosemide, the plasma concentrations of furosemide declined in a polyexponential fashion in all groups of rats.

Compared with control rats, changes in the pharmacokinetic parameters of furosemide were as follows: the AUC was significantly greater in troleandomycin- and cimetidine-treated rats (43.6 and 36.5% increase, respectively); MRT was significantly shorter (29.8% decrease) in quinine-treated rats; V_{ss} was significantly smaller (33.3%

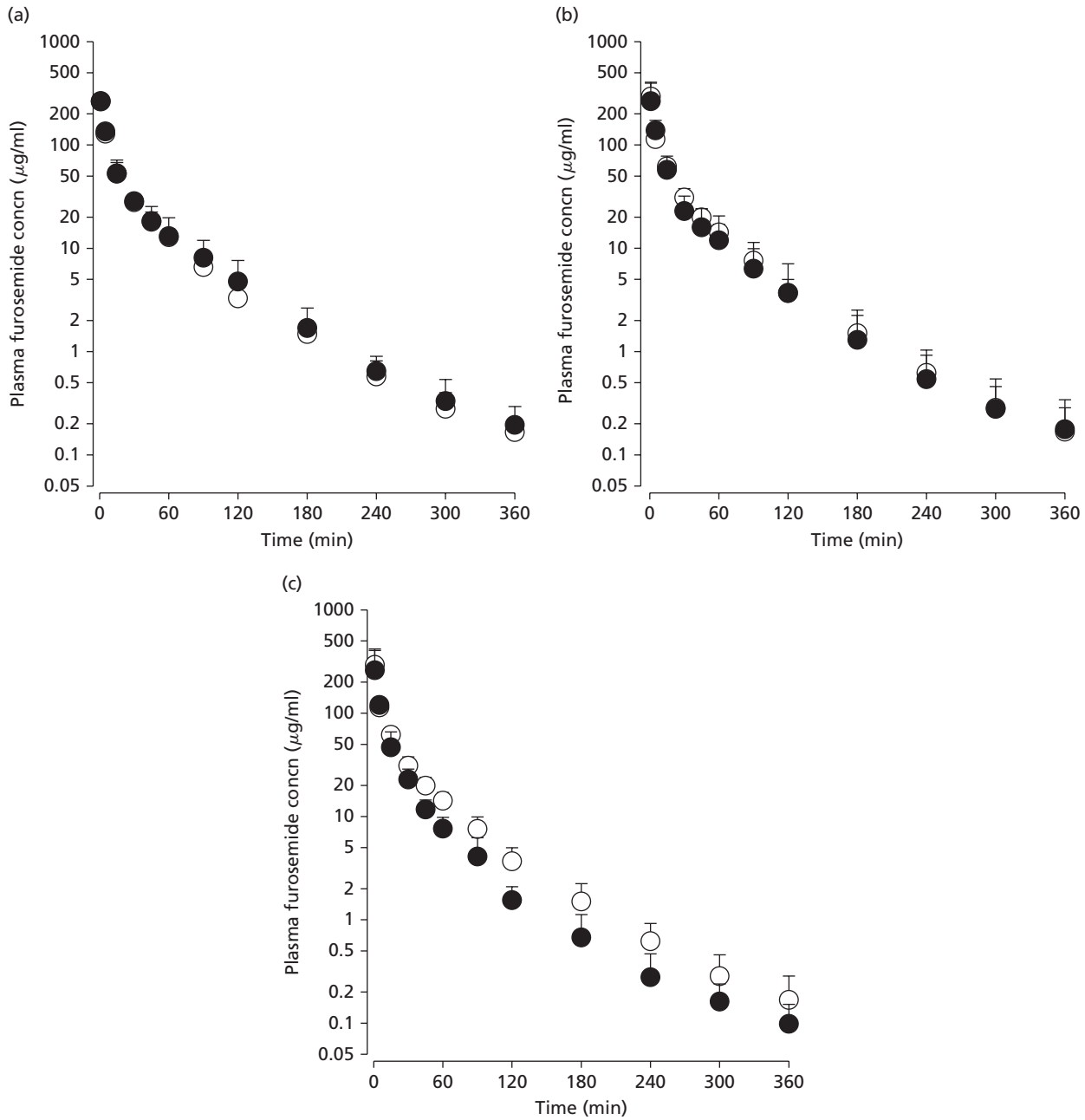

Figure 1 Mean arterial plasma concentration–time profiles of furosemide after intravenous infusion at a dose of 20 mg/kg to rats pretreated with enzyme inducers (●), 3-methylcholanthrene (a), orphenadrine (b) or isoniazid (c), and control rats (○). Vertical bars represent the SD.

decrease) in troleandomycin-treated rats; CL was significantly slower (23.8 and 28.2% decrease, respectively) in SKF-525A- and troleandomycin-treated rats; and CL_{NR} was significantly slower in SKF-525A-, troleandomycin- and cimetidine-treated rats (22.3, 22.7 and 38.5% decreases, respectively). Pharmacokinetic parameters of furosemide were not significantly different between sulfaphenazole-treated and control rats (Table 2).

Metabolism of furosemide by microsomes from baculovirus-infected insect cells expressing CYP2C11, 2E1, 3A1 and 3A2

Rates for the disappearance (primarily metabolism) of furosemide were: 10.3 ± 3.04, 9.12 ± 2.83, 13.9 ± 6.01 and 14.8 ± 6.70 nmol/pmol CYP/min for CYP2C11, 2E1, 3A1 and 3A2, respectively, suggesting that furosemide is metabolized via CYP2C11, 2E1, 3A1 and 3A2.

Table 1 Pharmacokinetic parameters of furosemide after intravenous infusion at a dose of 20 mg/kg to rats pretreated with 3-methylcholanthrene (MCT), orphenadrine (OPT) or isoniazid (INT), and control rats (MCC, OPC, and INC, respectively)

Parameter	MCC (n = 5)	MCT (n = 5)	OPC, INC (n = 7)	OPT (n = 5)	INT (n = 8)
Initial body weight (g)	244 ± 5.63	246 ± 7.76	254 ± 11.1	260 ± 6.12	251 ± 13.6
Final body weight (g)[a]	274 ± 10.8	278 ± 6.71	279 ± 8.52	256 ± 11.9	243 ± 16.0
AUC (μg/min/ml)[b]	3560 ± 628	3800 ± 812	3780 ± 311	3520 ± 1170	2840 ± 481
C_{max} (μg/ml)	271 ± 39.4	265 ± 40.6	293 ± 111	265 ± 161	260 ± 159
Terminal half-life (min)	69.0 ± 13.1	69.2 ± 9.29	74.9 ± 16.8	73.7 ± 21.9	63.5 ± 23.7
MRT (min)	37.5 ± 5.49	41.6 ± 11.0	38.4 ± 7.72	34.7 ± 11.9	28.3 ± 9.77
V_{ss} (ml/kg)	214 ± 37.5	217 ± 25.8	203 ± 32.4	199 ± 48.4	209 ± 103
CL (ml/min per kg)[b]	5.76 ± 1.04	5.44 ± 1.06	5.32 ± 0.430	6.08 ± 1.48	7.25 ± 1.34
CL_{NR} (ml/min per kg)[c]	2.66 ± 0.595	2.36 ± 0.321	1.95 ± 0.413	2.08 ± 0.590	3.04 ± 0.703
$Ae_{0-8\ h}$ (% of dose)[d]	54.1 ± 3.33	56.1 ± 3.58	63.6 ± 6.34	65.4 ± 5.39	58.2 ± 5.08

Values are mean ± SD.

AUC, total area under the plasma concentration–time curve from time zero to time infinity; C_{max}, peak plasma concentration; MRT, mean residence time; V_{ss}, apparent volume of distribution at steady state; CL, time-averaged total body clearance; CL_{NR}, time-averaged non-renal clearance; $Ae_{0-8\ h}$, percentage of the dose excreted in the 8 h urine.

[a]OPT and INT groups were significantly different ($P < 0.05$) from OPC and INC groups.

[b]INT group was significantly different ($P < 0.05$) from INC group.

[c]INT group was significantly different ($P < 0.05$) from INC and OPT groups.

[d]OPT group was significantly different ($P < 0.05$) from INT group.

Plasma protein binding of furosemide

Plasma protein binding (bound fraction) values for furosemide at 10 μg/ml were 90.8 ± 7.64% in quinine-treated rats compared with 87.2 ± 5.31% in the control rats (not significantly different).

Discussion

After intravenous administration of furosemide, the contribution of the gastrointestinal (including the biliary) excretion of unchanged drug to the CL_{NR} of furosemide is negligible: less than 5% of the intravenous dose was excreted in the 24 h bile[37] and recovered from the gastrointestinal tract at 24 h.[30] The small values were unlikely to be due to chemical and enzymatic degradation of furosemide in rat gastric juices. Furosemide is stable for up to 2 h when incubated with human gastric or duodenal fluids.[30,38] Thus, the CL_{NR} values for furosemide listed in Tables 1 and 2 represent the metabolic clearance of the drug, and changes in the CL_{NR} of furosemide represent changes in the metabolism of the drug in rats.

To find out whether hepatic CYP isozymes are involved in the metabolism of furosemide in rats, rats were pretreated with SKF-525A, a non-specific inhibitor of hepatic CYP isozymes. The CL_{NR} of furosemide was significantly slower than in control rats (Table 2), indicating that furosemide is metabolized via hepatic CYP isozymes in rats. Thus, various inducers (Table 1) and inhibitors (Table 2) of hepatic CYP isozymes were used to identify which hepatic CYP isozymes are involved in the metabolism of furosemide in rats. In rats pretreated with isoniazid (a main inducer of CYP2E1 in rats), the CL_{NR} of furosemide was significantly faster than that in control rats (Table 1). In contrast, in rats pretreated with cimetidine or troleandomycin, inhibitors of CYP2C11 and 3A1/2, respectively, in rats, the CL_{NR} of furosemide was significantly slower than in control rats (Table 2). These data suggest that hepatic CYP2E1, 2C11 and 3A1/2 contribute to the metabolism of furosemide in rats. The CL_{NR} of furosemide was similar in rats pretreated with orphenadrine citrate, a main inducer of CYP2B1/2 in rats, and control rats. However, Choi *et al.*[9] reported that the CL_{NR} of furosemide was significantly faster in rats pretreated with phenobarbital (a main inhibitor of CYP2B1/2 in rats). This may reflect the fact that phenobarbital induces CYP3A1 in addition to CYP2B1/2.[39] Metabolism of furosemide was also mediated via CYP3A1/2 in the present study.

To confirm the CYP isozymes responsible for the metabolism of furosemide, microsomes from baculovirus-infected insect cells expressing CYP2C11, 2E1, 3A1 and 3A2 were incubated for 30 min with furosemide. Furosemide was metabolized via CYP2C11, 2E1, 3A1 and 3A2.

After intravenous administration of furosemide to rats pretreated with quinine, the V_{ss} of furosemide was significantly smaller than in control rats (Table 2). However, this was not likely to be due to a significantly decreased free (unbound to plasma proteins) fraction of furosemide in plasma in the treated rats, as plasma protein binding values for furosemide were similar in quinine-treated and control rats. Although the exact reason is not clear, the smaller V_{ss} of furosemide in quinine-treated rats could have been due to decreased affinity of rat tissues for furosemide caused by quinine. Similar result have also been reported for the V_{ss} of metformin in quinine-treated rats, which decreased by 71.3%; the free fraction of metformin in quinine-treated rats increased by only 13.4%.[35]

In conclusion, furosemide was metabolized via CYP2C11, 2E1, 3A1 and 3A2 in rats. This result could help to explain the possible pharmacokinetic changes of furosemide in rat models of acute renal failure induced by uranyl nitrate,[40] diabetes mellitus induced by streptozotocin or alloxan,[41] protein/calorie malnutrition,[42] inflammation

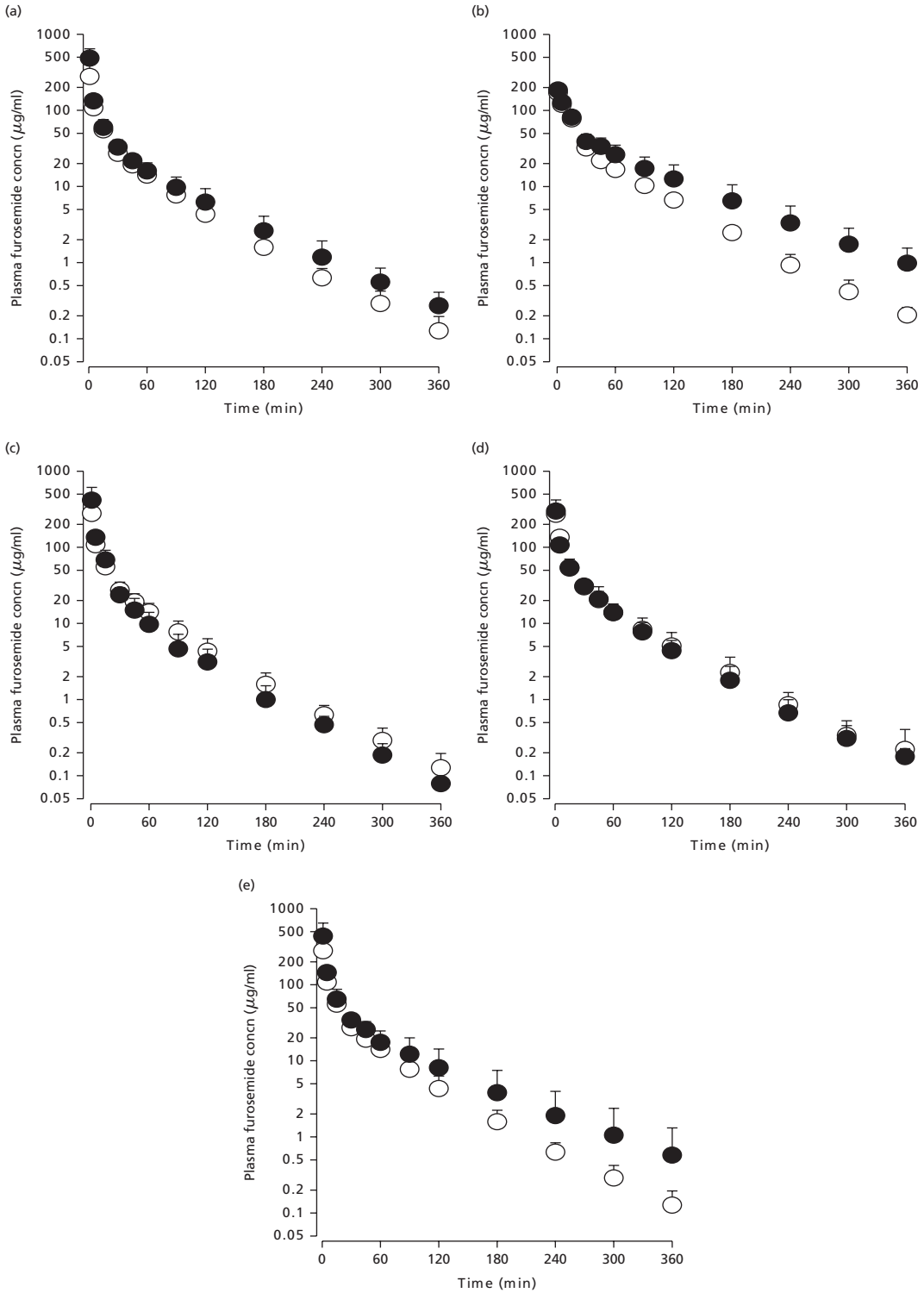

Figure 2 Mean arterial plasma concentration–time profiles of furosemide after intravenous infusion at a dose of 20 mg/kg to rats pretreated with enzyme inhibitors (●), SKF 525-A (a), cimetidine (b), quinine (c), sulfaphenazole (d) or troleandomycin (e), and control rats (○). Vertical bars represent SD.

induced by *Escherichia coli* lipopolysaccharide[43] or *Klebsiella pneumoniae* endotoxin,[44] dehydration,[45] and mutant Nagase analbuminaemic rats[46] in which the protein expression and/or mRNA levels of CYP2C11, 2E1, 3A1 and/or 3A2 are changed. Our findings could also explain possible drug–drug interactions between furosemide and other drugs (mainly metabolized via CYP2C11, 2E1, 3A1 and/or 3A2).

Table 2 Pharmacokinetic parameters of furosemide after intravenous infusion at a dose of 20 mg/kg to rats pretreated with SKF-525A (SKT), quinine (QNT), troleandomycin (TMT), sulfaphenazole (SPT) or cimetidine (CMT), and respective control rats (SKC, QNC, TMC, SPC, and CMC respectively)

Parameter	SKC, QNC, TMC (n = 7)	SKT (n = 7)	QNT (n = 8)	TMT (n = 7)	SPC (n = 7)	SPT (n = 6)	CMC (n = 6)	CMT (n = 6)
Body weight (g)	259 ± 12.4	259 ± 12.1	254 ± 12.9	264 ± 13.1	289 ± 12.8	288 ± 13.3	253 ± 8.45	251 ± 1.77
AUC (μg/min/ml)[a]	3580 ± 731	4660 ± 849	3780 ± 942	5140 ± 1500	3930 ± 927	3710 ± 674	4270 ± 602	5830 ± 1440
C_{max} (μg/ml)[b]	278 ± 133	485 ± 159	415 ± 197	435 ± 209	263 ± 39.5	274 ± 10.8	186 ± 9.68	171 ± 15.8
Terminal half-life (min)	51.5 ± 8.81	70.3 ± 27.5	59.5 ± 19.1	63.3 ± 11.8	56.1 ± 7.42	59.8 ± 14.8	50.2 ± 11.1	73.7 ± 34.0
MRT (min)[c]	39.2 ± 7.86	42.6 ± 10.2	27.5 ± 5.95	48.8 ± 20.1	42.0 ± 8.75	39.1 ± 7.61	45.3 ± 2.98	69.2 ± 16.0
V_{ss} (ml/kg)[d]	228 ± 68.3	187 ± 54.5	152 ± 41.9	190 ± 44.9	218 ± 43.4	213 ± 34.1	220 ± 19.2	241 ± 34.0
CL (ml/min per kg)[e]	5.79 ± 1.21	4.41 ± 0.778	5.56 ± 1.24	4.16 ± 1.12	5.31 ± 1.16	5.57 ± 1.17	4.87 ± 0.49	3.63 ± 0.94
CL_{NR} (ml/min per kg)[f]	2.38 ± 0.584	1.85 ± 0.209	2.18 ± 0.460	1.84 ± 0.298	2.15 ± 0.615	2.47 ± 0.547	2.52 ± 0.42	1.55 ± 0.34
$Ae_{0–8\ h}$ (% of the dose)	59.0 ± 3.84	57.3 ± 7.15	60.4 ± 3.60	53.8 ± 11.8	59.6 ± 5.61	55.6 ± 5.29	48.4 ± 5.31	56.4 ± 6.98

Values are mean ± SD.

AUC, total area under the plasma concentration-time curve from time zero to time infinity; C_{max}, peak plasma concentration; MRT, mean residence time; V_{ss}, apparent volume of distribution at steady state; CL, time-averaged total body clearance; CL_{NR}, time-averaged non-renal clearance; $Ae_{0–8\ h}$, percentage of the dose excreted in the 8 h urine.

[a]TMT group was significantly different ($P < 0.05$) from TMC and QNT groups. CMT group was significantly different ($P < 0.01$) from CMC group.

[b]SKT, QNT, and TMT groups were significantly different ($P < 0.05$) from SKC, QNC, and TMC groups.

[c]QNT group was significantly different ($P < 0.05$) from SKT and QNC groups.

[d]QNT group was significantly different ($P < 0.05$) from QNC group.

[e]TMT group was significantly different ($P < 0.05$) from QNT and TMC groups. SKT group was significantly different ($P < 0.05$) from SKC group.

[f]SKT and TMT groups were significantly different ($P < 0.05$) from SKC and TMC groups. CMT group was significantly different ($P < 0.01$) from CMC group.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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