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Effects of enzyme inducers and inhibitors on the pharmacokinetics of intravenous torasemide in rats

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Abstract

In order to find whether torasemide is metabolized via CYP isozymes in rats, torasemide at a dose of 2 mg/kg was infused in rats pretreated with SKF 525-A, a non-specific CYP isozyme inhibitor in male Sprague–Dawley rats. The total area under the plasma concentration–time curve from time zero to time infinity (AUC) of torasemide was significantly greater in rats pretreated with SKF 525-A (a non-specific CYP isozyme inhibitor in rats) than that in control rats (3570 versus 1350 μg min/ml). This indicated that torasemide is metabolized via CYP isozymes in rats. Hence, torasemide was infused in rats pretreated with various enzyme inducers and inhibitors to find what types of CYP isozymes are involved in the metabolism of torasemide in rats. The AUC values were not significantly different in rats pretreated with 3-methylcholanthrene, phenobarbital, isoniazid, quinine and troleandomycin (main inducers of CYP1A1/2, CYP2B1/2, and CYP2E1, and main inhibitors of CYP2D1 and CYP3A1/2 in rats, respectively) compared with those in respective control rats. However, in rats pretreated with dexamethasone (a main inducer of CYP3A1/2 in rats), the AUC was significantly smaller than that in control rats (1290 versus 1590 μg min/ml). Dexamethasone probably also induces rat CYP2C11; this could be due to an increase in CYP2C11 in rats pretreated with dexamethasone. It has been reported from our laboratories that in rats pretreated with sulfaphenazole (a main inhibitor of CYP2C11 in rats) the AUC was significantly greater than that in control rats (2970 versus 1610 μg min/ml). The above data suggested that torasemide could be metabolized in male rats mainly via CYP2C11.
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Keywords: Torasemide; CYP2C11; Enzyme inducers or inhibitors; Pharmacokinetics; Rats

1. Introduction

Torasemide (1-isoproryl-3-{[4-(3-methylphenyl-amino)pyridine]-3-sulfonyl} urea, Torem®) is a pyridine sulfonylurea loop diuretic closely resembling furosemide and bumetanide in its diuretic action (Knauf and Mutschler, 1998). It inhibits reversibly to Na^+–K^+–2 Cl^- transport carrier system in the

Abbreviations: AUC, total area under the plasma concentration–time curve from time zero to time infinity; CL, time-averaged total body clearance; CL_R, time-averaged renal clearance; CL_{NR}, time-averaged non-renal clearance; V_{ss}, apparent volume of distribution at steady state; MRT, mean residence time; $A_{e\,0–24\,h}$, total amount excreted in 24 h urine

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thick ascending limb of loop of Henle like other loop diuretics (Wittner et al., 1991). It was reported (Miners et al., 1995) that human hepatic microsomal cytochrome P450 (CYP) 2C9 catalyzes the rate-limiting pathway (tolylmethyl hydroxylation, the major biotransformation pathway of torasemide) of torasemide metabolism. It was also reported (Bae et al., 2004) that the total area under the plasma concentration–time curve from time zero to time infinity (AUC) of torasemide was significantly greater (2970 versus 1610 μg min/ml) after intravenous administration at a dose of 2 mg/kg to male Sprague–Dawley rats pretreated with sulfaphenazole (a main inhibitor of CYP2C11 in male rats; Ogiso et al., 1999) than that in control rats. Human CYP2C9 and male rat CYP2C11 proteins have 77% homology (Lewis, 1996). However, the effects of various enzyme inducers and inhibitors on the pharmacokinetics of torasemide seemed not to be published.

The aim of this paper is to report what types of CYP isozymes are involved in the metabolism of torasemide in male Sprague–Dawley rats. Torasemide at a dose of 2 mg/kg was infused over 1 min via the jugular vein of each rat pretreated with enzyme inducers, such as 3-methylcholanthrene, inducers of CYP1A1/2 (Correia, 1995; Spatzenegger et al., 2000) and 2A1 (Correia, 1995); phenobarbital, inducers of CYP2B1/2 (Correia, 1995; Kawamura et al., 1999) and 2C6, 2C7, and 3A1/2 (Correia, 1995); isoniazid, a main inducer of CYP2E1 (Correia, 1995; Hammond and Fry, 1997); dexamethasone, a main inducer of CYP3A1/2 (Halpert, 1988; Correia, 1995) in rats, and enzyme inhibitors, such as SKF 525-A, a non-specific CYP isozymes inhibitor (Correia, 1995); quinine, a main inhibitor of CYP2D1 (Tomkins et al., 1997; Tyndale et al., 1999); troleandomycin, a main inhibitor of CYP3A1/2 (Wrighton et al., 1985) in rats, and their respective control rats.

2. Materials and methods

2.1. Chemicals

Torasemide was donated from Roche Pharmaceutical Company (Mannheim, Germany). SKF 525-A, dexamethasone phosphate, isoniazid, troleandomycin, and quinine hydrochloride were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). 3-Methylcholanthrene and sodium phenobarbital were products from Wako Pure Chemical Industries (Osaka, Japan) and Dai Han Pharmaceutical Company (Seoul, South Korea), respectively. Polyethylene glycol 400 (PEG 400) was purchased from Duksan Chemical Company (Seoul, South Korea). Other chemicals were of reagent grade or high-performance liquid chromatographic (HPLC) grade, and therefore, were used without further purification.

2.2. Animals

Male Sprague–Dawley rats (weighing 230–320 g) purchased from Charles River Company Korea (Orient, Seoul, South Korea) were housed in a light-controlled room (light: 07:00–19:00, dark: 19:00–07:00) kept at a temperature of $22 \pm 2\,^{\circ}C$ and a relative humidity of $55 \pm 5\%$ (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea). Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under the supply of filtered pathogen-free air and with food (Samyang Company, Seoul, South Korea) and water ad libitum. The protocol of this study was approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University.

2.3. Pretreatment with enzyme inducers, such as 3-methylcholanthrene, phenobarbital, isoniazid, and dexamethasone, and enzyme inhibitors, such as SKF 525-A, quinine and troleandomycin

Rats received a single intraperitoneal injection of 50 mg (3.3 ml)/kg of SKF525-A (dissolved in 0.9% NaCl-injectable solution; Conney, 1971), 500 mg (5 ml)/kg of troleandomycin (dissolved in 0.9% NaCl-injectable solution acidified to pH 4.0 with HCl; Ogiso et al., 1999), or 20 mg (5 ml)/kg of quinine hydrochloride (dissolved in 0.9% NaCl-injectable solution; Tomkins et al., 1997); three daily intraperitoneal injections of 50 mg (5 ml)/kg of dexamethasone phosphate (dissolved in 0.9% NaCl-injectable solution; Arlotto et al., 1987; Ross et al., 1993) or 150 mg (3 ml)/kg of isoniazid (dissolved in 0.9% NaCl-injectable solution; Ryan et al., 1985); or four daily intraperitoneal injections of 80 mg (3.3 ml)/kg of sodium phenobarbital (dissolved in 0.9% NaCl-injectable

solution; Williams et al., 1979; Choi et al., 1991), 20 mg (3.3 ml)/kg of 3-methylcholanthrene (dissolved in corn oil; Williams et al., 1979; Choi et al., 1991), corn oil, 3.3 ml/kg (3-methylcholanthrene control group), or 0.9% NaCl-injectable solution, 5 ml/kg (other control groups). During the pretreatment, rats had free access to food (Samyang Company, Seoul, South Korea) and water.

2.4. Intravenous study

The procedures for the pretreatment of rats including the cannulation of the carotid artery (for blood sampling) and the jugular vein (for drug administration) were reported previously (Kim et al., 1993). An experiment was performed during the first 1 h for the SKF525-A (Arlotto et al., 1987) and quinidine (Tomkins et al., 1997), and 2 h for the troleandomycin (Conney, 1971), 4th day for dexamethasone and isoniazid groups (Ryan et al., 1985; Ross et al., 1993; Sinclair et al., 2000), and 5th day for control groups (Williams et al., 1979; Ryan et al., 1985; Choi et al., 1991; Ross et al., 1993; Sinclair et al., 2000) as the commencement of respective pretreatment.

Torasemide (dissolved in distilled water with a minimum amount of 10N NaOH and adjusted to a final pH of approximately 8 with HCl) at a dose of 2 mg/kg was administered intravenously over 1 min via the jugular vein of each group. Total injection volume was 2 ml/kg. Blood samples (approximately 0.22 ml) were collected via the carotid artery at 0 (to serve as a control), 1 (at the end of the infusion), 5, 15, 30, 60, 90, 120, 240, 360, 480, 600, and 720 min after the beginning of the infusion. Approximately, 0.25 ml aliquot of the heparinized 0.9% NaCl-injectable solution (20 units/ml) was used to flush the cannula after each blood sampling to prevent blood clotting. Blood samples were immediately centrifuged at $9000 \times g$ over 10 min and a 100 μl aliquot of each plasma sample was stored in a −70 °C freezer (Model DF8517; Ilshin Laboratory Company, Seoul, South Korea) until HPLC analysis of torasemide. At the end of 24 h, each metabolic cage was rinsed with 10 ml of distilled water and the rinsed material was combined with the urine sample. After measuring the exact volume of the combined urine sample, two 0.1 ml aliquots of the combined urine sample were stored in a −70 °C freezer until HPLC analysis of torasemide. At the same time, each rat was sacrificed by cervical dislocation and the entire gastrointestinal tract (including its contents and faeces) was removed, transferred into a beaker containing 50 ml of 0.01N NaOH (to facilitate the extraction of torasemide) and cut into small pieces using scissors. After stirring with a glass rod, two 100 μl aliquots of the supernatant were collected from each beaker and stored in a −70 °C freezer until HPLC analysis of torasemide.

2.5. HPLC analysis of torasemide

Concentrations of torasemide in the above sample were determined by an HPLC method developed from our laboratories. In a 2.2 ml eppendorf tube containing a 100 μl aliquot of sample, a 50 μl aliquot of methanol containing an internal standard (chlorzoxazone; 20 μg/ml) was added. After vortex mixing for 30 s, the mixture was extracted with 1 ml ethyl acetate. The organic phase was transferred into a clean eppendorf tube and evaporated under nitrogen gas at 55 °C. The residue was then reconstituted with a 100 μl aliquot of the mobile phase and 50 μl was injected directly onto the HPLC column. The mobile phase, phosphate buffer, 0.02 M NaH_2PO_4 (pH 4.5):acetonitrile (65:35 (v/v) for rat plasma and 70:30 (v/v) for rat urine and tissue samples) was run at a flow-rate of 1.0 ml/min and the column effluent was monitored by a UV detector set at 290 nm. The retention times of torasemide and an internal standard were approximately 6.3 and 8.9 min, respectively, in rat plasma and 8.3 and 11.6 min, respectively, in rat urine and tissue samples. The detection limits of torasemide in rat plasma and urine were 20 and 50 ng/ml, respectively. The inter- and intra-day coefficients of variation were generally low (below 9.88%).

The HPLC system consisted of a model P-580 pump (Jasco, München, Germany), a reversed-phase (C_{18}) Nucleosil column (4.6 mm, $l \times 250$ mm, i.d.; particle size, 5 μm; Macherey-Nagel, PA, USA), a model UV/VIS-151 detector (Gilson, Middleton, WI, USA) and a model Chromatocorder 21 integrator (SIC, Tokyo, Japan).

2.6. Pharmacokinetic analysis

The AUC was calculated by the trapezoidal rule-extrapolation method; this method utilized 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 datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal rate constant.

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

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

2.7. Statistical analysis

A P-value of less than 0.05 was considered to be statistically significant using the unpaired t-test. All results are expressed as mean ± S.D.

3. Results

3.1. Pharmacokinetics of torasemide in rats pretreated with enzyme inducers

The mean arterial plasma concentration–time profiles of torasemide after 1 min intravenous administration at a dose of 2 mg/kg to rats pretreated with 3-methylcholanthrene, phenobarbital, isoniazid and dexamethasone, and their respective control rats are shown in Fig. 1, and some relevant pharmacokinetic

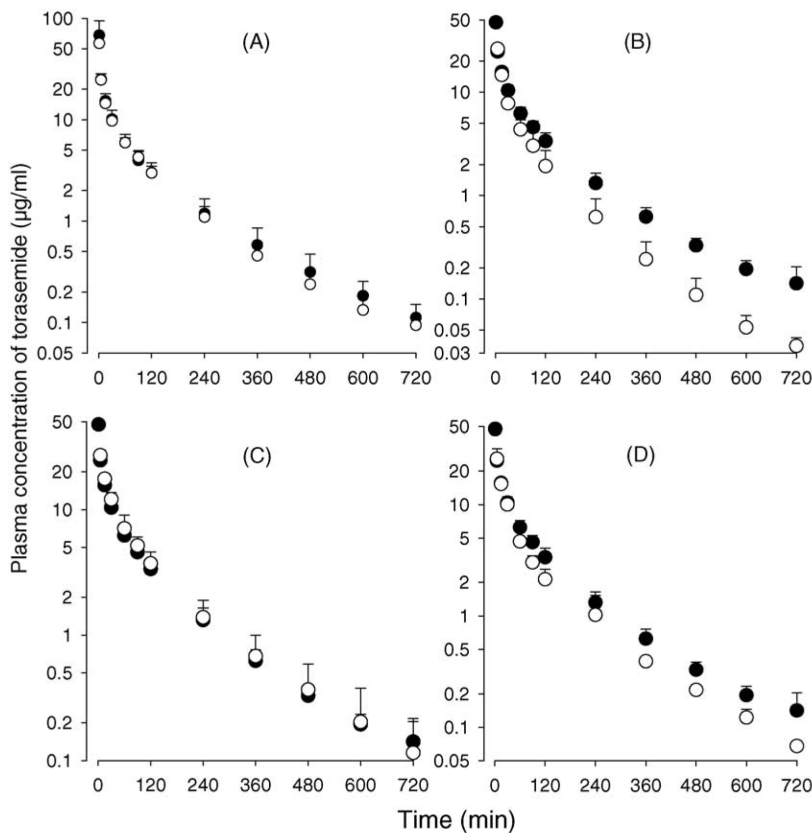

Fig. 1. Mean arterial plasma concentration–time profiles of torasemide after 1 min intravenous administration at a dose of 2 mg/kg to rats pretreated with enzyme inducers (open circle), 3-methaylcholanthrene (A), phenobarbital (B), isoniazid (C) and dexamethasone (D), and their respective control rats (closed circle). Vertical bars represent S.D.

parameters are listed in Table 1. After intravenous administration, the plasma concentrations of torasemide declined in a polyexponential fashion for all groups of rats.

In rats pretreated with 3-methylcholanthrene, the plasma concentrations of torasemide were similar compared with those in control rats (Fig. 1A). This resulted in similar AUC values between two groups of rats. Other pharmacokinetic parameters of torasemide listed in Table 1 were also not significantly different between two groups of rats. Similar results were also obtained from rats pretreated with phenobarbital and isoniazid. Note that body weight gain decreased significantly in rats pretreated with isoniazid (from 301 to 294 g) compared with that in control rats (from 298 to 313 g).

In rats pretreated with dexamethasone, the plasma concentrations of torasemide were lower than those in control rats (Fig. 1D). This resulted in a significantly smaller AUC (18.9% decrease) than that in control rats due to significantly faster CL (23.0% increase). The faster CL could be due to significantly faster CL_{NR} (31.9% increase), since CL_R was significantly slower (5.85% decrease) than those in control rats. Significantly slower CL_R could be due to significantly smaller (56.2% decrease) amount of unchanged torasemide excreted in 24 h urine ($A_{e\,0\text{–}24\,h}$, expressed in terms of intravenous dose of torasemide) in rats pretreated with dexamethasone. The significantly faster CL could be supported by significantly shorter MRT (24.4% decrease) in rats pretreated with dexamethasone. Note that body weight gain decreased significantly in rats pretreated with dexamethasone (from 297 to 276 g) compared with that in control rats (from 298 to 313 g) (Table 1).

3.2. *Pharmacokinetics of torasemide in rats pretreated with enzyme inhibitors*

The mean arterial plasma concentration–time profiles of torasemide after 1 min intravenous administration at a dose of 2 mg/kg to rats pretreated with SKF 525-A, quinine and troleandomycin, and their respective control rats are shown in Fig. 2, and some relevant pharmacokinetic parameters are listed in Table 2. After intravenous administration, the plasma concentrations of torasemide declined in a polyexponential fashion for all groups of rats.

Table 1

Pharmacokinetic parameters of torasemide after intravenous (i.v.) administration at a dose of 2 mg/kg to rats pretreated with 3-methylcholanthrene (MCT), phenobarbital (PBT), isoniazid (INT), and dexamethasone (DXT), and respective control rats (MCC, PBC, INC, and DXC)[a]

Parameter	MCC ($n = 7$)	MCT ($n = 7$)	PBC ($n = 8$)	PBT ($n = 10$)	INC, DXC ($n = 8$)	INT ($n = 9$)	DXT ($n = 9$)
Initial body weight (g)	302 ± 12.8	304 ± 11.9	240 ± 19.0	258 ± 35.8	298 ± 9.64	301 ± 11.1	297 ± 14.6
Final body weight (g)	321 ± 19.2	331 ± 16.0	280 ± 9.26	281 ± 30.4	313 ± 10.7	294 ± 15.2[b]	276 ± 8.82[c]
AUC (μg min/ml)	1540 ± 308	1450 ± 146	1350 ± 399	1210 ± 207	1590 ± 196	1790 ± 309	1290 ± 162[b]
Terminal half-life (min)	151 ± 25.0	188 ± 59.3	117 ± 63.5	107 ± 35.1	144 ± 87.6	137 ± 64.9	154 ± 31.9
V_{ss} (ml/kg)	149 ± 17.0	156 ± 13.4	147 ± 43.7	127 ± 18.6	148 ± 25.1	128 ± 20.7	134 ± 32.2
MRT (min)	114 ± 19.8	114 ± 6.53	110 ± 67.6	77.1 ± 7.35	120 ± 25.3	118 ± 34.5	90.7 ± 29.4[d]
CL (ml/min/kg)	1.30 ± 0.293	1.38 ± 0.142	1.48 ± 0.385	1.64 ± 0.281	1.26 ± 0.176	1.12 ± 0.205	1.55 ± 0.217[b]
CL_R (ml/min/kg)	0.0502 ± 0.0680	0.0643 ± 0.0590	0.0896 ± 0.0435	0.0883 ± 0.0872	0.0633 ± 0.0945	0.0403 ± 0.0592	0.0596 ± 0.0244[d]
CL_{NR} (ml/min/kg)	1.23 ± 0.265	1.28 ± 0.122	1.39 ± 0.368	1.52 ± 0.246	1.13 ± 0.152	1.04 ± 0.215	1.49 ± 0.209[b]
$A_{e\,0\text{–}24\,h}$ (% of i.v. dose)	6.06 ± 3.84	6.97 ± 3.16	6.39 ± 2.20	7.46 ± 3.87	9.64 ± 4.49	6.64 ± 4.28	4.22 ± 1.34[b]

[a] Each value represents the mean ± S.D.

[b] Significantly different ($P < 0.01$) from respective control.

[c] Significantly different ($P < 0.001$) from respective control.

[d] Significantly different ($P < 0.05$) from respective control.

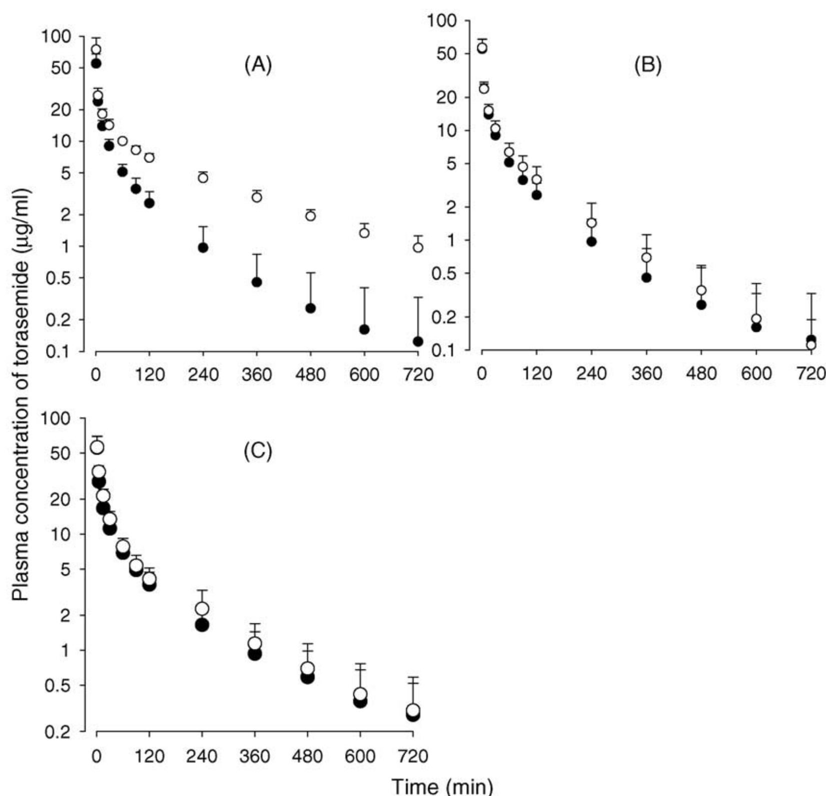

Fig. 2. Mean arterial plasma concentration–time profiles of torasemide after 1 min intravenous administration at a dose of 2 mg/kg to rats pretreated with enzyme inhibitors (open circle), SKF 525-A (A), quinine (B) and troleandomycin (C), and their respective control rats (closed circle). Vertical bars represent S.D.

In rats pretreated with SKF 525-A, the plasma concentrations of torasemide were higher than those in control rats (Fig. 2A). This resulted in a significantly greater AUC (164% increase) than that in control rats due to significantly slower CL (62.2% decrease). The slower CL could be due to significantly slower CL_{NR} (64.2% decrease) because CL_R values were not significantly different between two

Table 2
Pharmacokinetic parameters of torasemide after intravenous (i.v.) administration at a dose of 2 mg/kg to rats pretreated with SKF 525-A (SKT), quinine (QNT), and troleandomycin (TMT), and their respective control rats (SKC, QNC, TMC)[a]

Parameter	SKC, QNC ($n=8$)	SKT ($n=9$)	QNT ($n=7$)	TMC ($n=7$)	TMT ($n=6$)
Body weight (g)	280 ± 9.26	282 ± 11.2	286 ± 9.45	324 ± 3.78	320 ± 8.37
AUC (μg min/ml)	1350 ± 399	3570 ± 483[b]	1620 ± 424	1900 ± 547	2240 ± 541
Terminal half-life (min)	117 ± 63.5	217 ± 48.9[c]	126 ± 13.0	151 ± 74.9	142 ± 57.6
V_{ss} (ml/kg)	147 ± 43.7	149 ± 19.2	139 ± 11.2	150 ± 33.0	127 ± 25.9
MRT (min)	110 ± 67.6	268 ± 43.0[b]	114 ± 35.4	152 ± 70.0	149 ± 63.3
CL (ml/min/kg)	1.48 ± 0.385	0.560 ± 0.0833[b]	1.24 ± 0.476	1.05 ± 0.310	0.891 ± 0.237
CL_R (ml/min/kg)	0.0896 ± 0.0435	0.0395 ± 0.0492	0.0791 ± 0.0259	0.0453 ± 0.0514	0.0476 ± 0.0354
CL_{NR} (ml/min/kg)	1.39 ± 0.368	0.498 ± 0.0860[b]	1.15 ± 0.468	0.996 ± 0.270	0.838 ± 0.212
$A_{e\,0\text{–}24\,h}$ (% of i.v. dose)	6.39 ± 2.20	10.5 ± 7.34	6.80 ± 2.45	5.29 ± 2.53	6.16 ± 2.29

[a] Each value represents the mean ± S.D.
[b] Significantly different ($P<0.001$) from respective control.
[c] Significantly different ($P<0.05$) from respective control.

groups of rats. The significantly slower CL could be supported by significantly longer terminal half-life (85.5% increase) and MRT (144% increase) in rats pretreated with SKF 525-A (Table 2). In rats pretreated with quinine and troleandomycin, the pharmacokinetic parameters of torasemide were not significantly different compared with their respective control rats (Table 2).

4. Discussion

After intravenous administration of torasemide in rats, contribution of CL_R to CL of torasemide was not considerable; the $A_{e\ 0\text{–}24\,h}$ values were smaller than 10.5% for all rats studied (Tables 1 and 2). Contribution of gastrointestinal (including biliary) excretion of unchanged torasemide to CL_{NR} of torasemide was also negligible; torasemide was below the detection limit from the entire gastrointestinal tract at 24 h for all rats studied. Moreover, the percentage of intravenous dose of torasemide (at 10 mg/kg) excreted in 8 h bile as unchanged drug was less than $2.02 \pm 1.18\%$ (Kim and Lee, 2003). Torasemide was stable in human gastric juices and various pH solutions (Lee et al., in press). The above data indicated that torasemide was metabolized considerably after intravenous administration in rats, and the CL_{NR} values of torasemide listed in Tables 1 and 2 could represent metabolic clearances of torasemide. Hence, the CL_{NR} changes in Tables 1 and 2 could indicate changes in metabolism of torasemide in rats.

In order to find whether CYP isozymes are involved in the metabolism of torasemide in rats, SKF 525-A, a non-specific inhibitor of CYP isozymes in rats (Correia, 1995) was pretreated to rats. In rats pretreated with SKF525-A, the AUC of torasemide was significantly greater than that in control rats (Table 2), indicating that torasemide is metabolized via CYP isozymes in rats. Hence, various CYP enzyme inducers and inhibitors were pretreated in rats to find what types of CYP isozymes are involved in the metabolism of torasemide. In rats pretreated with 3-methylcholanthrene, a main inducer of CYP1A1/2 in rats (Spatzenegger et al., 2000), the AUC of torasemide was similar compared with that in control rats (Table 1). This suggested that CYP1A1/2 could not contribute considerably to the metabolism of torasemide in rats.

In rats pretreated with phenobarbital, inducers of CYP2B1/2, 2C6, 2C7, and 3A1/2 in rats (Correia, 1995; Kawamura et al., 1999), the AUC of torasemide was also similar compared to that in control rats (Table 1). This suggested that CYP2B1/2, 2C6, 2C7, and 3A1/2 also could not contribute considerably to the metabolism of torasemide in rats. In rats pretreated with quinine, a main inhibitor of CYP2D1 in rats (Tomkins et al., 1997; Tyndale et al., 1999), the AUC values of torasemide were comparable between two groups of rats (Table 2). This suggested that CYP2D1 could not contribute considerably to the metabolism of torasemide in rats. In rats pretreated with isoniazid, a main inducer of CYP2E1 in rats (Correia, 1995), the AUC values were not significantly different between two groups of rats (Table 1) suggesting that CYP2E1 may not contribute considerably to the metabolism of torasemide in rats.

In rats pretreated with dexamethasone, a main inducer of CYP3A1/2 in rats (Halpert, 1988), the AUC of torasemide was significantly smaller than that in control rats (Table 1). However, in rats pretreated with troleandomycin, a main inhibitor of CYP3A1/2 in rats (Wrighton et al., 1985), the AUC values of torasemide were not significantly different between two groups of rats (Table 2). The above data suggested that CYP3A1/2 could not contribute considerably to the metabolism of torasemide in rats. Although only 18.9% difference was observed, the significantly smaller AUC of torasemide in rats pretreated with dexamethasone could be due to enhanced expression of CYP2C11 by dexamethasone pretreatment (Liddle et al., 1992; Nakamura et al., 1994). It was reported (Bae et al., 2004) that the AUC of torasemide was significantly greater in male Sprague–Dawley rats pretreated with sulfaphenazole, a main inhibitor of CYP2C11 in male rats (Ogiso et al., 1999). The above data suggested that torasemide could be metabolized mainly via CYP2C11 in male rats.

The above results played an important role in explaining the pharmacokinetic changes of torasemide seen in various rat disease models where the CYP isozymes are changed. For example after intravenous administration of torasemide at a dose of 2 mg/kg to rats with protein–calorie malnutrition (PCM), the CL_{NR} of torasemide was significantly slower (56.7% decrease) than that in untreated rats (Bae et al., 2004), since CYP2C11 decreased in PCM rats (Cho et al., 1999).

After intravenous administration of torasemide at a dose of 2 mg/kg to rats with diabetes mellitus induced by alloxan (DMIA) or streptozotocin (DMIS), the CL_{NR} of torasemide was significantly slower (27.5 or 26.6% decrease, respectively) than that in untreated rats (Kim, 2005), since CYP2C11 decreased in DMIA and DMIS rats (Kim, 2005). In addition, it was reported that several drugs or foods could induce or inhibit CYP2C9. For example some drugs including rifampin (Gerbal-Chaloin et al., 2001), colchicine (Dvorak et al., 2003), and fluvastatin (Scripture and Pieper, 2001) can induce or inhibit CYP2C9. And some herbal extracts, such as grape fruit juice, peppermint oil, Devil's crow root extract and *Eucalyptus* oil inhibit several CYP isozymes including CYP2C9 (Unger and Frank, 2004), and many of the spices, herbal and black teas and soybean products have inhibitory activity of CYP2C9 (Foster et al., 2003). If our present rat study could be extrapolated to humans, the dosage modification of torasemide may be required in patients with various diseases who are concurrently taking drugs or foods that can induce or inhibit CYP2C9.

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