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Urinary Excretion Profile of Torasemide and its Diuretic Action in Dogs

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Abstract

The plasma concentration profile, urinary excretion rate and diuretic response were studied in anaesthetized dogs after an intravenous administration of torasemide or furosemide.

The urinary excretion rate of furosemide decreased rapidly after administration. The plasma concentration, which is related to the urinary excretion profile, also decreased rapidly. The diuretic response, which reflected the excretion rate, occurred rapidly after administration but lasted for a short time. The urinary excretion rate of torasemide was much lower than that of furosemide and decreased slowly after administration. The plasma concentration also decreased slowly. The diuretic response to torasemide occurred more slowly but lasted longer than the response to furosemide.

These results suggest that the diuretic response profile of either diuretic depends on their urinary excretion rate, and that the difference in the diuretic response between torasemide and furosemide may be explained by the different transfer rate of the drugs from the plasma to the nephron.

Diuretics are well known to exert their diuretic action at the nephron; thus the amount of diuretic in the tubular fluid rather than the plasma concentration is the main determinant of diuresis (Rose et al 1976). Therefore, it is important to measure the urinary concentration of drugs and to investigate the relationship between the urinary excretion rate and diuretic response.

Torasemide (1-isopropyl-3[(4-*m*-toluidino-3-pyridyl)sulphonyl]urea) is a new loop diuretic of the pyridyl sulphonylurea group with low kaliuresis (Ghys et al 1985a; Broekhuysen et al 1986).

The main site for the diuretic action of torasemide is the thick ascending limb of the loop of Henle (Hermes & Heidenreich 1985; Wittner et al 1986; Uchida et al 1991a).

A previous study has shown that the urinary concentration of torasemide was several times lower than that of furosemide in normal volunteers, whereas the cumulative urine output of torasemide was similar to that of furosemide (Dodion et al 1986).

The diuretic response of torasemide in early stages following an intravenous dose occurred more slowly (Ghys et al 1985b; Uchida et al 1991b) and lasted longer than that of furosemide (Ghys et al 1985a; Broekhuysen et al 1986).

There has not been a detailed pharmacokinetic study to explain the diuretic profile by analysing the urinary excretion rate.

The present study was designed to clarify the differences in the diuretic properties of torasemide and furosemide and to investigate the relationship between the urinary excretion rate and the diuretic response after a single intravenous administration of torasemide or furosemide to dogs.

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Materials and Methods

Materials

Torasemide (1-isopropyl-3[(4-*m*-toluidino-3-pyridyl)sulphonyl]-urea), BM15275 (1[4-(2-methoxy phenyl)-1-piperazinyl]-3-(1-naphthyloxy)-2-propanol) and AH6666 (*N*-[4-crotonylamino)phenylsulphonyl]-*N'*-butylurea) were kindly supplied by Boehringer Mannheim GmbH, Mannheim, Germany. Furosemide (Lasix Injection) was purchased from Hoechst. Piretanide was extracted from Arelix (Hoechst) in our laboratory.

Drugs

Torasemide was dissolved in a 0·1 M sodium bicarbonate solution (0·3 mg mL^{-1}), added to an equal volume of 0·1 M hydrochloric acid, and diluted with physiological saline. Furosemide was diluted with physiological saline (1·0 mg mL^{-1}).

Animals

Male and female mongrel dogs (11–15 kg, n = 4) were used for the study.

Dose and administration

The animals were anaesthetized with pentobarbitone sodium (25 mg kg^{-1}, i.v.). Right brachiocephalic vein and left ureter catheters were placed. Torasemide (0·3 mg kg^{-1}) or furosemide (1 mg kg^{-1}) was intravenously injected as a bolus through the venous catheter. Urine samples were collected from the left ureter every 5 min during the 0–30-min period and every 10 min during the 30–90-min period following the administration of drug. Blood samples were withdrawn from the right brachiocephalic artery at the middle point of each urine collection interval. Plasma samples were obtained by centrifugation at 3000 rev min^{-1} for 10 min. All samples were stored at −80°C until analysis.

Preparation of plasma and urine for furosemide determination
Each sample, containing 100 μL internal standard (piretanide: 0·1 mg mL^{-1}), was acidified with 50 μL 10 M hydrochloric acid and extracted with 10 mL diethylether on a mechanical shaker for 10 min. After a brief centrifugation (2500 rev min^{-1}) for 10 min, the upper diethylether phase was collected and evaporated to dryness under a nitrogen stream. The residue was dissolved in 200 μL HPLC mobile phase consisting of 0·02 M phosphoric acid, methanol and glacial acetic acid (57·6 : 40 : 2·4). Twenty microlitres of the solution was injected into the HPLC system.

Preparation of plasma for torasemide determination
Plasma samples were prepared by solid phase extraction using Bond Elut C8 and Si cartridges (Varian). The cartridge was previously washed with methanol and equilibrated with 0·2 M phosphate buffer (pH 3·0). One millilitre of a plasma sample, containing 100 μL internal standard (AH6666: 5 μg mL^{-1}), was applied to the washed Bond Elut C8 cartridge. The cartridge was washed with 10 mL 0·2 M phosphate buffer (pH 3·0) and then with 3 mL of distilled water. Torasemide was eluted with 5 mL methanol and the eluate was evaporated to dryness under a nitrogen stream at 40°C. The residue was dissolved in 2 mL 0·2 M phosphate buffer (pH 3·0) and the solution was applied to a Bond Elut Si cartridge (Varian). The cartridge was washed with 0·5 mL distilled water and then eluted with 2 mL dichloromethane-methanol (1 : 1). The eluate was evaporated to dryness under a nitrogen stream at 40°C. The residue was reconstituted in 200 μL HPLC mobile phase, 0·02 M phosphate buffer (pH 4·5)-acetonitrile (90 : 10). One hundred microlitres of the solution was injected into the HPLC system.

Preparation of urine for torasemide determination
One millilitre of a urine sample, containing 100 μL internal standard (BM15275: 50 μg mL^{-1}), was mixed with 1 mL 1 M phosphate buffer (pH 3·0). The contents were extracted with 5 mL diethylether containing tetrahexylammonium bromide (0·5 mg mL^{-1}) on a mechanical shaker for 10 min. After a brief centrifugation, the upper diethylether phase was transferred to another tube and extracted with 200 μL 0·1 M sulphuric acid. After a brief centrifugation, the organic phase was aspirated and discarded; 20 μL residual aqueous phase was injected into the HPLC system.

Drug assays
The concentrations of drugs in the plasma and urine were determined by high-performance liquid chromatography (HPLC). Furosemide concentrations were determined according to a previously described method (Miwa et al 1988). Briefly, an Inertsil C8 column (5 μM, 4·6 × 250 mm, GL Sciences) at 45°C was used. Furosemide was eluted at 0·7 mL min^{-1} in a linear gradient elution mode with a 0·02 M phosphoric acid, methanol and glacial acetic acid mixture (gradient from 57·6 : 40 : 2·4 to 38·4 : 60 : 1·6). A UV detector was used for monitoring at 340 nm. The concentration of furosemide was calculated from the peak height ratio of furosemide to the internal standard piretanide. For torasemide determination, a Nucleosil, 10C18 column (10 μM, 4·6 × 250 mm, Gasukuro Kogyo) at 45°C was used. Torasemide was eluted at 1·0 mL min^{-1} in a linear gradient elution mode from 10% acetonitrile/0·02 M phosphate buffer (pH 3·0) to 50% acetonitrile/0·02 M phosphate buffer (pH 3·0). A UV detector was used for monitoring at 290 nm. The concentration of torasemide was calculated from the peak-height ratio of torasemide to the internal standard AH6666.

Table 1. Pharmacokinetic parameters of torasemide and furosemide in the plasma after intravenous administration in dogs.

Parameters	Torasemide (n = 3)	Furosemide (n = 4)
$t_{\frac{1}{2}}$ (min)	120·54 ± 42·14**	9·76 ± 2·93
Vd (mL)	643·54 ± 105·81***	3171·69 ± 438·96
CL (mL min^{-1})	3·91 ± 0·91**	237·67 ± 60·73
AUC (μg min mL^{-1})	1040·99 ± 293·53***	58·57 ± 18·93

**$P < 0·01$, ***$P < 0·001$; significant difference from furosemide value. The data represent the mean ± s.d.

Protein binding
The concentrations of drugs present as the unbound drug in the plasma and urine were determined by the ultrafiltration method. Each sample was transferred to Minicent-30 cartridge (30 000 mol. wt, Tosoh). The cartridge was centrifuged at 5000 rev min^{-1} for 30 min. The concentration of drugs in the filtrate (unbound fraction) was measured by the drug assay method.

Pharmacokinetic analysis
The plasma concentration and the urinary excretion of both diuretics were analysed using a one-compartment model.

Statistical analysis
Student's *t*-test was used to evaluate differences between the mean of the data obtained with furosemide and torasemide in dogs. A *P*-value of less than 0·05 was considered to be significant.

Results

Pharmacokinetic studies
The pharmacokinetic parameters for both diuretics in plasma are shown in Table 1. The value of the half-lives ($t_{\frac{1}{2}}$) was significantly prolonged in the case of torasemide ($P < 0·01$). The distribution volume (Vd) of furosemide was five times larger than that of torasemide. These results show that furosemide concentration decreased rapidly in plasma

Table 2. Pharmacokinetic parameters of torasemide and furosemide in the urine after intravenous administration in dogs.

Parameters	Torasemide (n = 4)	Furosemide (n = 4)
k_{ex} (h^{-1})	0·12 ± 0·04***	1·03 ± 0·24
Ae(∞) (mg)	0·34 ± 0·13***	3·57 ± 0·76
f_e (%)	8·91 ± 3·04**	27·44 ± 6·12
CL_R (mL min^{-1})	[a]0·38 ± 0·23***	63·45 ± 12·77

**$P < 0·01$, ***$P < 0·001$; significant difference from furosemide value. The data represent the mean ± s.d. [a](n = 3).

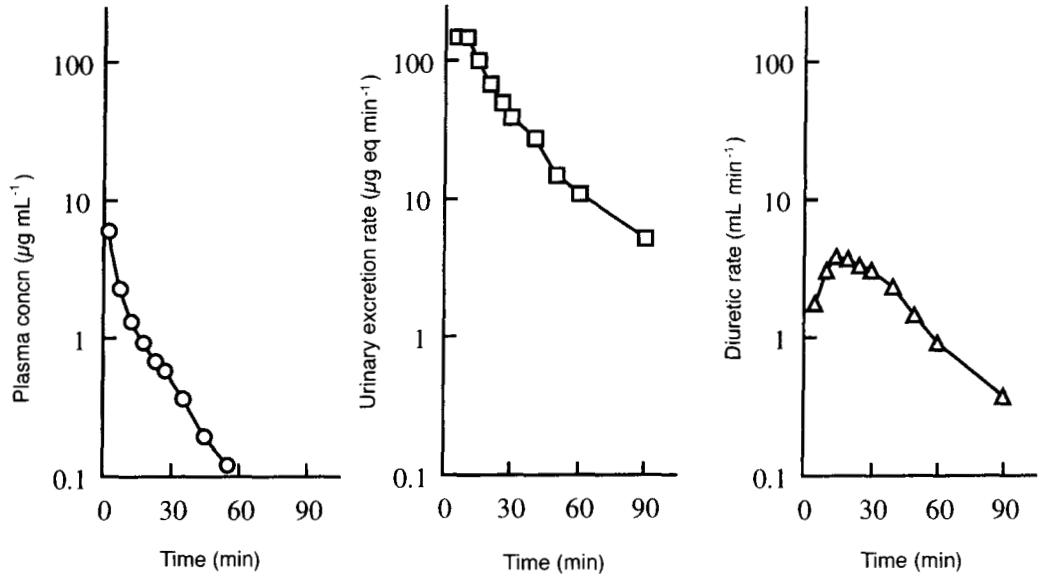

FIG. 1. Relationship between plasma concentration (○) and urinary excretion rate (□) and diuretic rate (△) of furosemide after an intravenous administration (1·0 mg kg^{-1}) to dogs. The data are expressed as the mean values of four animals.

and distributed quickly. The clearance (CL) of torasemide was significantly lower than that of furosemide because the Vd of torasemide was smaller than that of furosemide. There was also a significant difference ($P < 0.001$) in the area under the concentration–time curves (AUC) between furosemide and torasemide groups. Pharmacokinetic parameters of both diuretics in urine are shown in Table 2. The urinary excretion rate constant (k_{ex}) of furosemide was ten times larger than that of torasemide, showing that more furosemide was excreted in urine compared with torasemide. The cumulative excreted amount (Ae(∞)) of torasemide was essentially smaller than that of furosemide. The urinary recoveries (f_e) and the renal clearances (CL_R) of torasemide were significantly lower than those of furosemide ($P < 0.01$).

Diuretic rate and urinary excretion rate of the drugs

Fig. 1 shows the plasma concentration, diuretic rate and urinary excretion rate of furosemide. The plasma concentration was 5·89 μg mL^{-1}, 2·5 min after intravenous administration and decreased rapidly with a half-life of 9·76 min. The diuretic rate was 1·68 mL min^{-1} at 0–5 min after bolus injection, reached a peak of 3·69 mL min^{-1} at 10–15 min, and thereafter decreased rapidly to 0·35 mL

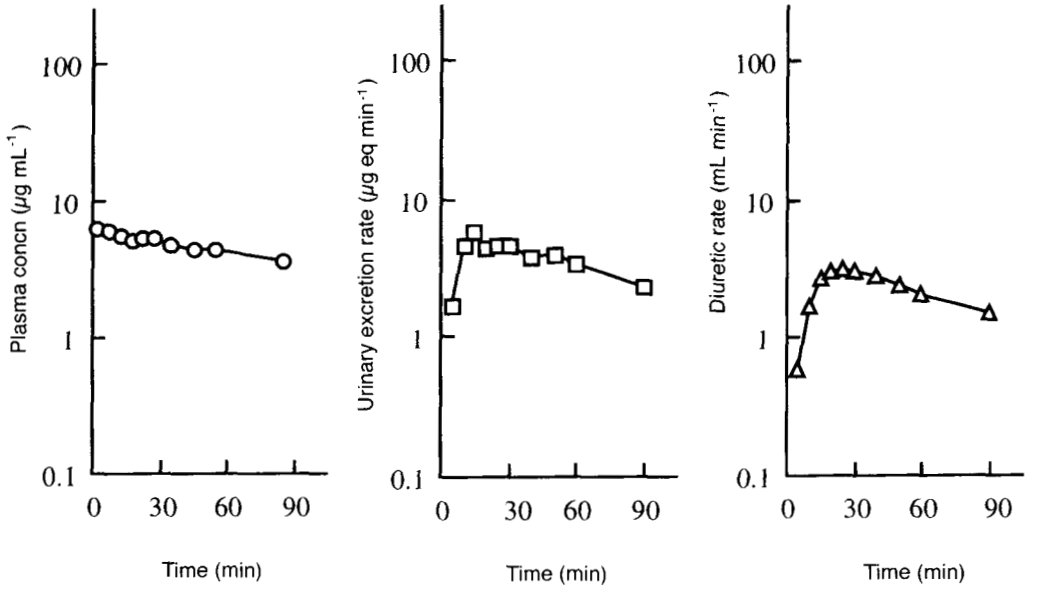

FIG. 2. Relationship between plasma concentration (○) and urinary excretion rate (□) and diuretic rate (△) of torasemide after an intravenous administration (0·3 mg kg^{-1}) to dogs. The data are expressed as the mean values of three or four animals.

min^{-1} at 80–90 min. No appreciable diuretic action of furosemide was observed at 80–90 min. The maximum urinary excretion rate (150 $\mu g\,min^{-1}$), which was about 25 times that of torasemide, was observed at 5–10 min and thereafter decreased rapidly to 3% of the peak level at 80–90 min.

Fig. 2 shows the plasma concentration, diuretic rate and urinary excretion rate of torasemide. The plasma concentration was 6·44 $\mu g\,mL^{-1}$ at 2·5 min and decreased with a half-life of 120·54 min. The diuretic rate was 0·56 mL min^{-1} at 0–5 min after administration, reached a peak of 3·03 mL min^{-1} at 20–25 min, and decreased gradually. The diuretic action was still observed at 80–90 min. These results show that the duration of the diuretic action of torasemide is longer than that of furosemide. The urinary excretion rate of torasemide was only 1·69 $\mu g\,min^{-1}$ at 0–5 min after intravenous administration, and peaked to 5·84 $\mu g\,min^{-1}$ at 10–15 min, then decreased gradually to 40% of the maximum level at 80–90 min.

Protein binding of the drugs in plasma and urine

Neither furosemide nor torasemide was detected as unbound drug in the plasma filtrate, although the detection level was 40 ng mL^{-1}. In the urine, virtually all of either drug was found in the filtrate. These findings demonstrate that torasemide and furosemide are highly bound to plasma proteins and are excreted as unbound drugs in the urine.

Discussion

The results obtained in the present experiments show that the difference in the diuretic profile of torasemide and furosemide may be explained by the differences in their urinary excretion rates.

The urinary excretion rate of furosemide was very high 0–10 min after intravenous administration and decreased rapidly. The plasma concentration, which reflects the urinary excretion profile, decreased rapidly after administration. The urinary excretion rate of furosemide is reported to be the prime determinant of the diuretic action (Hammarlund et al 1985). In this study, there was consistency between the diuretic rate and urinary excretion rate profiles, although there was a time-lag in the first 15 min after administration. In contrast, the urinary excretion rate of torasemide was much lower but gradually decreased. The plasma concentration of torasemide also decreased more slowly. The diuretic rate, which paralleled the urinary excretion rate, was lower but lasted longer (Fig. 2). These findings suggest that the diuretic profile of torasemide depends on its slower urinary excretion rate, which decreased more slowly with time.

Both diuretics, torasemide and furosemide, were found to have a high binding rate to plasma proteins in dogs (Cohen et al 1976; Ghys et al 1985a). Since their glomerular filtration was substantially limited, both diuretics were excreted by renal tubular secretion. Furosemide is a strong acid with pK_a of 3·9 and is secreted through the organic anion transport system, which is inhibited by *p*-amino hippuric acid (Hook & Williamson 1965; Hirsch et al 1975). Torasemide is a weak acid with pK_a of 7·1 and its transport in rat kidney vesicles is also inhibited by *p*-amino hippuric acid (Kramp et al 1985). These results suggested that both diuretics were transported through the same organic anion transport system. Anionic charge is important for affinity to the transport system and less specific structure is required (Møller & Sheikh 1983). Gutman et al (1960) reported that more acidic compounds (of lower pK_a) were excreted more rapidly in human or canine urine than less acidic compounds (of higher pK_a). A plausible mechanism is as follows. First, a less acidic compound, such as torasemide, would be largely reabsorbed (Gutman et al 1960), so that little torasemide is excreted in the urine.

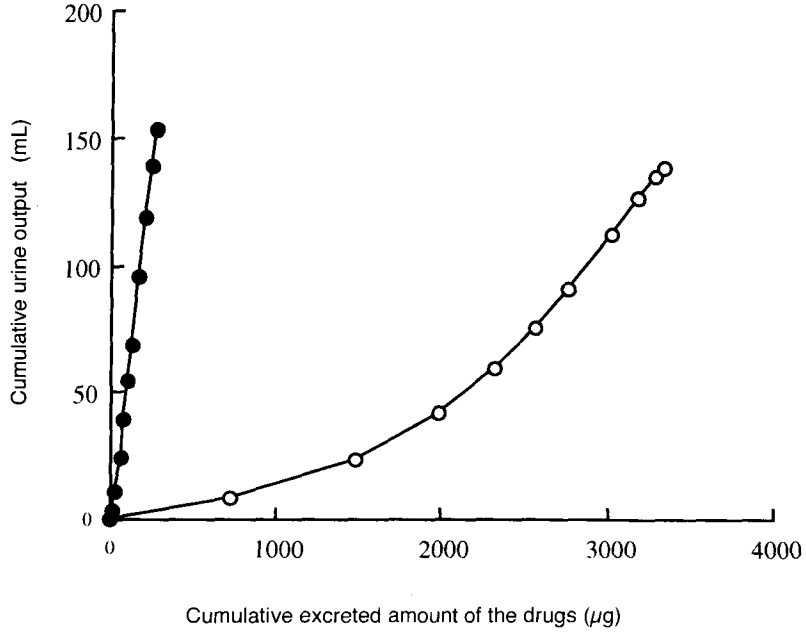

FIG. 3. Relationship between cumulative urine output and cumulative excreted amount of torasemide (●) and furosemide (○) after an intravenous administration to dogs. The data are expressed as the mean values of four animals.

Second, more furosemide in the ionic form exists in the plasma compared with torasemide and the ionized furosemide is primarily transported into the urine through the organic anion transport system, whereas torasemide is excreted more slowly.

There was a positive correlation between the cumulative excreted amount of torasemide or furosemide in the urine and the cumulative urine output. The cumulative excreted amount of torasemide in urine was much smaller than that of furosemide and the cumulative urine output was similar to that of furosemide within 90 min after an intravenous administration. This result shows that torasemide has a more potent diuretic action. Although it is suggested that the lower excretion of torasemide into the urine in normal volunteers compared with furosemide was due to its biotransformation to several metabolites (Dodion et al 1986), no active metabolites were observed.

Previous studies also reported that the inhibitory activity of torasemide on the luminal Na^+, Cl^-, K^+ cotransport system (Masereel et al 1992) and the diuretic action in anaesthetized dogs (Uchida et al 1992) were more potent than those of furosemide.

In conclusion, the present study provides evidence that the slower transfer rate from plasma to the nephron leads to the longer-lasting diuretic action of torasemide when compared with furosemide.

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