Bioavailability and Diuretic Effect of Furosemide Following Administration of Tablets and Retarded Capsules to Human Subjects

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Two kinds of dosage forms (tablets and retarded capsules) of furosemide (F) were compared in vitro dissolution profile and in vivo absorption studies. The dissolution of F from retarded capsules was extremely restricted in the first fluid of the JP XII disintegration test (within 0.8%), while the dissolution of F from tablets and retarded capsules in the second fluid of JP XII disintegration test were both complete.

Metabolite specific assay of F showed F, conjugation of F with glucuronic acid (FG) and acyl migration isomers of FG (FG-iso) in urine or plasma. The mean cumulative urinary excretion of F following administration of the tablets during 24 h was twice that of retarded capsules. The mean area under the plasma concentration-time curve (AUC) of F following administration of tablets was 1.5 times that of retarded capsules. The mean cumulative urinary volume during 24 h, however, was not significantly different between the two dosage forms. Clockwise hysteresis relationships between the diuretic response and the urinary excretion rate of F was observed after administration of retarded capsules. A straight relation between logarithm of the diuresis and logarithm of the urinary excretion of F was observed after maximum excretion rate of F following administration of both dosage forms.

Key words furosemide; tablet; retarded capsule; bioavailability; diuretic effect; furosemide glucuronide

Furosemide (F) has been widely used in the treatment of hypertensive crises in patients with acute pulmonary edema or renal failure.1,2,3) The dosage forms of F for oral administration were tablets or granules (rapid effect) and capsules or tablets (sustained release effect). Rapid effect of F often causes great fatigue by strong diuresis. The desirable therapy to maintain a continuous gentle diuresis requires the sustained release of F. The in vivo bioavailability and diuresis between tablets and retarded capsules of F have been reported.4,5,6)

The major metabolite of F in human is glucuronic acid conjugate (FG).7,8,9) We also found FG in human urine following oral administration of F tablets.10) We found that FG degraded easily to F and acyl migration isomers of FG (FG-iso) in bile following intravenous and intraduodenal administration of F to rabbits.10) We reported that F was very unstable in acidic media,11) and that FG was more unstable than F in acidic media and neutral in alkaline media.12) Maximum stability of FG was found at pH 3.212), moreover, F and FG were both sensitive to light.11) Correct determination of FG or F could be obtained if the biological specimens were stabilized by adjusting the pHs or were protected from light. The earlier reports4,5,6) lacked specific assay of F or metabolites.

Diuresis of F depends upon its concentration in urine rather than in plasma13) because F acts directly on renal tubule. Tablets and retarded capsules of F have been used clinically, and study of the pharmacokinetics and pharmacodynamics of F using the specific assay of metabolites in healthy human subjects could be valuable. We studied in vitro dissolution profiles of tablets and retarded capsules of F, and also studied in vivo absorption and diuresis following administration of these dosage forms.

MATERIALS AND METHODS

Materials F tablets (Lasix® tablet, 40 mg/tablet, lot No. 8L036) and F retarded capsules (Eutensin® capsule, 40 mg/capsule, lot No. 9A001) were obtained from Hoechst Japan, Ltd., Tokyo, Japan. F powder (lot No. 26F0636), bumetanide (lot No. 23H0487) and β-glucuronidase (from bovine liver, 5000 Sigma units/ml, lot No. 12F6171) were obtained from Sigma Chemical Co. St. Louis, MO, U.S.A. 4-Chloro-5-sulfamoylanilinic acid (CSA) (lot No. F1) was obtained from U.S. Pharmacopeia Inc., Rockville, MD, U.S.A. Acetonitrile was HPLC grade from Kanto Chemicals Co. Inc., Tokyo, Japan. FG was obtained as described in previous reports.10,12) All other chemicals were of reagent grade.

Dissolution Studies The dissolution profiles of F from tablets or capsules were obtained according to the paddle method of the JP XII dissolution test. The first (pH 1.2) and second (pH 6.8) fluids of the JP XII disintegration test were used as medium at 37 ± 0.1°C. An NTR-1000 system (Toyama Sangyo Co. Ltd., Osaka, Japan) was used in the dissolution study. Five hundred ml of the test fluid in a container was placed in a water bath and rotated at 100 rpm. A plastic cover was placed on the container to minimize the loss of test fluid. The amount of the F test sample was 40 mg. One tablet or one capsule was placed in the container and 2 ml of the fluid was withdrawn with a syringe at predetermined time intervals. Two ml of fresh fluid warmed to the same temperature was added to the container to maintain the original volume. The sample solution was filtered through a membrane filter (pore size 0.2 μm, HA type, Nihon Millipore Kogyo Co., Ltd., Yonezawa, Japan). After the filtration, the sample solution (1.0 ml) was added to 0.5 ml of the internal standard solution (100 ng/ml bumetanide solution) and F concentration was determined by the

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HPLC method. In the analysis in the first fluid, to the filtered sample (1.0 ml) was immediately added 0.2 N of sodium hydroxide (0.5 ml) to prevent the hydrolysis of F.

Subjects and Procedure Four male volunteers, aged between 22 and 46 (average 28.8) years and weighing between 63 and 85 (average 69.6) kg participated in this study. They were all healthy and informed consent was obtained from each of them.

The subjects were fasted for 10 h prior to administration of the drug. They were not permitted to take any beverage containing alcohol, caffeine or fruit juice for 24 h prior to the drug administration. Tablets or capsules (40 mg as F) were orally administered to the subjects together with 200 ml of water at 9:00 a.m. Food was offered 4 h later. Water or non-cafeinated beverage was allowed freely to prevent dehydration. The mean volumes of water intake were 126 ml at 0.8 h, 370 ml at 1.5 h, 265 ml at 2 h, 133 ml at 3 h, 128 ml at 4 h, 113 ml at 5 h, 104 ml at 6 h, 142 ml at 7 h and 120 ml at 8 h after administration of tablets (total 1501 ml by 8 h). After administration of capsules the mean volumes of water consumed were 122 ml at 0.8 h, 245 ml at 1.5 h, 240 ml at 2 h, 231 ml at 3 h, 160 ml at 4 h, 128 ml at 5 h, 123 ml at 6 h, 107 ml at 7 h and 126 ml at 8 h (total 1482 ml by 8 h). Without administration of any dosage form, the volume of urine was recorded at 2, 4, 6, 8, 12 and 24 h after 200 ml of water was allowed drunk at 9:00 a.m. (control). Subjects took 120 ml of water at each of 2, 4, 6 and 8 h; the volume of water intake was not recorded after 8 h. Urine was collected at 0 to 45, 45 to 105, 105 min to 2.5 h, 2.5 to 3.5, 3.5 to 4.5, 4.5 to 5.5, 5.5 to 6.5, 6.5 to 7.5, 7.5 to 9.5, 9.5 to 11.5 and 11.5 to 24 h. After urine volume was recorded, the pH was immediately adjusted to around 4 by addition of a small amount of 10% phosphoric acid to prevent acyl migration and the hydrolysis of FG.\textsuperscript{10,12} Blood samples (2.0 ml) following oral administration of tablets and capsules were collected at 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24 h and 1, 2, 3, 4, 6, 8, 12, 24 h, respectively. Blood was heparinized and cooled in an ice-water bath, then centrifuged at 3000 rpm for 10 min. The pH of the plasma was adjusted to around 4 by a small amount of 5% phosphoric acid. A portion of the urine and plasma samples were kept in the freezer until assayed. At least 10 d were allowed to pass following the experiment.

**Determination of Furosemide and Its Metabolites in Urine** Analytical procedures of F, (F+FG) and (F+FG+FG-isoo, total) fractions in urine were based on the previous study.\textsuperscript{10} For F assay, 1 ml of sample was added to 700 µl of acetate buffer (pH 4.8) and 300 µl of the internal standard solution (100 ng/ml bumetanide solution) and then passed through a membrane filter (pore size 0.5 µm, LCR-13-LH, Nihon Millapore Kogyo Co., Ltd.). For (F+FG) assay, 700 µl of β-glucuronidase solution (1000 Sigma units/ml, dissolved in acetate buffer at pH 4.8) was added to 1 ml of sample solution. For total fraction assay, 700 µl of 0.3 N sodium hydroxide was added to 1 ml of sample. Hydrolysis was made by incubating both fractions of (F+FG) and total at 37°C for 1 h. After the hydrolysis, internal standard solution (300 µl) added and passed through the membrane filter. The mixture was injected into the HPLC system. Each concentration of FG and FG-iso was calculated as F.

**Determination of Furosemide in Plasma** Plasma (0.4 ml) was added to 700 µl of a mixture of methanol and acetone (3:2) and centrifuged at 3000 rpm for 10 min. To the supernatant (400 µl) was added 50 µl of the internal standard solution (50 ng/ml bumetanide solution) and the mixture was passed through the membrane filter described above, then injected into the HPLC system.

**HPLC Conditions for Dissolution Study** The apparatus and conditions of HPLC for the F concentrations in vitro dissolution study were as follows: HPLC was performed on a Shim-pack CLC-ODS, with a 5 µm reversed-phase column (150 × 6 mm i.d.) and a Shim-pack G-ODS, with a 5 µm guard column (10 × 4 mm i.d.) (Shimadzu Co., Kyoto, Japan). The columns were maintained at 40 ± 0.1°C in the column oven (CTO-10A, Shimadzu). A spectrofluorometric detector (RF-550A, Shimadzu) was used. Excitation and emission wavelengths were 345 and 415 nm, respectively. The mobile phases were 20% acetonitrile containing 0.3% acetic acid (A) and 80% acetonitrile containing 0.3% acetic acid (B). From 0 to 5 min, the mobile phase consisted of 80% A and 20% B; 5 to 8 min, 40% A and 60% B; 8 to 12 min, 40% A and 60% B; 12 to 18 min, 80% A and 20% B. The flow rate was 1.0 ml/min. Sample solutions (10—30 µl) were automatically injected into the HPLC using a SIL-10A autoinjector (Shimadzu) and a LC-10AD pump (Shimadzu) using a system controller (SCL-10A, Shimadzu) with a run time of 18 min. A C-R6A computing integrator (Shimadzu) was used to calculate the area of the peaks of the chromatogram. In the chromatogram, peaks of CSA, F and the internal standard were observed at 3.6, 5.8 and 12.2 min, respectively. The detection limits of F in the first and second fluids of the JPXII disintegration test were 2 and 1 ng/ml, respectively.

**HPLC Conditions for Furosemide and Its Metabolites in Urine and Plasma** The apparatus was the same as described above except for the elute gradient program. The mobile phases of A and B were the same as described above. The gradient program was made as follows: from 0 to 5 min, the mobile phase was 88% A and 12% B; from 5 to 10 min, 60% A and 40% B; from 10 to 20 min, 50% A and 50% B; and from 20 to 30 min, 88% A and 12% B. Sample solutions (10—50 µl) were injected into the HPLC system with a run time of 30 min. In the chromatogram, peaks of F, FG, FG-iso and the internal standard were found at 13.8, 9.4, 8.8—9.2 and 17.4 min, respectively. The detection limits of F in urine and plasma were 5 and 1 ng/ml, respectively.

**Protection from Photodegradation** During the study all processes were protected carefully from photodegradation of F, FG and FG-iso by keeping them in a darkened room.\textsuperscript{11}

**Pharmacokinetic Analysis** The parameters of the area under the plasma concentration–time curve (AUC), the maximum plasma concentration (C_{max}) and the time (T_{max}) were estimated by the model-independent moment method\textsuperscript{12} using a micro-computer, model 9801 (NEC, Co., Tokyo, Japan). Statistical analysis was performed using a Student’s t-test with p < 0.05 as the minimal level
of significance.

RESULTS AND DISCUSSION

Dissolution Profiles of Furosemide from Tablets and Capsules

Figure 1 shows the dissolution profiles of F from tablets and retarded capsules in the first (a) and second fluid (b). The maximum percentage of dissolution of F from tablets was 14% in the first fluid. The concentrations of F from tablets decreased after 6 h and CSA, the hydrolyzed product of F, increased gradually. As the retarded capsules contain the enteric coated granules, the dissolution from capsules in the first fluid was greatly suppressed (maximum percentage was only 0.4%) and CSA increased gradually after 6 h. In contrast, F dissolved rapidly from tablets or capsules in the second fluid. CSA was not detected in the second fluid.

Stability of Furosemide Glucuronide in Human Urine

Figure 2 shows the time course of degradation of FG in urine at 37°C. Without adjustment of the pH (pH 5.1), FG degraded with apparent first-order kinetics. The half-life (t_{1/2}) was 30 h. The value was smaller than that in buffer solution at the same pH value (t_{1/2} was 390 h). Smith et al. reported a similar phenomenon in the degradation of zomepirac glucuronide. The component of urine might accelerate the degradation of FG. FG was stabilized by adjusting the pH of urine (pH 3.45) with t_{1/2} 550 h. The pH values of urine samples were between 5.08 and 7.23. The t_{1/2} was smaller when pH value of the urine was higher. FG degraded even in the freezer without adjustment of the pH. By adjusting the pH of the urine and keeping samples in the freezer, the recovery ratio of FG (n = 30, FG concentration as F, 50 ng/ml - 2.8 μg/ml) was 97-100% within one month.

Furosemide and Its Metabolites in Urine and Plasma

Following administration of tablets and capsules to human subjects, peaks of F, FG and FG-iso were observed in the HPLC chromatogram in urine and plasma. Figure 3b shows the peaks found in urine after 3.5 h following oral administration of capsule. The peaks 1 and 2 corresponded to FG and F, respectively. After the hydrolysis with β-glucuronidase, the peak area of FG decreased and the peak area of F increased (136% of the peak area in b). However, peak 1 remained small in the chromatogram as shown in Fig. 3c, and disappeared after the hydrolysis with 0.3 N sodium hydroxide (Fig. 3d); the peak area of F increased (141% of the peak area in b). Peak 1 was thus considered to be a mixture of FG and FG-iso. The peaks of FG and FG-iso were also detected in plasma but were negligible.

Urinary Excretion of Furosemide and Its Metabolites

Figure 4 shows the cumulative urinary excretion of F and its metabolites following oral administration of tablets and capsules. The mean cumulative urinary excretions of F, FG and FG-iso during the 24 h after tablet administration (Fig. 4a) were 13.8, 5.6 and 1.2 mg, respectively, corresponding to 34.5, 14.0 and 3.0% of the dose. The
total recovery was 51.6%, while those of retarded capsules were 6.1, 2.6 and 0.2 mg, respectively (Fig. 4b), corresponding to 15.0, 6.7 and 0.4% of the dose. The mean cumulative urinary excretion of F following administration of tablets was more than twice that of capsules.

These results showed the values of cumulative urinary excretion of F following oral administration of tablets and retarded capsules during a 24 h period both to be smaller than those reported previously (tablets 22.6 mg, capsules 8.5 mg). Moreover, values of the cumulative urinary excretion of the total fraction were similar to those of F which have been reported before. The reason for the difference between earlier results and ours might be the difference of analytical methods. In the previous reports, acidification of urine sample for the extraction of F was done prior to the fluorometric analysis. FG in the urine is easily hydrolyzed to F by acid. F itself is hydrolyzed to CSA in acidic solution. Not only F but also FG or CSA has fluorescence. The concentration of F reported previously thus might not be correct. By stabilizing FG or FG-iso, unchanged F concentrations were obtained in this study.

Figure 5 shows the time course of urinary excretion rate of F following oral administration of tablets and
capsules. The peak of these rates was found 1.3 h after administration of tablets and 3.0 h after administration of capsules. Delay in the urinary excretion of F was thought to be due to the low release of F in the stomach as shown in Fig. 1a. The value of the peak urinary excretion rate of F after administration of tablets was more than 3 times that of capsules.

**Plasma Concentration of Furosemide** Figure 6 shows the time course of plasma concentrations of F following oral administration of tablets and capsules. The pharmacokinetic parameters are shown in Table 1.

The mean AUC of F following tablet administration was about 1.5 times larger than that of capsules. Though the value was not directly proportional to that of urinary excretion, large AUC following administration of tablets reflected larger cumulative urinary excretion of F (Fig. 4). The Cmax after administration of tablets was 3 times that of capsules. The Tmax following capsule administration was significantly delayed compared to that of tablets.

**Diuretic Response by Furosemide Following Oral Administration of Tablets and Capsules** Pharmacodynamic studies of the diuretic effects of F were evaluated by the cumulative urinary volume (a) and the diuretic rate (b) as shown in Fig. 7. These diuretic effects are summarized

![Fig. 5. Urinary Excretion Rate of Furosemide Following Oral Administration of 40 mg Furosemide to Human Subjects](image)

- ○, tablets; □, retarded capsules. Each point represents the mean ± S.E. of four determinations.

![Fig. 6. Plasma Concentration of Furosemide Following Oral Administration of 40 mg Furosemide to Human Subjects](image)

Symbols are the same as Fig. 5. Each point represents the mean ± S.E. of four determinations.

<table>
<thead>
<tr>
<th></th>
<th>Tablets</th>
<th>Retarded capsules</th>
</tr>
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<tbody>
<tr>
<td>AUC (ng·h/ml)</td>
<td>2530.5 ± 224.5</td>
<td>1703.0 ± 235.2</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.13 ± 0.40</td>
<td>2.27 ± 0.11</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>1038.7 ± 412.5</td>
<td>340.3 ± 89.3</td>
</tr>
</tbody>
</table>

Each data represents the mean ± S.E. of four subjects. *p < 0.05 as compared with tablets.

![Fig. 7. Cumulative Urinary Volume (a) and Diuretic Rate of Urine (b) Following Oral Administration of 40 mg Furosemide to Human Subjects](image)

- ○, tablets; □, retarded capsules; △, control. Each point represents the mean ± S.E. of four determinations.
in Table 2.

The mean cumulative urinary volumes during the 24 h of the control and following administration of tablets and capsules were 1281, 3947 and 3319 ml, respectively. There was no significant difference between the two dosage forms. The maximum diuretic rate after administration of tablets was 1.8 times greater than that of capsules.

Figure 8 shows the relationships between the diuretic response and the urinary excretion rate of F after administration of tablets (a) and capsules (b), respectively. A counter-clockwise hysteresis loop and clockwise hysteresis loop were observed between diuretic rate and logarithm of excretion rate of F following tablet and retarded capsule administration, respectively.

Hammarlund et al. reported a clockwise hysteresis relationship between the diuretic response and the urinary excretion of F. They reported that a clockwise hysteresis loop was observed following administration of F tablets or solution after food intake, but was not observed in the fasting state. Though they did not mention the reason, food might decrease the gastric emptying rate of F and result in a slow absorption of F from the intestinal tract.

Figure 9 shows the relationship between logarithm of diuretic rate and logarithm of excretion rate of F following the administration of tablets and capsules. After oral administration of tablets, rapid diuresis was observed before the maximum excretion rate of F; the diuretic rates then decreased with the decrease in excretion rates of F. A good straight line was observed (\(y = 0.631x + 2.517, r^2 = 0.972\)) after the maximum excretion rate of F. Before this maximum rate was occurred, the effect of the F excretion rates on diuresis were below the line. One possible explanation for this might be that inhibition of the reabsorption of sodium and chloride at the ascending limb of Henle's loop after administration of F tablets was delayed because the fast absorption of F resulted in such rapid excretion. Following the administration of retarded capsules, before the maximum diuresis, F with smaller excretion rates showed an apparent diuretic effect. After F reached maximum excretion rate, diuretic rates decreased as these excretion rates fell. Again a good straight line was observed (\(y = 0.662x + 2.585, r^2 = 0.954\)). The straight lines for both tablets and capsules were almost equal. The combined line of the data points of tablets and capsules after the maximum excretion rate of F showed: \(y = 0.640x + 2.521, r^2 = 0.962\).

Table 2. Diuretic Effects Following Administration of 40 mg Furosemide to Human Subjects

<table>
<thead>
<tr>
<th></th>
<th>Tablets</th>
<th>Retarded capsules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>FG</td>
</tr>
<tr>
<td><strong>Cumulative amount of</strong></td>
<td><strong>F (0–24 h, mg) (as F)</strong></td>
<td><strong>F (0–24 h, mg)</strong></td>
</tr>
<tr>
<td>Cumulative amount of F</td>
<td>13.81 ± 1.27</td>
<td>5.61 ± 0.96</td>
</tr>
<tr>
<td>Percent dose (%)</td>
<td>34.53 ± 3.17</td>
<td>14.03 ± 2.41</td>
</tr>
<tr>
<td>Maximum excretion rate</td>
<td>4.65 ± 1.07</td>
<td>1.70 ± 0.58</td>
</tr>
<tr>
<td>Cumulative urinary</td>
<td>3947 ± 537.4</td>
<td></td>
</tr>
<tr>
<td>volume (0–24 h, ml)</td>
<td>885.0 ± 97.0</td>
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</table>

Each data represents the mean ± S.E. of four subjects. *p < 0.05 as compared with each fraction of tablets.

![Graph](a) ![Graph](b)

Fig. 8. Relationship between Diuretic Response and Logarithm of Urinary Excretion Rate of Furosemide Following Oral Administration of Tablets (a) and Retarded Capsules (b) of 40 mg Furosemide to Human Subjects

Open circles and closed circles represent the diuretic response before and after the peak of diuresis, respectively.
reaching a maximum, the excretion rates of F acted on the diuresis in different ways. Even lower excretion rates of F caused diuresis, indicating that the dose-response of F might depend on the water-regulation in the body. The efficiency of F excretion rate in the urine was not proportional to the diuresis. Homeostatic balance for cells is controlled by extracellular fluid, which is further divided into intravascular fluid (plasma), interstitial fluid and transcellular fluid. Transcellular fluid is the smallest portion of extracellular fluid, composed of cerebrospinal, pericardial, pleural, synovial, and intraocular fluids, sweat and digestive secretions. In healthy humans, the balance of interstitial fluid is controlled by water in plasma via urinary excretion. Large loss of water in plasma could change the water balance in interstitial fluid. Before the maximum excretion rate of F, the diuresis might be due to water in the plasma or a partial portion of the interstitial fluid. Before the maximum excretion rate of F following administration of tablets and capsules, the mean cumulative amount of F in urine was 6.63 and 2.55 mg, respectively, and the corresponding cumulative urine volume was 1186 and 1297 ml. There was no significant difference in cumulative urine volume between them. The straight portion in Fig. 9 might be the relation between the diuretic rates and excretion rates of F after the change of water balance in interstitial fluid when the diuresis had been completed. Similar lines of the tablets and capsules might mean that the excretion rate of F acted on the diuretic rate in a similar manner. As the excretion rates of F after 4 h following administration of retarded capsules were almost equal to those of tablets, the diuretic effects at that time were also similar. This might be the reason why retard capsules having small AUC and tablets having large AUC showed equal diuresis.

The average diuretic rate in control (without F) was 54.0 ± 9.1 ml/h (logarithmic value 1.73). Smaller diuretic rates than control were observed in the region where excretion rates of F were under 60 μg/h. These results were reasonable because the volume of water in interstitial fluid might be smaller than that of control.

F is an acidic drug with a pKa value of 3.8. After dissolution from enteric coated granules in retarded capsules, most of the F molecules might exist in an ionized form at the absorption site. The extent of absorption of F might thus be limited and show the small AUC values. The selection of coating material or development of other sustained released formulation of F may result in larger AUC or sustained diuresis.

REFERENCES AND NOTES

1. A part of this study was presented at the 11th Annual Meeting of Japan TDM, Sapporo, June 1994.