Bioavailability and Diuretic Effect after Administration of Retarded Capsules of Bumetanide in Human Subjects¹⁾

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Retarded capsules containing 1 mg bumetanide (BN) were prepared and their *in vivo* absorption and diuretic effect after oral administration in human subjects were studied. For comparison, commercially available tablets of BN (rapid effect) were administered orally. The mean value of the area under the plasma concentration time curve (AUC) after administration of retarded capsules was about one half that of the tablets. The mean maximum plasma concentration ($C_{\max}$) and the mean maximum urinary excretion rate of BN after administration of retarded capsules were also about one half compared to those of the tablets. Cumulative urinary volumes for 24 h, however, were not significantly different between retarded capsules and tablets. Peak times for the urinary excretion rate of BN, urine flow rate and the $C_{\max}$ after administration of retarded capsules were significantly delayed compared to those of tablets. Clockwise hysteresis relationships between the urine flow rate and plasma concentration or urinary excretion rate of BN were observed after administration of retarded capsules. From these studies, retarded capsules of BN possessed a mild diuresis and its diuretic effect was maintained for a few hours after administration.

Key words bumetanide; retarded capsule; tablet; bioavailability; diuretic effect; clockwise hysteresis

Bumetanide (BN), 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoic acid, has been widely used as a loop diuretic agent.^{3–6)} The available dosage form of BN for oral administration is as a tablet. Due to the rapid effect of BN tablet, patients often feel great fatigue caused by rapid and strong diuresis.^{6,7)} We previously compared the *in vitro* dissolution, *in vivo* bioavailability and diuretic effect between tablets (Lasix[®]) and retarded capsules (Eutensin[®]) of furosemide.⁸⁾ The subjects who were administered retarded capsules of furosemide experienced hardly any of the feelings described above. But the peak time of the diuresis was not significantly different between tablets and retarded capsules. The absorption of furosemide from retarded capsules was smaller than that of tablets because granules in retarded capsules were the enteric-coated type. The dissolution of furosemide from granules in the stomach was suppressed.⁸⁾

In the previous report we prepared sustained release granules of BN to obtain mild diuresis for a few hours to avoid urination during the night.⁹⁾ *In vitro* dissolution studies, the dissolution behavior of BN from a preparation with a composition of BN: γ -cyclodextrin (γ -CD): ethylcellulose (EC) (1:4:2, weight ratio) showed favorable sustained release in JP XIII disintegration media No. 1 and No. 2.⁹⁾

In this study, we investigated the pharmacokinetics and pharmacodynamics of BN after oral administration of capsules (retarded capsules) containing sustained release granules in healthy human subjects. The relationships between plasma concentration or urinary excretion rate of BN and diuretic effect were also evaluated. For comparison, conventional tablets of BN were administered orally to human subjects.

MATERIALS AND METHODS

Materials BN tablets (Lunetoron[®] tablet, 1 mg/tablet, lot No. T110P) were obtained from Sankyo Co., Tokyo, Japan. BN powders, γ -CD, EC, piretanide powders and acetonitrile

used were the same as in the previous report.⁹⁾ All other chemicals were of reagent grade.

The preparation of sustained release granules of BN was the same as in the previous report with a weight ratio for BN, γ -CD and EC of 1:4:2, respectively.⁹⁾

Subjects and Procedure Three male volunteers, each 24 years old and weighing between 70 and 86 (average 77.7) kg participated in this study. They were all healthy and informed consent was obtained from each one of them. Food was restricted for 10 h prior to the drug administration. Sustained release granules containing 1 mg BN were filled in JP No. 2 capsules (Kobayashi Capsule Manufactory, Himeji, Japan) and used the *in vivo* studies (retarded capsules). The dissolution behavior of BN using JP disintegration media No. 1 and No. 2 showed no difference between capsules and granules. Lunetoron[®] tablets or retarded capsules (1 mg as BN) were orally administered to the subjects together with 200 ml of water. Water or non-caffeinated beverage (100 to 250 ml) was taken after every urination to prevent dehydration. Food was not allowed for 4 h after administration. The schedule for collection of blood and urine is shown in Fig. 1. One week was allowed for the wash-out of the drug following the experiment. Blood was heparinized immediately and centrifuged at 3000 rpm for 10 min. The pH of the plasma was adjusted to around 5 by a small amount of 5% phosphoric acid to prevent acyl migration of the metabolites.^{10,11)} After urine volume was recorded, the pH was adjusted to around 5 by addition of a small amount of 10% phosphoric acid. A portion of plasma and urine samples was kept in the freezer until assayed.

Determination of BN in Plasma and Urine Plasma (0.4 ml) was added to 0.5 ml of acetonitrile and centrifuged at 3000 rpm for 10 min. To the supernatant (0.5 ml) was added 0.3 ml of the internal standard solution (50 μ g/ml piretanide dissolved in 0.1 M sodium hydroxide) and the mixture was passed through a membrane filter (pore size 0.5 μ m, HPLC Sample Prep LCR 13-LH, Nihon Millipore Kogyo

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Fig. 1. Schedule for Collection of Blood and Urine

Blood samples after oral administration of tablets and capsules were collected at 0.5, 1, 1.5, 2, 4, 6 and 8 h and 0.5, 1, 2, 3, 4, 6 and 8 h, respectively. 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. Control urine means urine without administration of any dosage form.

Fig. 2. Plasma Concentration of 1 mg BN after Administration of Tablets and Retarded Capsules in Human Subjects

●, tablets; ○, retarded capsules.

Co., Ltd., Yonezawa, Japan). The solution (10–50 μ l) was injected into the HPLC system. Urine (1 ml) was added to 0.5 ml of the internal standard solution (50 μ g/ml piretanide) and then passed through the membrane filter described above. The solution (10–50 μ l) was injected into the HPLC system.

HPLC Conditions for BN in Plasma and Urine The apparatus and conditions for HPLC for the BN concentrations were the same as in the previous report.⁹⁾ The detection limits for BN in plasma and urine were 2 and 10 ng/ml, respectively.

During the study, all processes were carefully conducted in a darkened room to protect from photodegradation of BN.

Pharmacokinetic Analysis The parameters of the area under the plasma concentration time curve (AUC), the mean residence time (MRT), the maximum plasma concentration (C_{max}) and the time (T_{max}) were estimated by the model-independent moment method¹²⁾ 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

Plasma Concentration of BN Figure 2 shows the time course of plasma concentrations of BN after oral administration of Lunetoron[®] tablets and retarded capsules. The pharmacokinetic parameters are shown in Table 1. A rapid increase of the plasma concentration of BN was observed after administration of tablets. Plasma concentrations from 0.5 to 2 h after administration of tablets were high compared to those of the retarded capsules in all of the subjects. The mean values of the AUC and the C_{max} after administration of tablets averaged 1.8 and 2.5 times those of retarded capsules, respectively. The mean T_{max} after administration of retarded capsules (3.0 h) was significantly delayed compared to that of tablets (0.8 h). The mean MRT after administration of retarded capsules tended to be larger than that of tablets.

Diuretic Effect by BN Figure 3 shows the cumulative amount of BN in urine (a) and the cumulative urinary volume (b) after administration of tablets or retarded capsules. The cumulative amounts of BN in urine after administration of tablets were larger at every time compared to those of re-

tarded capsules in the three subjects. The cumulative urinary volume, however, was not different between tablets and retarded capsules after 3.5–5.5 h. The mean cumulative urinary volume during 24 h after administration of tablets, retarded capsules and control (urine without administration of BN) were 2110, 2380 and 1300 ml, respectively.

Figure 4 shows the urinary excretion rate of BN (a) and the urine flow rate (b) after administration of tablets and retarded capsules. These diuretic effects are summarized in Table 2.

The mean maximum urinary excretion rate of BN after administration of tablets ($286 \mu\text{g/h}$) was 2.6 times larger than that of retarded capsules ($108 \mu\text{g/h}$). This ratio was almost the same as that in the C_{max} . The mean peak time of urinary excretion rate of BN after administration of retarded capsules (2.8 h) was delayed compared to that of tablets (1.3 h). On the other hand, the mean maximum urine flow rate of tablets and

retarded capsules were 867 and 493 ml/h (1.8 times that of retarded capsules), respectively. The diuretic effect after administration of tablets occurred rapidly and was short. When the tablets were administered, the peak times of the urinary excretion rate of BN and urine flow rate were almost equal (1 h). On the other hand, the mean peak time of the urine flow rate was found earlier (2.3 h) than those of the plasma concentration (3.0 h) or the urinary excretion rate of BN (2.8 h) after administration of retarded capsules. The urine flow rates after administration of retarded capsules were larger at 3 and 4 h compared to those of tablets in all of the subjects. The mean urine flow rate in control was 53 ml/h. Therefore, the diuretic effect by BN after administration of tablets and retarded capsules seemed to end at 3 and 5 h (4 and 6.5 h in subject A), respectively. Thus the time course of the urine flow rate after administration of retarded capsules was found to be different from those of plasma concentration and urinary excretion rate of BN.

Figures 5 and 6 show the relationships between plasma concentrations and urinary excretion rates of BN urine flow rates, respectively, after administration of tablets and retarded capsules.

Counter-clockwise and clockwise hysteresis loops were observed after administration of tablets and retarded capsules, respectively, in the relationship between plasma concentration of BN and urine flow rate (Fig. 5). As shown in Fig. 6, however, clockwise hysteresis loops were found in the relationship between urinary excretion rate of BN and urine

Table 1. Pharmacokinetic Parameters after Oral Administration of BN in Human Subjects

Parameters	Tablets	Capsules
AUC (ng·h/ml)	106.7 ± 11.1	$59.0 \pm 6.8^*$
MRT (h)	1.9 ± 1.2	3.3 ± 0.2
C_{max} (ng/ml)	47.0 ± 0.2	$18.7 \pm 2.9^*$
T_{max} (h)	0.8 ± 0.5	$3.0 \pm 0.0^*$

Each data represents the mean $\pm$ S.E. of three subjects. * Significantly different from tablets at $p < 0.05$.

Fig. 3. Cumulative Amount of BN in Urine (a) and Cumulative Urinary Volume (b) of 1 mg BN after Administration of Tablets and Retarded Capsules in Human Subjects

Symbols are the same as Fig. 2. □, control urine.

Fig. 4. Urinary Excretion Rate of BN (a) and Urine Flow Rate (b) of 1 mg BN after Administration of Tablets and Retarded Capsules in Human Subjects
Symbols are the same as Fig. 3.

Table 2. Diuretic Effects after Oral Administration of BN in Human Subjects

	Tablets	Capsules
Urinary excretion of BN		
Cumulative amount (0–24 h, μg)	476.8 ± 37.8	$328.5 \pm 21.6^*$
Percent of dose (%)	47.7 ± 3.8	$32.9 \pm 2.2^*$
Peak time (h)	1.3 ± 0.3	$2.8 \pm 0.3^*$
Maximum excretion rate of BN ($\mu\text{g/h}$)	285.64 ± 4.1	$108.4 \pm 12.8^*$
Diuresis		
Cumulative urinary volume (0–24 h, ml)	2110.2 ± 182.4	2377.5 ± 156.6
Peak time (h)	1.0 ± 0.0	$2.3 \pm 0.3^*$
Maximum urine flow rate (ml/h)	866.7 ± 88.2	$492.5 \pm 39.0^*$

Each data represents the mean $\pm$ S.E. of three subjects. * Significantly different from tablets at $p < 0.05$.

flow rate after administration of both of dosage forms. When the retarded capsules were administered, apparent diuresis occurred by the smaller plasma concentration or urinary excretion rate of BN.

DISCUSSION

For a comparison of tablets versus retarded capsules of BN, plasma concentration, urinary excretion of BN and the diuretic effect after administration were investigated in human subjects. The C_{max} and the maximum urinary excretion rate of BN after administration of tablets were more than twice those of retarded capsules. The diuretic effect after administration of tablets was strong and rapidly decreased. Counter-clockwise and clockwise hysteresis loops were ob-

served in the relationships between plasma concentration and urinary excretion rate of BN urine flow rate, respectively, after administration of tablets (Figs. 5a, 6a). It is considered that there might be a time lag between plasma concentration and diuretic effect in the case of tablets because BN acts on the renal tubule directly. On the other hand, clockwise hysteresis loops were observed after administration of retarded capsules as shown in Figs. 5b and 6b. The maximum of urine flow rate was observed before the maximum of plasma concentration or urinary excretion rate of BN in retarded capsules. Hammarlund *et al.*¹³⁾ has explained that a clockwise hysteresis loop might indicate an acute tolerance development to diuresis. These two kinds of hysteresis loops may be due to the differences of the activate site of BN or the absorption rate from the gastro intestinal tract after oral admin-

Fig. 5. Relationships between Plasma Concentration of BN and Urine Flow Rate after Administration of Tablets (a) and Retarded Capsules (b) in Human Subjects

Open circles and closed circles represent the diuretic response before and after the peak of urine flow rate, respectively. Each point represents the mean $\pm$ S.E. of three experiments.

Fig. 6. Relationships between Urinary Excretion Rate of BN and Urine Flow Rate after Administration of Tablets (a) and Retarded Capsules (b) in Human Subjects

Each point represents the mean $\pm$ S.E. of three experiments. Symbols are the same as Fig. 5.

istration.

Very few drugs have been reported where two different types of hysteresis loops are observed by the same administration method.^{8,14} We reported the relationship between urine flow rate and urinary excretion rate of furosemide after administration of tablets and retarded capsules of furosemide.⁸ The relationship described above also showed a counter-clockwise and a clockwise hysteresis loop, respectively. Gourlay *et al.*¹⁴ reported that a clockwise and a counter-clockwise hysteresis loop were observed between venous and arterial plasma concentration of nicotine heart rate response, respectively, after intravenous administration of nicotine. They considered that the site of blood sample can profoundly alter the interpretation of pharmacokinetic/pharmacodynamic relations.

The maximum urinary excretion rate of BN after administration of tablets and retarded capsules was observed at 1.3 and 2.8 h, respectively. At these times, the mean cumulative amounts of BN in urine after administration of tablets and re-

tarded capsules were 168.7 and 99.8 μ g, and the corresponding mean cumulative urine volume was 745 and 711 ml, respectively. Thus a sufficient diuretic effect was obtained after administration of retarded capsules in spite of plasma and urinary concentrations of BN being significantly smaller compared to those of tablets. Delay of the peak times in the plasma concentration, urinary excretion rate of BN and urine flow rate after administration of retarded capsules might suggest the slow release of BN in the gastro intestinal tract.

All of the subjects felt much fatigue or lassitude by the rapid and strong diuretic response after administration of tablets. On the other hand, they had no such feelings after administration of retarded capsules. From these results, retarded capsules of BN showed a mild diuresis and maintained suitable time periods in human subjects. The usefulness of a sustained release of BN is suggested by this study. Further investigation might also show clinical usefulness.

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