Enhanced Absorption of Bumetanide from Suppositories Containing Weak Acids in Rabbits

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The *in vitro* release of bumetanide from macrogol suppositories with and without weak acids (citric acid and tartaric acid) was studied. The release of bumetanide was not affected when weak acids were added to the suppositories. The *in vivo* rectal absorption of bumetanide from the suppositories was evaluated in rabbits. The bioavailability (absolute), expressed as the ratio of the area under the plasma concentration-time curve (AUC) following oral administration of bumetanide, was 39% that of intravenous administration. The value in bumetanide following rectal administration of the suppositories without weak acids was 32%. Each absolute bioavailability following rectal administration of the suppositories with 5% citric acid and 5% tartaric acid was 52% and 42%, respectively. These values were significantly larger than those of rectal administration of the suppositories without weak acids. Particularly, the bioavailability following rectal administration of the suppositories containing citric acid was significantly different from even those of oral administration. The absorption rate constants of bumetanide from the suppositories with weak acids were significantly larger than those following oral administration. These results indicated the possibilities of the rectal route of administration of drugs which are weak organic acids and show low or variable bioavailability following oral administration.

Keywords: bumetanide; bioavailability; suppository; rectal administration; citric acid; tartaric acid

Bumetanide, 3-(butylamino)-4-phenoxy-5-sulfamoylbenzaic acid, is one of the most potent loop diuretics used for the treatment of edema associated with congestive heart failure, hepatic and renal disease, including the nephrotic syndrome. It exhibits the rapid onset and short duration of action. Pharmacological and clinical studies have shown that 1 mg of bumetanide has a diuretic potency equivalent to approximately 40 mg of furosemide. It is believed to act at the luminal surface of the nephron where it inhibits the active reabsorption of chloride in the ascending limb of the loop of Henle. Bumetanide is a weak organic acid ($pK_a = 7.7$). The available dosage forms of bumetanide today are tablets and injections which are similar to those of other diuretic agents. Development of the new administration route may expand the clinical uses of diuretics. The rectal route is not the favorable site for the absorption of weak organic acids of which the $pK_a$ value is below the pH of the rectum because most of the molecule is dissociated. Takubo et al. studied the effect of buffer components on the absorption of several sulphonamides in rabbits and found that the absorption of sulphonamides were well correlated to the increase of the dissolution rates of sulphonamides. Böttger et al. studied the rectal absorption of sodium benzoate from aqueous solution at different pHs in humans and found that the absorptions of the drug were increased at pHs where the drug molecule existed in a unionized form. In this study the authors studied the possibilities of the rectal absorption of bumetanide from suppositories in rabbits. Weak acids, such as citric acid and tartaric acid, were used as the buffer components in the suppositories to keep the pHs lower at the absorption site in the rectum in order to make the drug molecule a unionized form which is favorable for absorption.

**Experimental**

**Materials** Bumetanide powder (lot No. D61), with an original particle size of $5.8 \pm 0.14 \mu$m (mean $\pm$ S.D., Green diameter), and injection (Lunetron® injection, 0.5 mg/ml lot No. L0009Y) were supplied by Sankyo Co., Ltd., Tokyo, Japan. Piretanide (lot No. 700 L023), supplied by Hoechst Japan Ltd., Tokyo, Japan, was dissolved in phosphate buffer at pH 7.4 and used as an internal standard. Macrogol 1540 and macrogol 4000 were obtained from Kanto Chemical Co., Inc., Tokyo, Japan. Acetonitrile was HPLC grade from J. T. Baker Inc., Phillipsburg, NJ. All other chemicals were of reagent grade.

**Preparation of Suppositories** The suppository base was a mixture of macrogol 1540 and macrogol 4000 in a weight ratio of 1:4. After the base had been melted in the beaker at 58±1°C, bumetanide and weak acids (citric acid or tartaric acid) were dissolved in the base. The solution was poured into a suppository mold (Kemoto Scientific Technology Co., Ltd., Tokyo, Japan) cooled at 0°C. The mold was kept in the refrigerator (4°C) for 24 h and the suppositories were obtained. The mean weight of suppositories (in each case) was 2.1 g and contained 1 mg/g of bumetanide. The suppositories were wrapped with aluminum foil and kept in the refrigerator until used.

**Extraction of Bumetanide from Aqueous Solution and Solution Containing Macrogol** The extraction experiment was done in order to discuss the lipophilicity of bumetanide in the acidic region and around the pH (about 7.3) in the rectum when macrogol was added to an aqueous buffer solution. Five ml of aqueous buffer solution or 60% macrogol solution at different pHs containing bumetanide (1 µg/ml) were added to an equi-volume of ethyl acetate at room temperature. The buffer systems used were 0.1 M citric acid buffer (pH 1–2), 0.1 M acetate buffer (pH 3–5), 0.1 M phosphate buffer (pH 6–8), 0.1 M KH₂PO₄-0.05 M borax buffer (pH 9) and 0.1 M Na₂HPO₄-0.05 M NaOH buffer (pH 11–12).

**Release of Bumetanide from the Suppositories** An apparatus for the measurement of bumetanide release from the suppositories (2.1 g of weight, 2.1 mg as bumetanide), model TMS-103, Toyama Sangyo Co., Osaka, Japan, was used according to the procedure reported by Muranishi et al. The temperature of the water bath was maintained at 37±0.1°C. Phosphate buffer solution at pH 7.3 (0.01 M) was employed as the release medium. A Millipore filter, SSWP 04700 (Nihon Millipore Kogyo Co., Ltd., Yonezawa, Japan), pore size 3 µm, was employed as an artificial membrane. A rotation rate of the steel rod (3.4 cm i.d.) was 25 rpm. The receptor solution at pH 7.3 (0.01 M phosphate buffer, 300 ml) was stirred by the magnetic stirring bar (30 mm length) at 100 rpm. Following the dissolution of the suppository into the release medium, 1 ml of receptor solution was withdrawn at appropriate time intervals. An equi-volume of new medium was replaced into the receptor solution. After the release study was finished, the pH of the receptor solution was recorded.

**In Vivo Absorption of Bumetanide in Rabbits** Male albino rabbits weighing 2.5–3.5 kg were fasted for 24 h prior to drug administration. The dose of bumetanide was 0.5 mg/kg body weight. For intravenous administration, Lunetron® was injected into the ear vein. For oral
administration, gastric-emptying time controlled rabbits were employed in the study. Suspension of bumetanide in 0.5% methylecellulose solution (bumetanide, 0.5 mg/ml) was directly administered into the stomach using a stomach tube with a further 10 ml of distilled water. For rectal administration, suppositories containing bumetanide (1 mg/g) were used. They were prepared using an oil soluble suppository base (a mixture of suppository bases containing 5% citric acid, and suppositories containing 5% tartaric acid. For rectal administration, the size of the suppositories were reduced appropriately (1.5–1.7 g) by cutting down the original suppositories according to the weight of the rabbits. The rabbit was secured in a supine position. The suppository was inserted into the rectum at about 3 cm depth from the anus. Then, the anus was closed with an adhesive tape to prevent leakage. The order of administration of these dosage forms were designed with the cross-over design. An interval of more than one week was allowed between the experiments. Following administration of these dosage forms, each ml of blood was withdrawn from the ear vein at appropriate time intervals. The blood was heparinized and a small amount of phosphoric acid was added to adjust the pH around 4 to prevent the acyl migration or hydrolysis of glucuronide conjugate of bumetanide in plasma. Plasma was obtained by centrifuging the blood at 3000 rpm for 10 min. The plasma samples were kept in the freezer until assayed.

**Assay Procedure for Bumetanide**

The assay for bumetanide was made by the HPLC method. HPLC was performed on a Shim-pack CLC-ODS, with a 5 μm reversed-phase column (15 cm × 6 mm i.d.) and a Shim-pack CLC-ODS, with 30 μm guard column (50 × 2.1 mm i.d.) from Shimadzu Co., Ltd., Kyoto, Japan. The columns were maintained at 40 ± 0.5°C in the column oven (CTO-6A, Shimadzu, Co., Ltd.). A fluorescence spectrophotometer Model RF-535 (Shimadzu, Co., Ltd.) was used as a detector. Excitation and emission wavelengths were 335 and 415 nm, respectively. The mobile phase used was 44% acetonitrile containing phosphoric acid (0.1%, pH 3.5). The flow rate was 1.0 ml/min. Samples were automatically injected onto HPLC using a SLC-6A autosampler (Shimadzu, Co., Ltd.) and a LC-6A pump (Shimadzu, Co., Ltd.) with a run time of 15 min using a SCL-6A system controller (Shimadzu, Co., Ltd.). A C-R6A computing integrator (Shimadzu, Co., Ltd.) was used to obtain the areas of the peaks from the chromatogram.

**Results and Discussion**

**Extraction of Bumetanide from Aqueous Solution and Solution Containing Macrogol**

Figure 1 shows the extraction of bumetanide into ethyl acetate from aqueous solution or 60% macrogel aqueous solution. The extraction percent of bumetanide below pH 7.3 where the bumetanide molecule existed in a unionized form were mostly larger than those in the alkaline media. The relationship between the extraction percent and pH of bumetanide was closed to the pKₐ curve of bumetanide reported by Orita et al. On the other hand, when macrogel was present in the solution, the extraction profile was somewhat broad and did not exhibit the clear curve. The extraction percent around pH 7.3 was only 40%, while those in the acidic regions were more than 70%. These results indicated the decrease of absorption of bumetanide when macrogel is present in the solution.

**The Change of pH and Irritation on the Rectum by Suppositories**

The change of the pH in the rectum after the administration of the suppositories were measured by the pH test paper (Universal pH Stick, Kanto Chemical, Co., Inc.) through the glass applicator (2.5–3.0 cm length). The irritation effect of the suppositories on the rectum was observed after 5 h post-administration. The rectum was obtained by the conventional method and observed directly. The tissue was also observed by the microscope (x 400) after dyeing with hematoxylin-eosin. The evaluation of the irritation was made by the method (the levels of five steps from − to + + + + + ) reported by Satoh et al.

**Pharmacokinetic Analysis**

The pharmacokinetic parameters were estimated by the Gauss-Newton method using MULTI, which is based on an ordinary nonlinear-squares (OLS), with a microcomputer, model 9801 (NEC Co., Tokyo, Japan). The plasma data following oral or rectal administration were analyzed using a two-compartment model including the absorption process by simultaneously fitting with the plasma data of intravenous administration.

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**Fig. 1. pH Profiles of the Extraction Ratio of Bumetanide from Buffer Solutions into Ethyl Acetate**

- ○, control; ●, 60% of macrogel. Each point represents the mean ± S.E. of three experiments.

**Fig. 2. Effects of Concentration of Acids on the pH after Dissolution from Suppositories in 0.1 N (a) and 0.01 N (b) Phosphate Buffer (pH 7.3) at 37°C**

- ○, citric acid; ●, tartaric acid.
absorption medium. However, these results also indicated the possible absorption of bumetanide from the rectal tract by adding weak acids into the macrogel base if the pH of the absorption site is suitable for the pH-partition hypothesis, although the extraction percents were smaller than those without macrogel (control) in the acidic regions.

**Release of Bumetanide from the Suppositories**  Figure 2 shows the effect of the concentration of citric acid and tartaric acid on the pH of 0.1 N phosphate buffer (a) and 0.01 N phosphate buffer (b). The change of pH in 0.1 N phosphate buffer was small, even the concentrations of the weak acids were 5%. On the other hand, when the medium was 0.01 N phosphate buffer, the pH apparently declined with increasing weak acid concentration. From the study of the change of pH in the rectum in rabbits following the administration of the suppositories containing weak acids, the changes of pH were well reflected in the case of 0.01 N phosphate buffer (discussed later). The buffer capacity of rectal medium was low in humans.\(^{14,15}\)

So, for the study of the release of bumetanide from the suppositories, 0.01 N phosphate buffer at pH 7.3 was used as a medium.

Figure 3 shows the release of bumetanide from the suppositories containing citric acid (a) and tartaric acid (b). When the weak acids were added to the suppositories, the releases of the drug were initially lower and were later higher than the control suppositories.

Table 1 shows the final pH of the test solution and final disintegration time of the suppositories. The final disintegration times of the suppositories containing citric acid or tartaric acid were longer than the control suppositories. The release of bumetanide in the initial periods was followed by the disintegration time of the suppository base. These results were well reflected in the lower release of the drug from the suppositories containing weak acids in the initial periods. On the other hand, the release of bumetanide from the control suppositories was lower than those containing weak acids by 3 h. The reason may be attributed to remains of bumetanide on the membrane filter due to the rapid disintegration of the suppository base. The final pH apparently declined, but the release of the drug was not affected when containing percents of weak acids were increased. The decrease of the pH of the test solution after the dissolution of weak acids might increase the presence of a unionized form of bumetanide.

**Bioavailability of Bumetanide from the Suppositories in Rabbits** Plasma concentrations of bumetanide following intravenous administration, oral administration of aqueous suspension, rectal administration of the suppositories were measured to evaluate the absorption characteristics of each administration route.

Figure 4 shows the plasma bumetanide concentrations following intravenous and oral administration. Plasma concentration–time curve following intravenous administration well fitted the two-compartment open model. The AUC following oral administration was 39% of that following intravenous administration.

Figure 5 shows the plasma bumetanide concentrations following rectal administration of the control suppositories and the suppositories containing 5% citric acid (a) and 5% tartaric acid (b).

The absorption of bumetanide in the rectum from the control suppositories was as well as that following oral

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**Table I. Final pH of Test Solution and Final Time of Disintegration of Suppositories**

<table>
<thead>
<tr>
<th>Suppository</th>
<th>Final pH</th>
<th>Final time of disintegration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.40</td>
<td>33.3</td>
</tr>
<tr>
<td>Citric acid</td>
<td>7.25</td>
<td>53.0</td>
</tr>
<tr>
<td>1%</td>
<td>6.90</td>
<td>52.0</td>
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<tr>
<td>3%</td>
<td>6.54</td>
<td>42.7</td>
</tr>
<tr>
<td>5%</td>
<td>7.26</td>
<td>46.3</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>6.91</td>
<td>48.7</td>
</tr>
<tr>
<td>1%</td>
<td>6.58</td>
<td></td>
</tr>
<tr>
<td>3%</td>
<td>6.58</td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>6.58</td>
<td></td>
</tr>
</tbody>
</table>
administration in rabbits. The AUC following administration of the control suppositories was 88% that following oral administration. The values of AUC are not significantly different between oral administration and administration of the control suppositories. The absorption of bumetanide increased when the suppositories containing citric acid or tartaric acid were administered. Pharmacokinetic parameters are summarized in Table II. Bioavailability parameters were also calculated and shown in Table III.

The absorption rate constant ($k_a$), AUC, maximum plasma concentration ($C_{\text{max}}$), and the time ($T_{\text{max}}$) from the control suppositories were not significantly different from those of oral administration. The absorption rate constants of the suppositories containing citric acid and tartaric acid were almost 2.8 times ($p<0.05$) and 3.2 times ($p<0.05$) larger than those obtained from oral administration, respectively. Moreover, the values in the suppositories containing citric acid and tartaric acid were almost 3.6 and 4.1 times larger than those of the control suppositories, respectively. Plasma concentrations of bumetanide from the suppositories containing weak acids were significantly higher during the initial 60 min period than those from the control suppositories. $T_{\text{max}}$ were significantly shortened (about one second of the control suppositories) and $C_{\text{max}}$ increased significantly (about 3 times of the control suppositories) in the suppositories containing weak acids. The absolute bioavailability of bumetanide from the suppositories containing citric acid and tartaric acid was 52% and 42%, respectively. These values were significantly larger than those of the rectal administration of the control suppositories (32%). The relative bioavailability of bumetanide from the suppositories containing citric acid and tartaric acid also increased to 139% and 122% compared to oral administration, respectively. Particularly, the values in bioavailability following the rectal administration of the suppositories containing citric acid were signi-
significantly different from even those of oral administration ($p<0.05$). The values in bioavailability of bumetanide from the control suppositories were decreased compared to those of oral administration. These results show that bumetanide is absorbed more rapidly and efficiently from the suppositories containing weak acids in the rectum.

The Change of pH and Irritation on the Rectum by Suppositories Table IV summarizes the change of pH in the rectum following administration of suppositories. Following administration of the control suppositories, the apparent change of the pH was not observed. On the other hand, the pH was lowered by the suppositories containing citric acid and tartaric acid by 30 min. The apparent start of the recovery of the pH was observed at 45 min. The pH was recovered to the normal value at 60 min. These results well reflected that $T_{\text{max}}$ was significantly shortened following rectal administration of the suppositories containing weak acids in Table III.

The irritation effect of the suppositories on the rectal membrane is summarized in Table V. In all cases, mild or moderate irritation was observed. Satoh et al.\textsuperscript{12)} reported the irritation effect of suppositories in rabbits. They found the irritation by macrogol suppository base in rabbit rectum. In our study, a similar observation was noticed, but the irritation effects were not increased by the addition of weak acids.

Thus, the absorption of bumetanide from the suppositories might become available especially by the addition of weak acids to lower the pHs in the rectum. It has recently been reported that carboxylic acids serve to make the intercellular space more accessible by eliminating Ca ions of rectal mucosa.\textsuperscript{16,17} It has also been known that the gastrointestinal absorption of metal ions such as Fe, Al or Ca is enhanced by citric acid or tartaric acid.\textsuperscript{18,19} The possibilities might also be considered as to the absorption-promoting effects in the rectum by adding citric acid or tartaric acid. Further studies might be necessary to examine the effect of the pH decline and rectal absorption-promoting effects of citric acid or tartaric acid. Though the dosage forms were different, these results indicated the possibilities of the presence of the first-pass effect of metabolism of bumetanide when the drug was administered orally as well as furosemide reported by Lee et al.\textsuperscript{20)}

References and Notes

1) A part of this study was presented at the 112th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, March 1992.
14) K. Kakemi and S. Muranishi, Yakuzaiyaku, 26, 94 (1966).