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# Effect of Dissolution Rate and Food on Oral Bioavailability of Bropirimine Tablets in Dogs

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**ABSTRACT**

*Three different tablet formulations of bropirimine were evaluated in an in vitro dissolution study. Further, the effect of dissolution rate of bropirimine and food on the bioavailability after oral administration of the tablets was investigated in dogs. A tablet formulation with lower bropirimine content percent and smaller tablet size showed faster in vitro dissolution rate due to the larger tablet surface area per unit mass of bropirimine and the higher ratio of hydrophilic excipients in a tablet. In the fasted state, the bioavailability of bropirimine after oral administration of tablets tended to reflect the in vitro dissolution characteristics. The bioavailability after administration of tablets with slow in vitro dissolution rate was increased by food intake due to the in vivo dissolution increased in the fed state, while the postprandial effect on the bioavailability of tablets with fast in vitro dissolution rate was not clearly observed. In the fed state, there were no differences in the plasma concentration profile and pharmacokinetic parameters of bropirimine between the tablets with a slow and a fast in vitro dissolution rate. This suggests that the postprandial administration of bropirimine tablets may maximize the bioavailability without distinction of the in vitro dissolution rate.*

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## INTRODUCTION

Bropirimine, 2-amino-5-bromo-6-phenyl-4(3*H*)-pyrimidinone, is a biological response modifier (immune modulator) which has established induction of interferon, modulation of other lymphokines, and antiviral and antitumor activity in various animal models (1–5). It is an orally active agent when given at high doses, but is poorly water soluble.

In the development of oral bropirimine tablets required to be administered at high doses, reductions in the number and size of tablets dosed were desired to improve the compliance; i.e., tablets with high potency and high content percent of bropirimine in a tablet were desired. Because of the poorly aqueous solubility of bropirimine, however, it was considered that the content percent and the tablet size might significantly influence the dissolution rate of bropirimine. Further, a previous study has reported that the rate-determining step for the absorption of bropirimine from the rat small intestine after dosing in suspension is the dissolution process (6). Therefore, it was possible that the bioavailability of bropirimine after oral administration of tablets might depend on the dissolution rate. It has been reported also that the bioavailability of poorly water-soluble drugs is often increased when dosed under the postprandial condition (7–9). Indeed, it has been shown that that bioavailability of bropirimine in dogs after oral administration of a certain tablet formulation is increased by food intake (10).

In the present study, the evaluation of bropirimine tablets differing in the content percent and the tablet size was performed in an in vitro dissolution study. Further, the effect of dissolution rate of bropirimine and food on the bioavailability after oral administration of the tablets was investigated in dogs.

## MATERIALS AND METHODS

### Materials

The three different formulations of bropirimine evaluated were circular, flat-face, and disintegrating tablets, i.e., bropirimine 50 mg tablet (B-50 CT), bropirimine 125 mg tablet (B-125 CT), and bropirimine 250 mg tablet (B-250 CT). The formulation compositions are given in Table 1. The formulation of B-50 CT was obtained by substituting 75 mg of hydrophilic excipients for the same amount of bropirimine in the formulation of B-125 CT. The tablet diameter and thickness were 7.5 and 2.7 mm for both B-50 CT and B-125 CT, and 9.5 and 3.4 mm for B-250 CT, respectively. The tablets were made by a wet granulation method as follows: Bropirimine and intragranular excipients were granulated, and then the dry granules were mixed with extragranular excipients and compressed into tablets. B-125 CT and B-250 CT were made from the same lubricated granules. Other reagents used were of analytical grade.

### In Vitro Testing

Tablet disintegration tests were carried out using the JP XII disintegration test apparatus (Toyama Sangyo, Japan). Water kept at 37°C was used as a disintegration medium, and the basket was raised and lowered at a constant frequency of 30 cycles per min. Six tablets were tested for each formulation.

*Table 1*

*Formulation Compositions of Bropirimine Tablets*

| Component | Amount per Tablet (mg) | | |
|---|---|---|---|
| | B-50 CT | B-125 CT | B-250 CT |
| Bropirimine | 50.00 | 125.00 | 250.00 |
| Cornstarch | 26.75 | 7.50 | 15.00 |
| Mannitol | 62.00 | 6.25 | 12.50 |
| Hydroxypropyl cellulose | 5.00 | 5.00 | 10.00 |
| L-HPC[a] | 7.50 | 7.50 | 15.00 |
| Microcrystalline cellulose | 8.00 | 8.00 | 16.00 |
| Magnesium stearate | 0.75 | 0.75 | 1.50 |
| Total | 160.00 | 160.00 | 320.00 |

[a]Low-substituted hydroxypropyl cellulose.

Dissolution tests were performed according to the paddle method (JP XII). The apparatus employed consisted of a dissolution tester (NTR-VS6P; Toyama Sangyo), an autosampler (PAS-615; Toyama Sangyo), an ultraviolet (UV) spectrophotometer (UV-160A; Shimazu, Japan) with a cellpositioner (CPS-240B; Shimazu), and a personal computer (NEC, Japan). Nine hundred milliliters of the JP XII disintegration first medium (pH 1.2) was used at 37°C, and the paddle was rotated at 100 rpm. Five tablets of B-50 CT, 2 tablets of B-125 CT, or 1 tablet of B-250 CT was used in each dissolution test to equalize the final amount of bropirimine dissolved in the medium. At 2, 4, 7, 10, 15, 20, 30, and 60 min, bropirimine concentration was monitored automatically at 231 and 296 nm. Six replications of tests were made for each formulation.

### Animals

Male beagle dogs (12 to 14 kg) were used throughout the studies. In the study under the fasting condition, the dogs were fasted for 18 hr before dosing; and at 4 hr after dosing they were fed 250 g of food (LABO D STOCK; Nihon Nousan, Japan). In the study under the postprandial condition, the dogs were given 250 g of food at 30 min before dosing, which was consumed within 30 min. Water was freely available in both conditions.

### Bioavailability Testing

Ten tablets of B-50 CT, 4 tablets of B-125 CT, or 2 tablets of B-250 CT were administered orally to 5 dogs under the fasting condition in a cross-over design with 1 week between doses. Dosing was followed by 50 ml of water. Three milliliters of blood sample was withdrawn from the femoral vein with a heparinized syringe before dosing and at designated time intervals after dosing. The blood sample was centrifuged (1000 *g*, for 10 min) to collect the plasma. The plasma sample was then transferred to a container and frozen at -20°C until analysis. In the other study 10 tablets of B-50 CT or 2 tablets of B-250 CT were administered orally to 6 dogs under the postprandial condition in a cross-over design with 1 week between doses.

### Assay of Bropirimine by HPLC

Concentration of bropirimine in plasma was measured by high-performance liquid chromatography (HPLC) according to the method reported previously (10).

### Kinetic Analysis

The mean in vitro dissolution time (*MDT*) was calculated by model-independent moment analysis (11) according to Eq. (1).

$$MDT = \int_0^\infty t(dm/dt)\ dt / \int_0^\infty (dm/dt)\ dt \quad (1)$$

where $m$ is the mass of drug dissolved at time $t$. The maximum plasma concentration ($C_{max}$) and the time to reach $C_{max}$ ($T_{max}$) were obtained directly from the plasma concentration profile data. The terminal elimination half-life ($t_{1/2\beta}$) was calculated from the terminal elimination rate constant ($\beta$) estimated by least squares linear regression of the terminal log-linear region of the plasma concentration–time curves.

$$t_{1/2,\beta} = \ln 2/\beta \quad (2)$$

The area under the plasma concentration–time curve (*AUC*) and the mean residence time (*MRT*) were obtained by model-independent moment analysis (12) using Eqs. (3) and (4), respectively.

$$AUC = \int_0^\infty C_p\ dt \quad (3)$$

$$MRT = \int_0^\infty tC_p\ dt / \int_0^\infty C_p\ dt \quad (4)$$

where $C_p$ is the drug concentration in plasma at time $t$.

### Statistical Analysis

Statistical analysis was performed by Fisher's pairing $t$ test.

## RESULTS AND DISCUSSION

As shown in Table 2, the in vitro disintegration rates of the three tablet formulations were in the following order: B-50 CT > B-125 CT > B-250 CT, which did also reflect the in vitro dissolution characteristics. The tablet surface areas per unit mass of bropirimine were 3.04, 1.22, and 0.97 $mm^2/mg$ for B-50 CT, B-125 CT, and B-250 CT, respectively. In comparing B-125 CT and B-250 CT, which were made from the same lubricated granules, the in vitro tablet performance seems to

*Table 2*

*In Vitro Disintegration and Dissolution Characteristics of Bropirimine Tablets*

| Formulation | Disintegration Time[a] (min) | % Dissolved[b] 2 min | % Dissolved[b] 7 min | % Dissolved[b] 15 min | *MDT*[b] (min) |
|---|---|---|---|---|---|
| B-50 CT | 1.9 ± 0.3 | 84 ± 6 | 100 ± 3 | 102 ± 3 | 1.7 ± 0.2 |
| B-125 CT | 5.6 ± 0.6[c] | 36 ± 7[c] | 83 ± 4[c] | 95 ± 3[c] | 5.2 ± 1.2[c] |
| B-250 CT | 8.1 ± 0.3[c,d] | 18 ± 4[c,d] | 72 ± 9[c,d] | 91 ± 4[c] | 7.4 ± 1.3[c,d] |

[a]Values are expressed as mean ± *SD* of 6 tablets.
[b]Values are expressed as mean ± *SD* of 6 tests.
[c]$p < 0.01$, vs. B-50 CT.
[d]$p < 0.01$, vs. B-125 CT.

depend on the tablet surface area per unit mass of bropirimine. B-250 CT showed a disintegration time and MDT 1.4 times longer than B-125 CT, which had 1.3 times the surface area of B-250 CT. On the other hand, in comparing B-50 CT and B-125 CT, the rate of increase in the in vitro disintegration and dissolution rate is somewhat higher than that in the tablet surface area per unit mass of bropirimine; i.e., the surface area ratio, the disintegration time ratio and the *MDT* ratio of B-50 CT to B-125 CT were 2.5:1, 1:2.9, and 1:3.1, respectively. The additional increase in the in vitro rates of B-50 CT is considered to be attributed to the higher ratio of hydrophilic excipients in a tablet, which may overcome the poor aqueous solubility of bropirimine. From these results, it is indicated that the lower potency and content percent of bropirimine, and the higher content percent of hydrophilic excipients in a tablet result in the faster in vitro disintegration and dissolution rate of bropirimine tablets.

In dogs under the fasting condition, higher plasma concentration, $C_{max}$, and *AUC* after oral administration of B-50 CT were observed in comparison with those after administration of either B-125 CT or B-250 CT, while there were no significant differences in $C_{max}$, $T_{max}$, *AUC*, *MRT*, and $t_{1/2,\beta}$ among the three tablet formulations due to the large standard deviations (Fig. 1 and Table 3). The $C_{max}$ and *AUC* after oral administration of bropirimine tablets in the fasted state have a tendency to reflect the in vitro disintegration and dissolution characteristics. This is supported by the previous report that the rate-determining step for the absorption of bropirimine from the rat small intestine after dosing in suspension is the dissolution process (6).

In comparing the *AUCs* of B-250 CT (Tables 3 and 4), it is estimated but not statistically shown that the *AUC* in the fed state was twice greater than that in the fasted state. This corresponds with the result of the previous study in which the postprandial effect on the bioavailability of B-250 CT was investigated in dogs according to a cross-over design (10). The previous study reported that the longer gastric residence time and larger volume of the gastric fluid induced by food intake increase the in vivo dissolution of B-250 CT and consequently the bioavailability. However, there were no great differences in $C_{max}$ and *AUC* after oral administration of B-50 CT between the fasted state and the fed state. This can be explained by the mechanism of postprandial effect reported in the previous study as follows: Even in the fasted state, B-50 CT with fast in vitro disintegration and dissolution rate showed as high in vivo dissolution as in the fed state, and therefore, a clear increase in the bioavailability by food intake was not observed.

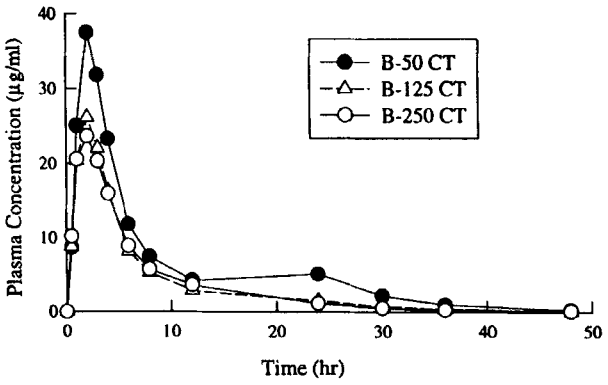


**Figure 1.** Mean plasma concentrations of bropirimine under the fasting condition ($n = 5$).

*Table 3*

*Pharmacokinetic Parameters of Bropirimine Tablets Under Fasting Condition*[a]

| Parameter | Formulation | | | Paired t-test |
|---|---|---|---|---|
| | B-50 CT | B-125 CT | B-250 CT | |
| $C_{max}$ (μg/ml) | 38.2 ± 14.9 | 26.9 ± 3.8 | 24.6 ± 6.5 | NSD[b] |
| $T_{max}$ (hr) | 1.8 ± 0.4 | 1.8 ± 0.4 | 1.6 ± 0.5 | NSD[b] |
| *AUC* (μg h/ml) | 279.3 ± 154.2 | 170.3 ± 32.1 | 170.1 ± 58.3 | NSD[b] |
| *MRT* (hr) | 9.4 ± 4.7 | 8.0 ± 2.0 | 7.5 ± 2.4 | NSD[b] |
| $t_{1/2,\beta}$ (hr) | 6.3 ± 1.3 | 6.7 ± 1.5 | 7.1 ± 1.6 | NSD[b] |

[a]Values are expressed as mean ± *SD* of 5 dogs.
[b]NSD = No significant difference.

*Table 4*

*Pharmacokinetic Parameters of Bropirimine Tablets Under Postprandial Condition*[a]

| Parameter | Formulation | | Paired *t*-test |
|---|---|---|---|
| | B-50 CT | B-250 CT | |
| $C_{max}$ (μg/ml) | 36.4 ± 2.9 | 36.3 ± 7.5 | NSD[b] |
| $T_{max}$ (hr) | 2.2 ± 1.9 | 2.0 ± 0.6 | NSD[b] |
| *AUC* (μg hr/ml) | 347.6 ± 76.2 | 332.2 ± 68.9 | NSD[b] |
| *MRT* (hr) | 10.0 ± 1.4 | 11.0 ± 1.3 | NSD[b] |
| $t_{1/2,\beta}$ (hr) | 7.4 ± 0.9 | 8.9 ± 0.8 | $p<0.05$ |

[a]Values are expressed as mean ± *SD* of 6 dogs.
[b]NSD = No significant difference.

In the fed state, the plasma concentration profile and pharmacokinetic parameters of bropirimine after oral administration of B-50 CT were consistent with those after administration of B-250 CT (Fig. 2 and Table 4); i.e., no difference in the bioavailability of bropirimine under the postprandial condition was observed between the tablets with slow and fast in vitro disintegration and dissolution rates. Thus, it is suggested that the postprandial administration of bropirimine tablets may maximize the bioavailability without distinction of the in vitro disintegration and dissolution rate.

B-50 CT with lower bropirimine content percent and smaller tablet size showed faster in vitro disintegration and dissolution rate. B-50 CT also showed higher bioavailability even in the fasted state. In addition, the bioavailability of B-50 CT was hardly influenced by food intake. However, the lower drug content percent in a tablet and the smaller tablet size result in a remarkable increase in the number of tablets dosed because bropirimine is required to be administered at high doses, and increasing the number of tablets dosed is considered to lower the compliance. Therefore, B-50 CT is not a desirable tablet formulation for bropirimine. As described above, the postprandial administration of bropirimine tablets is expected to maximize the bioavailability without distinction of the in vitro disintegration and disso-

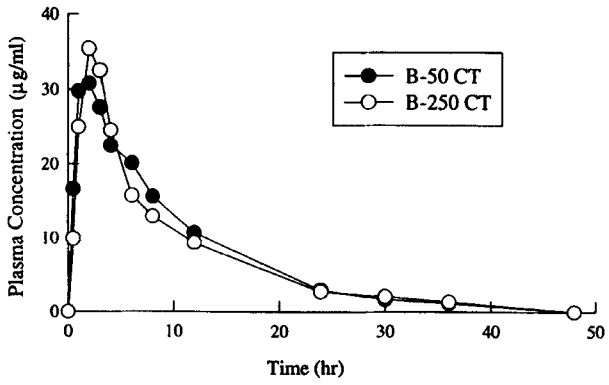


**Figure 2.** Mean plasma concentrations of bropirimine under the postprandial condition ($n$ = 6).

lution rate. Thus, B-250 CT is recommended as a bropirimine tablet formulation based on the compliance and on the postprandial dosing.

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