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Bioavailability of Bropirimine 250 mg Tablet in Dogs: Effect of Food

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Abstract

The postprandial effect on the bioavailability of bropirimine in dogs after oral administration of bropirimine tablets (Bropirimine 250 mg Tablet) was investigated.

At a dose of 500 mg bropirimine (two tablets of bropirimine 250 mg), the maximum plasma concentration under the postprandial condition was about twice that observed under the fasting condition, and the area under the plasma concentration vs time curve under the postprandial condition was also twice that under the fasting condition. The absolute oral bioavailabilities of bropirimine were 41·1% under the fasting condition and 83·5% under the postprandial condition.

It is considered that the longer gastric residence time and larger volume of the gastric fluid induced by food-intake caused the increase in dissolution of bropirimine which increased the bioavailability after oral dosing of bropirimine 250-mg tablets.

Bropirimine, 2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone, is a biological response modifier (immune modulator), which involves induction of interferon, modulation of other lymphokines, antiviral and antitumour activity in various animal models (Stringfellow et al 1980; Hamilton et al 1982; Wierenga 1985; Chang et al 1986; Eggermont et al 1986). The bioavailability of drugs after oral dosing often depends on the solubility and loading dose of drugs, and the bioavailability of poorly water-soluble drugs is often influenced when dosed under the postprandial condition (Aoyagi et al 1982). Because bropirimine is poorly water-soluble at neutral pH (Emori et al 1995) and because a high dose is required, the effect of food on the bioavailability of tablets with a high content of bropirimine (Bropirimine 250 mg Tablet) should be a factor in the choice of the dosage regimen in clinical trials.

The effect of food on the bioavailability of various drugs has been reviewed (Welling 1977; Melander 1978; Toothaker & Welling 1980). Food-intake may influence the bioavailability of drugs by changing circumstance of the gastrointestinal tract. Because positive or negative postprandial effects after oral dosing is dependent on drug properties and formulation types, an investigation may be necessary for each drug and each formulation type (Melander 1978; Liedholm et al 1982; Byrne et al 1984). In the present study, the postprandial effect on the bioavailability of bropirimine in dogs after oral administration of bropirimine 250-mg tablets was investigated in order to support the determination of the dosage regimen in clinical trials.

Materials and Methods

Materials and formulations

Bropirimine and 2-amino-5-bromo-6-(3-fluorophenyl)-4(3H)-pyrimidinone (ABFPP) were supplied by The Upjohn Co. (MI, USA). Other reagents used were of analytical grade. Bropirimine 250 mg Tablet used was a flat-face, round and disintegrating tablet with a diameter of 9·5 mm. The drug content and tablet weight were 250 and 320 mg, respectively. Bropirimine solution for intravenous dosing was prepared by dissolving 1 g of the bulk drug in 20 mL polyethylene glycol 400 (Wako Pure Chemical Industries, Ltd, Osaka, Japan).

Animals

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

Bioavailability of bropirimine 250-mg tablets in dogs

In the first series of experiments, to investigate the bioavailability of bropirimine after oral dosing vs intravenous dosing under the fasting condition, bropirimine was administered orally or intravenously to six dogs in a two-treatment, two-period, cross-over, Latin-square design (in triplicate) with one week between doses. Two tablets of bropirimine 250 mg were administered orally, or 2 mL bropirimine solution (100 mg bropirimine) was administered intravenously into the femoral vein. Oral dosing of bropirimine was followed by 50 mL water. Three millilitres of blood 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*, 10 min) to collect the plasma. The plasma sample was then transferred to a container and frozen at −20°C until analysis.

In the second series of experiments, to investigate the bioavailability of bropirimine after oral dosing vs

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intravenous dosing under the postprandial condition, either one tablet, two tablets or three tablets of bropirimine 250 mg were administered orally, or 2 mL bropirimine solution (100 mg bropirimine) was administered intravenously to eight dogs in a four-treatment, four-period, cross-over, Latin-square design (in duplicate) with one week between doses.

In the third bioavailability study of bropirimine, two tablets of bropirimine 250 mg were orally administered to six dogs under the fasting condition or the postprandial condition in a two-treatment, two-period, cross-over, Latin-square design (in triplicate) with one week between doses.

Assay of bropirimine by HPLC

Concentration of bropirimine in plasma was measured by HPLC as follows. Two hundred microlitres of an internal standard solution (10 μg mL^{-1} ABFPP in methanol) was added to a test-tube, and the solvent was evaporated completely under nitrogen gas flow at 50°C. Two hundred microlitres of the plasma sample and 3 mL pH 7 phosphate buffer (0·1 M) were added to the residue in the test-tube and mixed vigorously for 30 s, followed by sonication in an ultrasonic cleaner (47 kHz; Yamato Scientific Co., Ltd, Tokyo, Japan) for 5 min. The mixture was passed through a conditioned solid phase column (Bond Elut, C18, 3 cc; Analytichem International, USA), and the bonded phase was washed with one column volume of water three times. One millilitre of 80% methanol was passed through the column, and the eluate was collected. Fifty microlitres of the eluate was injected onto the HPLC column. The HPLC system consisted of a liquid chromatograph (LC-6A; Shimadzu, Kyoto, Japan), an auto injector (SIL-6A; Shimadzu) with a system controller (SCL-6A; Shimadzu), a UV spectrophotometric detector (SPD-6A; Shimadzu) and a chromatopac (C-R6A; Shimadzu). For the stationary phase, a reverse-phase column (STR ODS-H, 15 cm-4 mm; Shimadzu Techno Research, Kyoto, Japan) was used. The mobile phase was a mixture of water, acetonitrile and acetic acid (79:20:1, v/v/v) with a flow rate of 0·8 mL min^{-1}. Detection was performed at 254 nm. The plasma bropirimine concentrations were correlated in a linear fashion with the peak area ratios of bropirimine to the internal standard from 0·1 to 50 μg mL^{-1} (r = 0·999). Regarding the reproducibility of the assay, the mean result ± s.d. was 9·3 ± 0·6 μg mL^{-1} for 10 μg mL^{-1} bropirimine.

Pharmacokinetic analysis

The maximum plasma concentration (C_{max}) and the time (T_{max}) to reach C_{max} were obtained directly from the plasma concentration profile data. The terminal elimination half-life ($t_{\frac{1}{2}\beta}$) was calculated from the terminal elimination rate constant (β) determined by least squares linear regression of the terminal log-linear region of the plasma concentration vs time curve using a personal computer:

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

The other pharmacokinetic parameters of bropirimine after administration were estimated by model-independent moment analysis (Yamaoka et al 1978). The area under the plasma concentration vs time curve (AUC) and mean residence time (MRT) were calculated using equations 2 and 3, respectively:

$$AUC = \int_0^\infty C_p\, dt \quad (2)$$

$$MRT = \int_0^\infty tC_p dt \Big/ \int_0^\infty C_p dt \quad (3)$$

where C_p is the drug concentration in plasma at time t. AUC was calculated by the trapezoidal integration method and one-exponential extrapolation of terminal phase using a personal computer. MRT was also calculated using a personal computer. The absolute bioavailability (F) was determined using equation 4:

$$F = 100\ AUC_{p.o.}\ Dose_{i.v.}/AUC_{i.v.}\ Dose_{p.o.} \quad (4)$$

where $AUC_{p.o.}$, $Dose_{p.o.}$, $AUC_{i.v.}$ and $Dose_{i.v.}$ are the AUC and dose after oral and intravenous administrations, respectively. The total body clearance (CL_{tot}) and steady-state volume of distribution (Vd_{ss}) after intravenous dosing were obtained using equations 5 and 6, respectively:

$$CL_{tot} = Dose/AUC \quad (5)$$

$$Vd_{ss} = CL_{tot}\ MRT \quad (6)$$

The mean absorption time (MAT) was obtained using equation 7:

$$MAT = MRT_{p.o.} - MRT_{i.v.} \quad (7)$$

where $MRT_{p.o.}$ and $MRT_{i.v.}$ are the MRT after oral and intravenous administrations, respectively. The absorption ratio of bropirimine to dose as a function of time after oral dosing of bropirimine 250-mg tablets was calculated from the plasma concentrations after oral and intravenous administrations by a deconvolution method using a personal computer (Rescigno & Segre 1966). The plasma concentration after intravenous administration was analysed according to the two-compartment model, and the function was used for the deconvolution.

Statistical analysis

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

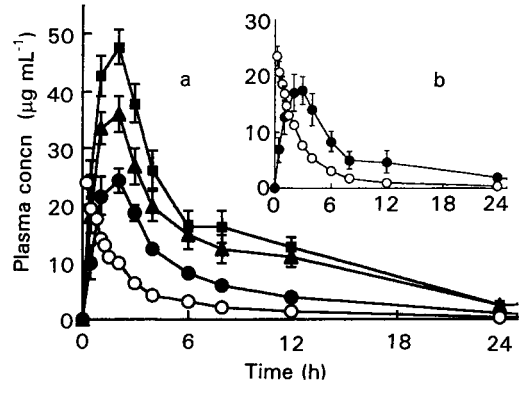

FIG. 1. a. Plasma concentrations of bropirimine in dogs under the postprandial conditions after intravenous administration of bropirimine in solution (○ 100 mg) or oral administration of 250-mg tablets (● 250, ▲ 500, ■ 750 mg). b. Plasma concentrations of bropirimine in dogs under fasting conditions after intravenous administration of bropirimine in solution (○ 100 mg) or oral administration of 250-mg tablets (● 500 mg).

Table 1. Pharmacokinetic parameters of bropirimine in dogs for the first and second series of experiments.

Parameter	Fasting condition[a]			Postprandial condition[b]		Tablet p.o.
Route	Solution i.v.	Tablet p.o.	Solution i.v.	Tablet p.o.	Tablet p.o.	
Dose (mg)	100	500	100	250	500	750
C_{max} ($\mu g\,mL^{-1}$)	—	20·9(29·2)	—	26·3(24·7)	37·7(22·3)	50·4(15·9)
T_{max} (h)	—	2·3(43·5)	—	1·8(38·9)	1·5(33·3)	1·8(38·9)
AUC ($\mu g\,h\,mL^{-1}$)	83·4(16·4)	171·0(48·2)	79·3(19·2)	165·3(15·3)	326·4(30·8)	398·0(23·3)
MRT (h)	6·7(28·4)	11·8(50·8)	6·2(25·8)	8·0(22·5)	9·4(24·5)	8·8(20·5)
MAT (h)	—	5·1(96·1)	—	1·8(55·6)	3·2(59·4)	2·6(53·8)
F (%)	—	41·1(42·6)	—	85·8(23·8)	83·5(33·3)	67·4(16·8)
$t_{1/2\beta}$ (h)	9·3(38·7)	11·6(91·4)	8·5(28·2)	8·3(19·3)	7·0(21·4)	6·5(26·2)
CL_{tot} ($L\,h^{-1}$)	1·2(16·7)	—	1·3(15·4)	—	—	—
Vd_{ss} (L)	8·3(32·5)	—	7·8(14·1)	—	—	—

[a] Values are expressed as mean (RSD, %) of six dogs. [b] Values are expressed as mean (RSD, %) of eight dogs.

Results

The mean plasma concentration profiles of bropirimine in dogs after oral administration of tablets or intravenous administration of solution are shown in Fig. 1 for the first and second series of experiments. The pharmacokinetic parameters of bropirimine are also given in Table 1. In the first series of experiments under the fasting condition, the F value of bropirimine after oral administration at a dose of 500 mg was about 40%. In the second series of experiments under the postprandial condition, the F value at a dose of 250 or 500 mg was about 80%. These results indicated that food-intake just before oral dosing of bropirimine 250-mg tablets increased the bioavailability of bropirimine. However, the F value had a tendency to decrease by increasing the dose to 750 mg even under the postprandial condition. There were no significant differences in the T_{max}, MRT and $t_{\frac{1}{2}\beta}$ values after oral administration between the three different dosing amounts of 250, 500 and 750 mg. There was no significant difference in the MAT obtained under the postprandial condition between the three different dosing amounts. The relative standard deviations (RSD) for the AUC, MRT and MAT obtained in the postprandial condition were smaller than those obtained in the fasting condition. There were significant differences in the dose-normalized C_{max} between doses of 250 and 500 mg and between doses of 250 and 750 mg under the postprandial condition ($P < 0.01$), in spite of no significant difference in the F value.

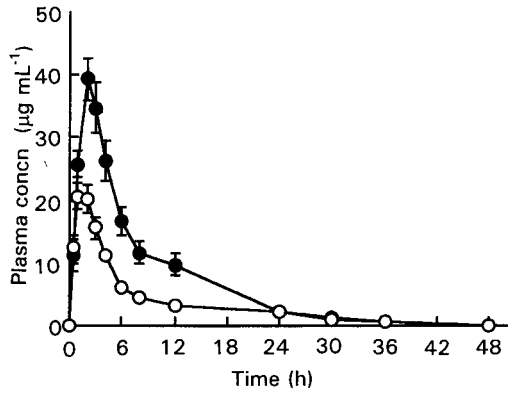

FIG. 2. Plasma concentrations of bropirimine in dogs under fasting conditions (○) or postprandial conditions (●) after oral administration of 250-mg tablets at a dose of 500 mg.

The cross-over study in the third series of experiments was conducted to reconfirm the postprandial effect on the bioavailability of bropirimine in dogs after oral administration of bropirimine 250-mg tablets, because the absolute bioavailability studies of bropirimine were performed in different dog groups between the fasting and postprandial conditions. The plasma concentration of bropirimine was markedly increased by food-intake (Fig. 2). The pharmacokinetic parameters of bropirimine are listed in Table 2. The C_{max} under the postprandial condition was about twice that observed under the fasting condition. The AUC under the postprandial condition was also twice greater than that under the fasting condition. This result was consistent with the results obtained in the first and second series of experiments. There were no significant differences observed for the values of T_{max} and MRT between the postprandial and fasting conditions, but the RSD values under the postprandial condition were smaller. The $t_{\frac{1}{2}\beta}$ under the postprandial and fasting conditions were consistent.

The absorption ratio of bropirimine to dose as a function of time after oral dosing of bropirimine 250-mg tablets was investigated with the data shown in Fig. 1. As shown in Table 3, the absorption of bropirimine occurred predominantly for the first 4 h after oral dosing both under the fasting and postprandial conditions. There were significant differences in the absorption ratio for the first 2 h between the fasting and postprandial conditions ($P < 0.01$).

Discussion

The bioavailability of bropirimine in dogs after oral administration of bropirimine 250-mg tablets was increased by food-intake. This result suggests that the postprandial dosing of bropirimine 250-mg tablets may maximize the bioavailability in man.

There was no difference in the $t_{\frac{1}{2}\beta}$ after oral administration of bropirimine 250-mg tablets to dogs at a dose of 500 mg between the postprandial and fasting conditions in the cross-over study (Table 2), and the plasma profile and pharmacokinetic parameters of bropirimine after intravenous administration of bropirimine solution to dogs in the postprandial condition were consistent with those in the

Table 2. Pharmacokinetic parameters of bropirimine in dogs for the third series of experiments[a].

Parameter	Fasting condition	Postprandial condition	Paired *t*-test
C_{max} ($\mu g\,mL^{-1}$)	21·3(23·0)	39·6(20·5)	< 0·01
T_{max} (h)	1·5(40·0)	2·0(30·0)	NSD[b]
AUC ($\mu g\,h\,mL^{-1}$)	160·7(27·4)	327·0(28·2)	< 0·01
MRT (h)	10·3(40·8)	9·3(30·1)	NSD[b]
$t_{2\beta}^{1}$ (h)	8·0(48·8)	8·0(58·8)	NSD[b]

[a] Values are expressed as mean (RSD, %) of six dogs. [b] No significant difference.

Table 3. Absorption ratios (%) of bropirimine to dose as a function of time in dogs after oral administration of bropirimine 250-mg tablets.

Time after dosing (h)	Fasting condition[a] 500 mg	Postprandial condition[b]		
		250 mg	500 mg	750 mg
0–2	21·2 ± 6·6*	75·0 ± 25·9**	53·5 ± 7·9	47·8 ± 9·6
2–4	8·9 ± 9·7	6·3 ± 7·2	5·5 ± 6·0	5·5 ± 6·0
4–6	0·1 ± 0·2	1·0 ± 2·5	3·5 ± 6·0	0·3 ± 0·7
6–8	0·6 ± 1·3	1·9 ± 3·1	3·1 ± 3·9	4·7 ± 6·4
8–12	1·8 ± 3·0	1·3 ± 1·9	4·0 ± 5·0	1·7 ± 1·5
12–24	1·2 ± 2·8	0	0	0

[a] Values are expressed as mean ± s.d. of six dogs. [b] Values are expressed as mean ± s.d. of eight dogs. *$P < 0·01$ compared with 250, 500 and 750 mg under the postprandial condition. **$P < 0·01$ compared with 500 and 750 mg under the postprandial condition.

fasting condition (Fig. 1, Table 1). From these results, it is considered that the fed state did not affect the disposition of bropirimine after oral administration of bropirimine 250-mg tablets to dogs. As shown in Table 3, the difference of the absorption ratio for the first 2 h was observed between the fasting and postprandial conditions. Thus, it is suggested that the increase in the bioavailability of bropirimine in dogs after oral administration of bropirimine 250-mg tablets with food-intake was caused by the increase in the extent of bropirimine absorbed into the systemic circulation for the first 2 h.

According to the reviews about the influence of food on the bioavailability of drugs (Welling 1977; Melander 1978; Toothaker & Welling 1980), gastric emptying rate, gastrointestinal motility, splanchnic blood flow, bile secretion and acid secretion are given as the possible mechanisms of the increase in the extent of bropirimine absorbed into the systemic circulation by food-intake. Because the CL_{tot} of bropirimine in dogs determined in this study is considerably lower than the canine hepatic plasma flow (Bischoff et al 1971); i.e. 1·2 vs 13·2 $L\,h^{-1}$, the hepatic extraction ratio (the ratio of the hepatic clearance to the hepatic plasma flow) is assumed to be low. Therefore, the change in the splanchnic blood flow seems not to influence the absorption of bropirimine by changing the drug clearance during the first pass through the hepatoportal system. The effect of an increase in the bile secretion with food-intake on the oral bioavailability of bropirimine 250-mg tablets is not considered, because the intestinal absorption of bropirimine in not only solution but also suspension was suppressed by sodium glycocholate in rats (Emori et al 1995).

It has been reported that the canine gastric pH is not influenced by food-intake, probably due to the higher peak acid output in dogs which cancels the buffering action of food (Dressman 1986). According to this report, the solubility change of bropirimine by food-intake may not occur in dogs. However, an increase in the gastric fluid by food-intake may increase the oral bioavailability of bropirimine by increasing the total amount of bropirimine dissolved. It has been reported also that the gastric residence time of small particles in dogs is significantly longer after feeding than in the fasted state (Itoh et al 1986). The longer gastric residence time may result in the higher dissolution of bropirimine because of the higher solubility of bropirimine at gastric pH. Thus, it is considered that the longer gastric residence time and larger volume of the gastric fluid induced by food-intake caused the increase in dissolution of bropirimine which increased the bioavailability after oral dosing of bropirimine 250-mg tablets. The gastrointestinal motility activated by food-intake may also contribute to the increases in the in-vivo dissolution and bioavailability of bropirimine. The conclusion is supported by the previous data in which bropirimine itself in solution was absorbed rapidly from the small intestine in rats (Emori et al 1995).

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