Effect of Gastric Acidity on Bioavailability of *N,N*-Dimethylcarbamoylmethyl α,2-Dimethyl-5*H*-[1]benzopyrano[2,3-*b*]pyridine-7-acetate, a New Prodrug-Type Anti-inflammatory Agent

Ichimaro YAMADA,* Tomoko GODA, Miwako KAWATA, Hiroaki MIZUTA, Kenji OGAWA and Testuo YOKOBE

Research Laboratories, Yoshitomi Pharmaceutical Industries Ltd., 955 Koiwai, Yoshitomi-cho, Chikujo-gun, Fukuoka 871, Japan. *Received May 1, 1989*

The effect of gastric acidity on the bioavailability of *N,N*-dimethylcarbamoylmethyl α,2-dimethyl-5*H*-[1]benzopyrano[2,3-*b*]pyridine-7-acetate (1), a new anti-inflammatory agent, was investigated in gastric acidity-controlled beagle dogs.

The dissolution rates of this compound in media of pH 1.2 and 3.0 were greater than those in media of pH 5.0 and 6.8. Reflecting these dissolution characteristics, the peak plasma concentration (C_{max}) and the area under the plasma concentration-time curve (AUC_{0-12h}) were reduced by shifting the gastric acidity to low levels (more than pH 6) with omeprazole treatment.

In designing dosage forms of 1, it is necessary to develop pharmaceutical preparations whose bioavailability is not affected by the gastric acidity.

Keywords weak base; pH; dissolution; gastric acidity-controlled beagle dog; gastric acidity; bioavailability

N,N-Dimethylcarbamoylmethyl α,2-dimethyl-5*H*-[1]-benzopyrano[2,3-*b*]pyridine-7-acetate (**1**) in Fig. 1, is a prodrug-type agent developed as a new non-steroidal anti-inflammatory drug. Although, compound **1** is pharmacologically inactive *per se*, it is rapidly hydrolyzed to an active metabolite (M1) following its absorption and exhibits a strong anti-inflammatory activity.[1]

It seemed likely that **1** would be released from its preparation in a pH-dependent manner because it is a weakly basic compound.

In dosage form design, it is very important to understand and evaluate the physico-chemical and bioavailability properties of the drug as well as the preparation characteristics of its various experimental dosage forms. The bioavailability of weakly basic and weakly acidic drugs is considered to be affected by various physiological factors, especially by the gastric acidity.[2] Clinically, inconsistent bioavailability often brings about unfavorable fluctuation in the efficacy and safety of a drug. Recently, there has been an increasing requirement for pharmaceutical preparations whose drug bioavailability is not affected by the gastric acidity.

From this viewpoint, prior to designing dosage forms of **1**, we determined the pH-solubility profile and dissolution characteristics of this compound in media of various pH values. Based on the results obtained, the effect of gastric acidity on the bioavailability of **1** was assessed. The bioavailability of **1** was studied in gastric acidity-controlled beagle dogs as reported previously.[3]

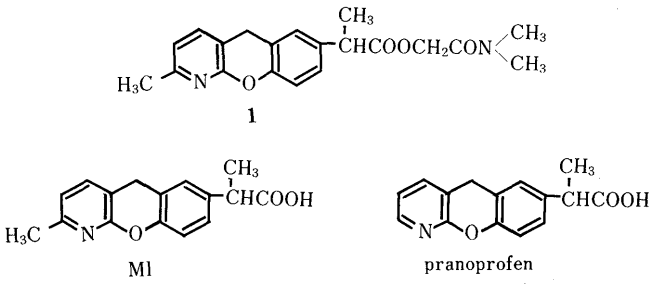

Fig. 1. Chemical Structures of **1**, M1 and Pranoprofen

Experimental

Materials Compound **1**, M1 and α-methyl-5*H*-[1]benzopyrano[2,3-*b*]-pyridine-7-acetic acid (pranoprofen) were synthesized in our laboratories. Figure 1 shows the chemical structures of these three compounds. Omeprazole((±)-5-methoxy-2-[[(4-methyl-3,5-dimethyl-2-pyridinyl)-methy]sulfinyl]benzimidazole) was used as received from AB Hässle, Sweden. All other chemicals were standard commercial products of analytical grade.

Solubility The solubility of **1** at pH values of 1.2 (the 1st fluid, JP XI disintegration test), 3.0 (0.001 N HCl), 4.0 (0.05 M acetic buffer), 5.0 (0.05 M phosphate buffer), 6.8 (the 2nd fluid, JP XI disintegration test) was determined at 25 °C. An excessive amount of the drug was placed in an Erlenmeyer flask along with 20 ml of the medium. The flask was tightly stoppered and shaken to equilibrate the content. The equilibrium solubility was determined by ultraviolet (UV) spectrophotometry using a Shimadzu UV-240 spectrophotometer.

Dissolution Studies The paddle method (JP XI dissolution method) was employed for investigating the dissolution behavior of **1**. Ten milliliters of 0.5% (v/v) methyl cellulose suspension containing 25 mg of **1**, the dosage form used in the bioavailability study was dispersed in 900 ml of the medium of pH 1.2, 3.0, 5.0, or 6.8 at 37.0±0.5 °C. The paddle was rotated at 50 or 100 rpm. Samples (5 ml each) were removed at suitable intervals and filtered through membrane filters with a pore size of 0.45 μm (Advantec Toyo, Japan). The concentrations of **1** were determined by UV spectrophotometry (wavelength: at 310 nm for pH 1.2 and at 277 nm for the others) using a Shimadzu UV-240 spectrophotometer. All dissolution experiments were carried out in triplicate and the results were highly reproducible. Thus only mean values are reported.

Animals Six healthy 2-year-old male beagle dogs weighing between 9.5 and 11.0 kg were used. They were fasted for 24 h before drug treatment. All dogs were allowed free access to water but no food was given until the experiment was finished.

Procedure for the Gastric Acidity Control The gastric acidity of beagle dogs was controlled with omeprazole to low levels (more than pH 6) as reported previously.[3]

Effect of Omeprazole on Distribution, Metabolism, and Excretion of M1 The beagle dogs were divided into two groups of three dogs. A solution of M1, corresponding to 2 mg/ml in terms of **1**, was dissolved in 0.1% (v/v) sodium hydrogencarbonate. This solution was intravenously administered at a dose corresponding to 1 mg/kg in terms of **1** to 24-h-fasted beagle dogs of the following two groups: 1) a group given omeprazole pretreatment; 2) a group without omeprazole treatment. In group 1, M1 was intravenously administered at 1 h after the omeprazole treatment. Venous blood samples were taken at 10, 20 and 40 min and 1.0, 1.5, 2.0, 4.0 and 8.0 h after the intravenous administration of M1. The blood samples were centrifuged for 15 min at 3000 rpm to obtain plasma, which was kept frozen at −20 °C until analysis.

Bioavailability Study Beagle dogs were divided into two groups of three dogs according to a latin-square cross-over design. Compound **1** was suspended in an aqueous solution of 0.5% (v/v) methyl cellulose (3 mg/ml) and was orally administered at a dose of 3 mg/kg to animals of the following two groups: 1) a group given omeprazole pretreatment; 2) a

group without omeprazole treatment. In group 1, **1** was orally administered at 1 h after the omeprazole treatment. A washout period of two weeks was allowed between two treatments of the same animal with **1**. Venous blood samples were taken at 20 and 40 min and 1.0, 1.5, 2.0, 4.0, 8.0 and 12 h after oral administration of **1**. The samples were stored at −20 °C until analysis as mentioned above.

Analytical Method The plasma concentration of M1 was determined by high-performance liquid chromatography (HPLC). A mixture of 0.5 ml of plasma sample, 2.0 μg of pranoprofen as an internal standard, 0.5 ml of 0.1 M citric acid and 2.0 ml of toluene was shaken for 10 min. After centrifugation, 1.6 ml of the organic layer was taken and evaporated at about 40 °C. The residue was dissolved in 0.1 ml of the mobile phase described below, and aliquots were used for the analysis.

Apparatus and Chromatographic Conditions The HPLC system used consisted of a LC-6A, a RF-530 fluorescence detector and a C-R3A integrating recorder (Shimadzu, Kyoto, Japan). The excitation and emission wavelengths of the detector were set at 298 and 350 nm, respectively. Samples were injected *via* a Rheodyne 7125 injector fitted with a 20 μl loop. Separations were performed on a TSK gel ODS 120T column (15 cm × 4.6 mm i.d., 5 μm particle size; Tosoh, Tokyo, Japan) with a mobile phase of methanol–0.1 M ammonium acetate (5 : 3 (v/v), adjusted to pH 3.0 with perchloric acid) at ambient temperature and a flow rate of 1.0 ml/min.

Data Analysis Plasma concentration was plotted against time, and the peak plasma concentration (C_{max}) and the time taken to attain the peak concentration (T_{max}) were determined directly from the graphs. The total area under the plasma concentration–time curve from time 0 to 12 h after drug administration (AUC_{0-12h}) was calculated according to the trapezoidal rule. Analysis of variance (ANOVA) was carried out for the pharmacokinetic parameters.

Results

Solubility Figure 2 shows the pH-solubility profile of **1** in the physiological pH range. Compound **1** dissolved very easily at pH 1.2 (3.70 mg/ml), but the solubility was 0.07 mg/ml at pH 3.0 and was reduced further in the higher pH range.

Dissolution Studies Figure 3 shows the pH-dissolution profiles of **1** determined by the paddle method at various pH values. Comparable dissolution profiles of **1** were obtained at 50 and 100 rpm paddle speeds. The dissolution rates of **1** in media of pH 1.2 and 3.0 were greater than those in media of pH 5.0 and 6.8.

Effect of Omeprazole on Distribution, Metabolism and Excretion of M1 It was reported that omeprazole interferes with the elimination of diazepam and phenytoin through inhibition of the drug-metabolizing monooxygenase system.[4] Therefore, the effect of omeprazole on elimination of M1 was investigated. Figure 4 shows the plasma concentration–time curves of M1 after its intravenous administration at a dose corresponding to 1 mg/kg in terms of **1** with and without omeprazole pretreatment. The plasma concentration-time curves declined similarly irrespective of omeprazole pretreatment. In addition, pharmacokinetic parameters calculated according to a two-compartment open model were not significantly altered by the pretreatment ($p > 0.05$).

These results suggest that the distribution, metabolism and excretion of M1 are not affected by omeprazole.

Bioavailability Studies Figure 5 shows the plasma concentration–time curves of M1 after oral administration of **1** at a dose of 3 mg/kg to groups of beagle dogs with and without omeprazole pretreatment. The mean plasma concentration–time curve until 2 h after administration of **1** was reduced slightly in the group of animals whose gastric acidity was shifted to low levels with omeprazole when compared with the animals without omeprazole pretreatment. The ANOVA for a cross-over design was carried out for the C_{max}, T_{max} and AUC_{0-12h} as shown in Table I. The ANOVA for all the parameters showed no significant difference in group or sequence ($p > 0.10$), confirming that the cross-over study had been properly done. There were no

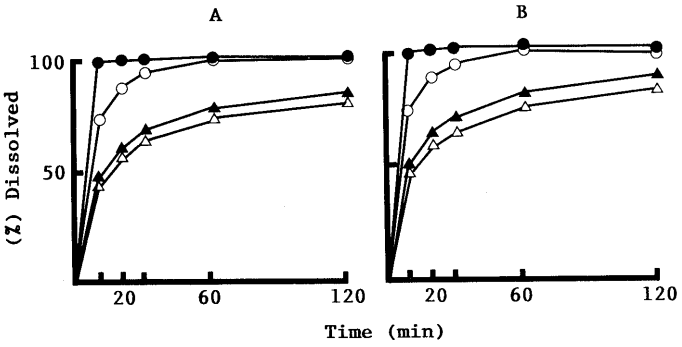

Fig. 3. Dissolution Profiles of Compound **1** from 0.5% Methylcellulose Suspension Using the Paddle Method at Various pH Values

●, pH 1.2; ○, pH 3.0; ▲, pH 5.0; △, pH 6.8. The paddle was rotated at 50 (A) or 100 (B) rpm. Each point represents the mean of 3 tests.

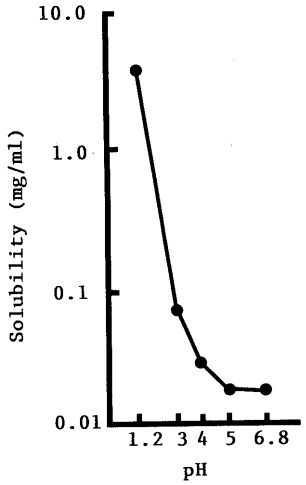

Fig. 2. pH-Solubility Profile of Compound **1**

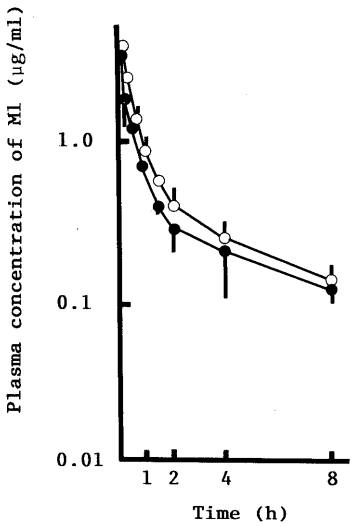

Fig. 4. Effect of Omeprazole on Plasma Concentrations of M1 after Intravenous Administration of M1 at a Dose of 1 mg/kg in terms of **1** in Dogs

●, control; ○, omeprazole pretreatment. Each point represents the mean of 3 dogs and the vertical bar indicates the S.D.

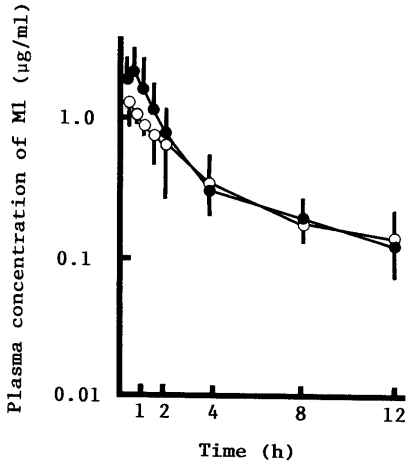

Fig. 5. Effect of Omeprazole on Plasma Concentrations of M1 after oral administration of Compound **1** at a Dose of 3 mg/kg in Dogs

●, control; ○, omeprazole pretreatment. Each point represents the mean of 6 dogs and the vertical bar indicates the S.D.

TABLE I. Effect of Omeprazole on C_{max}, T_{max} and AUC_{0-12h} of M1 after Oral Administration of Compound **1**

Parameter	Control	Omeprazole pretreatment	ANOVA results
T_{max} (h)	0.6±0.2	0.8±0.8	N.S.[a]
C_{max} (μg/ml)	2.29±0.84	1.41±0.24	N.S.
AUC_{0-12h} (μg·h/ml)	5.62±2.26	4.34±1.13	N.S.

Each value represents the mean±S.D. of 6 dogs. *a*) Not significant at the 0.05 level.

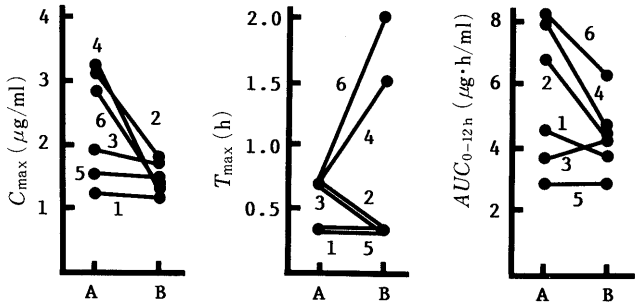

Fig. 6. Effect of Omeprazole on Individual C_{max}, T_{max} and AUC_{0-12h} of M1 after Oral Administration of Compound **1** at a Dose of 3 mg/kg in Dogs

A, control; B, omeprazole pretreatment.

significant differences in the C_{max}, T_{max} and AUC_{0-12h} between the two groups ($p>0.05$).

The effects of gastric acidity on C_{max}, T_{max} and AUC_{0-12h} in individual beagle dogs were investigated, as shown in Fig. 6. The C_{max} was reduced in all animals by shifting the gastric acidity to low levels. In particular, the values of dogs Nos. 2, 4 and 6 were remarkably reduced from 3.10 to 1.70 μg/ml, from 3.17 to 1.16 μg/ml and from 2.81 to 1.28 μg/ml, respectively. The AUC_{0-12h} was also reduced in 4 of 6 dogs. The reduction was marked in dogs Nos. 2, 4 and 6, as in the case of C_{max} (No. 2; 6.76→4.34 μg·h/ml, No. 4; 7.93→4.71 μg·h/ml, No. 6; 8.07→6.23 μg·h/ml). On the other hand, T_{max} values were prolonged only in dogs Nos. 4 and 6.

Discussion

It is thought that the bioavailability of a drug is influenced by various physiological factors such as gastric acidity, intestinal transit time and gastric emptying rate.[2,5] Prior to designing dosage forms of **1**, its solubility and dissolution characteristics were investigated. Moreover, in view of the results obtained, the effects of the gastric acidity on the bioavailability of **1** were investigated. The pH-solubility profiles of **1** seem to be typical of those of weakly basic drugs. Therefore, the dissolution study was carried out to estimate the variation in the bioavailability of **1**. The pH of the medium was adjusted to 1.2, 3.0, 5.0 and 6.8 to simulate *in vivo* variation in the gastric pH. The paddle was rotated at 50 and 100 rpm to examine the effect of variation in gastrointestinal motility on the bioavailability. Compound **1** dissolved rapidly at pH 1.2 and dissolved completely in 10 min. However, the dissolution was very slow at pH 5.0 and 6.8. D_{10} (% dissolved in 10 min) values of **1** at pH 5.8 and 6.8 were less than 50%, and the dissolution percentage did not reach 100% within 120 min at either pH. The dissolution of **1** was very fast under acidic conditions and very slow under mildly alkaline conditions. This may be due to the pH-dependent solubility of **1**. The dissolution behavior of **1** was not affected by the paddle speed. These results suggest that the bioavailability of **1** is affected by the variation in the gastric acidity rather than by that in gastrointestinal motility.

The effect of the gastric acidity on the bioavailability of **1** was assessed in gastric acidity-controlled beagle dogs in view of the above-mentioned findings. The plasma concentration-time curve up to 2 h after oral administration of **1** was slightly reduced by shifting the gastric acidity with omeprazole to low levels. In terms of the effect of the gastric acidity on the bioavailability of **1**, the beagle dogs were classified into two groups, *i.e.* animals which were affected remarkably (Nos. 2, 4 and 6) and those which were little affected (Nos. 1, 3 and 5) by omeprazole pretreatment. In the former group, C_{max} and AUC_{0-12h} were reduced by about 46 and 67%, respectively. We reported that the gastric pH of fasting beagle dogs varied not only from day to day but also within the same day. According to the fasting gastric pH, the animals were classified into two groups, *i.e.* those having a high acidity (less than pH 3) and those having a low acidity (more than pH 5).[3] As mentioned above, compound **1** showed a pH-dependent dissolution and the rate was greater at ambient pH < 3.0 than at pH > 5.0. The high bioavailability observed in beagle dogs Nos. 2, 4 and 6 without omeprazole pretreatment might be explained as follows: these three animals belonged, fortuitously, to the former group, and **1** dissolved rapidly in the stomach. On the other hand, in beagle dogs Nos. 1, 3 and 5, the gastric pH values were probably higher than 5.0 and the dissolution of **1** in the stomach was insufficient. These results suggest that the bioavailability of **1** is dependent on the dissolution process in the stomach and is markedly influenced by the gastric acidity.

Previous studies have suggested that the gastric pH of humans in the fasting state varies from person to person.[6] In addition, the proportion of persons having a low gastric acidity is considered to increase with age.[7] In designing dosage forms of **1**, it is necessary to develop pharmaceutical preparations whose bioavailability is not affected by the

gastric acidity.

Beagle dogs have been thought to be unsuitable for testing the bioavailability of drugs which are weakly basic or weakly acidic. However, beagle dogs whose gastric acidity is controlled with omeprazole appear to be useful animal models to evaluate the biological absorption behavior of drugs at low acidity levels.

References

1) K. Kawasaki, I. Yamada, T. Oe, M. Tsuruda, M. Terasawa, I. Imayoshi, Y. Yasunaga and K. Goto, *Yakugaku Zasshi*, **109**, 827 (1989).
2) H. Ogata, N. Aoyagi, N. Kaniwa, M. Koibuchi, T. Shibazaki and A. Ejima, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **20**, 166 (1982); N. Aoyagi, H. Ogata, N. Kaniwa and A. Ejima, *ibid.*, **23**, 469 (1985); H. Ogata, N. Aoyagi, N. Kaniwa, T. Shibazaki, A. Ejima, Y. Takagishi, T. Ogura, K. Tomita, S. Inoue and M. Zaizen, *Int. J. Pharmaceut.*, **23**, 277 (1985); H. Ogata, N. Aoyagi, N. Kaniwa, A. Ejima, N. Sekine, M. Kitamura and Y. Inoue, *ibid.*, **29**, 113 (1986).
3) I. Yamada, H. Mizuta, T. Goda, K. Haga and K. Ogawa, *Chem. Pharm. Bull.*, **37**, 2539 (1989).
4) R. Gugler and J. C. Jensen, *Gastroenterology*, **89**, 1235 (1985); D. J. Prichard, R. P. Walt, G. K. Kitchingman, K. W. Somerville, M. J. S. Langman, J. Williams and A. Richens, *Br. J. Clin. Pharmacol.*, **24**, 543 (1987).
5) K. K. Adjepon-Yamoah, D. B. Scott and L. F. Prescott, *Eur. J. Clin. Pharmacol.*, **7**, 397 (1974); J. Nimmo, R. C. Heading, P. Tothill and L. F. Prescott, *Br. Med. J.*, **1**, 587 (1973); R. C. Heading, J. Nimmo, L. F. Prescott and P. Tothill, *Br. J. Pharmacol.*, **47**, 415 (1973); P. Mojaverian, M. L. Rocci, D. P. Conner, W. B. Abrams and P. H. Vlasses, *Clin. Pharmacol. Ther.*, **1**, 11 (1987); M. L. Rocci, P. Mojaverian, R. J. Davis, R. K. Ferguson and P. H. Vlasses, *ibid.*, **1**, 45 (1987).
6) P. Finholt and S. Solvang, *J. Pharm. Sci.*, **57**, 1322 (1968); R. Iinuma and T. Toyama, *Yakuzaigaku*, **21**, 48 (1961).
7) H. Ogata, N. Aoyagi, N. Kaniwa, A. Ejima, K. Suzuki, T. Ishioka, M. Norishita, K. Ohta, Y. Takagishi and T. Ogura, *J. Pharmacobio-Dyn.*, **7**, 656 (1984).