# Gastric Acidity-Dependent Bioavailability of Commercial Sustained Release Preparations of Indomethacin, Evaluated by Gastric Acidity-Controlled Beagle Dogs

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**The relationship between gastric acidity and the bioavailability of two kinds of sustained-release indomethacin (IM) formulations was investigated in gastric acidity-controlled beagle dogs, and compared with that of rapid-release IM formulations.**

**All test dosage forms were more rapidly dissolved in simulated intestinal fluid than in simulated gastric fluid. Gastric acidity did not affect the bioavailability of IM from the rapid-release formulation. However, the bioavailability of IM from the two kinds of sustained-release formulations were markedly influenced by gastric acidity. The rates of IM bioavailability from both of the sustained-release formulations were faster under low acidity conditions than under high acidity conditions ($p<0.01$). In addition, $T_{max}$ and mean residence time ($MRT$) were approximately the same for the rapid-release and sustained-release formulations under low acidity conditions.**

**These results suggest that the IM sustained-release formulations showed a rapid-release profile under low acidity conditions.**

**Keywords** indomethacin; rapid-release formulation; sustained-release formulation; dissolution behavior; bioavailability; gastric acidity effect; beagle dog

Indomethacin (IM) is widely used in the treatment of inflammatory disorders.[1] However, it has a relatively short elimination half-life and a narrow range of effective plasma concentration in humans.[2] Therefore, a sustained-release dosage form of IM has been developed in an attempt to reduce the frequency of drug administration and the incidence and intensity of gastrointestinal and centrally-mediated side-effects.[3] However, such an advantage cannot be warranted if its bioavailability is influenced by a change in physiological functions. Therefore, it is important to study the relationship between the physiological factors and the bioavailability of the sustained-release formulations.[4] Some studies have evaluated the effect of gastric pH on bioavailability of sustained-release formulations.[5]

The aim of the present study is to determine whether gastric acidity influences the bioavailability of a sustained-release IM capsule relative to a rapid-release one. The bioavailability of IM formulations were studied in gastric acidity-controlled beagle dogs as reported previously.[6,7]

## Experimental

**Materials** IM was supplied by Wako Pure Chemical Industries, Ltd. Omeprazole was used as received from AB Hässle, Sweden. Pentagastrin and diazepam were purchased from Sigma, USA. All other reagents were commercially available products of analytical grade.

**Formulation** One rapid-release formulation A (lot No. NN416, diameter < 0.10 mm) and two sustained-release formulations B (lot No. NU466, diameter 0.50—0.85 mm) and C (lot No. 15B9B, diameter 0.35—0.85 mm) marketed in Japan were used in the study. The labeled amount of the drug in the products was 25 mg.

**Animals** Six healthy 3-year-old male beagle dogs weighing between 11.0 and 12.5 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 the beagle dogs was controlled at low levels (pH > 6) by intravenous administration of omeprazole at a dose of 1 mg/kg.[6] On the other hand, the gastric acidity of the animals was controlled at high levels (pH < 2) by intramuscular administration of pentagastrin at doses of 10 $\mu$g/kg (2 times at 1 h intervals).[7]

**Assay for IM Canine Plasma** High-performance liquid chromatography (HPLC) reported by Ohnishi *et al.*[8] was applied with a minor change. A mixture of 0.5 ml plasma sample, 2.0 $\mu$g of diazepam as an internal standard, 0.2 ml of 0.1 M citric acid and 2.0 ml of an organic mixture of ethyl acetate–*n*-hexane (1 : 9, v/v) was shaken for 10 min. After centrifugation, 1.6 ml of the organic layer was taken and evaporated at 25 °C. The residue was dissolved in 50 $\mu$l of the mobile phase described below and aliquots were used for the analysis. The HPLC system used consisted of a Shimadzu LC-6A, SPD-6A (wave length 254 nm) and C-R3A integrator recorder. Separations were performed on a Shim pack CLC ODS (15 cm × 4.6 mm, 5 $\mu$m particle size; Shimadzu) with a mobile phase of 0.1 M acetic acid–acetonitrile (2 : 3, v/v) and a flow rate of 1.0 ml/min. The lower limit of the assay of IM in plasma was 0.1 $\mu$g/ml.

**Dissolution Studies** The paddle method (JP XI dissolution method) was employed to investigate the dissolution behavior of IM from the capsules. The rotation speed was 100 rpm and the dissolution medium was 900 ml of simulated gastric fluid (pH 1.2, the 1st fluid, JP XI disintegration test) or simulated intestinal fluid (pH 6.8, the 2nd fluid, JP XI disintegration test), maintained at 37.0 ± 0.5 °C. Release of IM into the medium was determined by measuring the absorbance at 254 nm using a Shimadzu UV-240 spectrophotometer. All dissolution experiments were carried out in triplicate.

**Effect of Omeprazole on Distribution, Metabolism and Excretion of IM** The beagle dogs were divided into two groups of three dogs. IM was dissolved in an aqueous solution of 0.1% (w/v) sodium hydrogen carbonate. This solution (10 mg/ml) was intravenously administered at a dose of 1 mg/kg to dogs of the following two groups: 1) a group given omeprazole pretreatment; 2) a group without omeprazole treatment. Heparinized venous blood samples were taken at 0.25, 0.5, 1, 2, 4 and 8 h after administration. The blood samples were centrifuged at 1300 *g* for 15 min, and the plasma fraction was frozen and stored until assay.

**Bioavailability Studies** The bioavailability of IM formulation from the two treatments was studied by using a crossover design. Each capsule was orally administered to dogs of the following two groups: 1) a group given omeprazole pretreatment; 2) a group given pentagastrin pretreatment. Each dog received 30 ml of water immediately after oral administration. Experiments were carried out at 1-week intervals. To determine absorption profiles of IM from the gastrointestinal tract, the drug was administered intravenously at a dose of 10 mg/dog. Heparinized venous blood samples were taken at 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12 h after each administration. The blood samples were treated as described above.

**Pharmacokinetic Analysis** Plasma concentration was plotted against time. The peak plasma concentration ($C_{max}$) and the time taken to attain the peak concentration ($T_{max}$) were determined directly from the graphs. The area under the plasma concentration–time curve from 0 to 12 h after administration ($AUC_{0-12h}$) was calculated according to the linear trapezoidal rule. The mean residence time ($MRT$) was calculated by the following equation[9]:

$$MRT = \int_0^t tCdt / \int_0^t Cdt \quad (1)$$

where $C$ is the concentration of the drug in the plasma at any time $t$. The

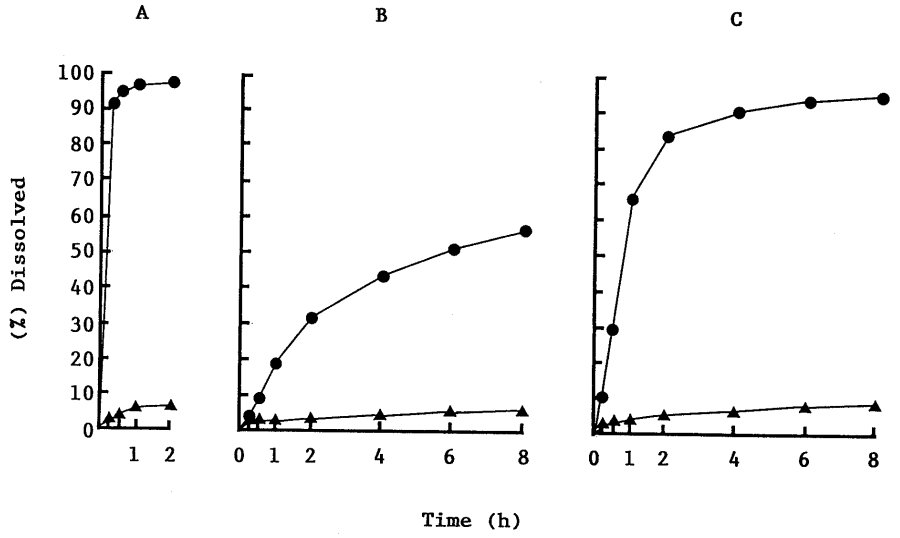


Fig. 1. Mean Dissolution Profiles for Formulations A, B and C in Simulated Gastric Fluid (▲) or in Simulated Intestinal Fluid (●)
The pH values of simulated gastric fluid and simulated intestinal fluid are 1.2 and 6.8, respectively.

cumulative fractions of the drug absorbed as a function of time were calculated from intravenous and oral administration data by the deconvolution method.[10] In general, if the drug followed linear kinetics, the input–response relationship could be described by the following convolution integral:

$$Y(t)=\int_0^t In(x)G(t-x)dx \qquad (2)$$

where $In(x)$ is the input function of drug into the body, $Y(t)$ is the resulting response function; $G(t)$ is the weighing function, which is the reponse following a unit dose impulse input (such as a rapid intravenous injection). Equation 2 provides the basis for the estimation of the absorption rate when $Y(t)$ and $G(t)$ are known. This is achieved by deconvolution.

**Statistical Analysis** A three-way analysis of variance following the Dunn procedure was used in statistical analysis of the pharmacokinetic parameters. A $p$ value of $<0.05$ was considered statistically significant.

## Results

**Dissolution Studies** Figure 1 shows the dissolution profiles of IM from the three commercial capsules in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8). All test dosage forms were more rapidly dissolved in simulated intestinal fluid than in simulated gastric fluid. The dissolution rates of IM in simulated intestinal fluid were in the following order: capsule A>C>B.

**Effect of Omeprazole on Distribution, Metabolism and Excretion of IM** It was reported that omeprazole interferes with the elimination of diazepam and phenytoin.[11] Therefore, the effect of omeprazole on the elimination of IM was investigated. Figure 2 shows the plasma concentration–time curves of IM after intravenous administration with and without omeprazole pretreatment. The plasma concentration–time curves declined similarly, regardless of omeprazole pretreatment. Pharmacokinetic parameters of IM were not significantly altered by omeprazole pretreatment ($p>0.05$).

**Effect of Gastric Acidity on Bioavailability of IM Formulations** Figure 3 shows the plasma concentration–time curves of IM after oral administration of capsule A to the high and low gastric acidity-controlled beagle dogs, respectively, and Table I shows the $C_{max}$, $T_{max}$, $AUC_{0-12h}$ and $MRT$ in the two groups. The $C_{max}$ was significantly

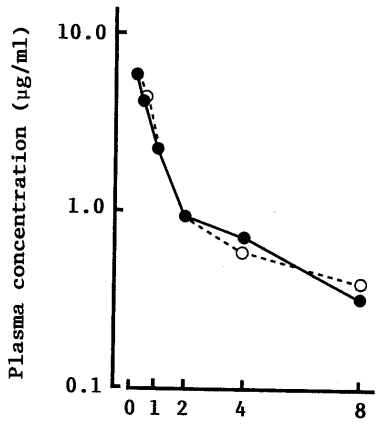


Fig. 2. Effect of Omeprazole on Plasma Concentrations of IM after Oral Administration at a Dose of 1 mg/kg to Dogs
●, control; ○, omeprazole pretreatment. Each point represents the mean of 3 dogs.

higher in the low acidity group than in the high acidity one ($p<0.05$), although the $T_{max}$, $AUC_{0-12h}$ and $MRT$ did not differ between the two groups ($p>0.05$).

Figures 4 and 5 show the plasma concentration–time curves after oral administration of sustained-release IM capsules B and C respectively to the gastric acidity-controlled beagle dogs. In the high acidity group, a double maxima phenomenon was observed in the plasma concentration–time curves of capsule C, but not in those of capsule B. The pharmacokinetic parameters are summarized in Table II and III. In the case of capsule B, the $AUC_{0-12h}$ was significantly greater in the high acidity group than in the low acidity group ($p<0.01$), and the $T_{max}$ and $MRT$ in the former were significantly longer than those in the latter ($T_{max}$; $p<0.01$, $MRT$; $p<0.01$). However, the $C_{max}$ was not different between the two groups ($p>0.05$). Capsule C also showed significantly greater $AUC_{0-12h}$ in the high acidity group than in the low acidity group ($p<0.01$). On the other hand, the $C_{max}$ of the high acidity group was significantly lower than that of the low acidity one. The $MRT$ was significantly longer in the high acidity

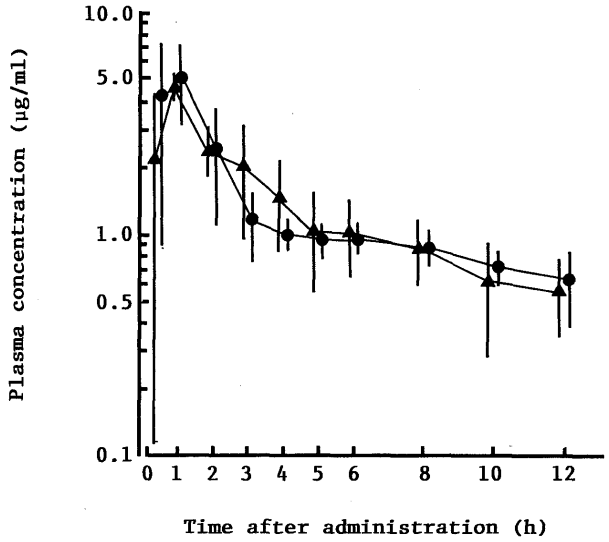


Fig. 3. Plasma Concentrations of IM after Oral Administration of Rapid-Release Formulation, Capsule A, to Gastric Acidity-Controlled Beagle Dogs

●, low gastric acidity; ▲, high gastric acidity. Each point represents the mean of 6 dogs and the vertical bar indicates S.D.

TABLE I. Effect of Gastric Acidity on Pharmacokinetic Parameters of IM after Oral Administration of Capsule A

| Parameter | Gastric acidity | |
|---|---|---|
| | Low | High |
| $C_{max}$ (μg/ml) | 6.49±1.53 | 4.78±0.66[a] |
| $T_{max}$ (h) | 0.7±0.3 | 0.7±0.3 |
| $AUC_{0-12h}$ (μg·h/ml) | 16.68±2.50 | 16.72±3.92 |
| *MRT* (h) | 4.0±0.3 | 4.1±0.4 |

Each value represents the mean±S.D. of 6 dogs. *a*) Significance ($p<0.05$).

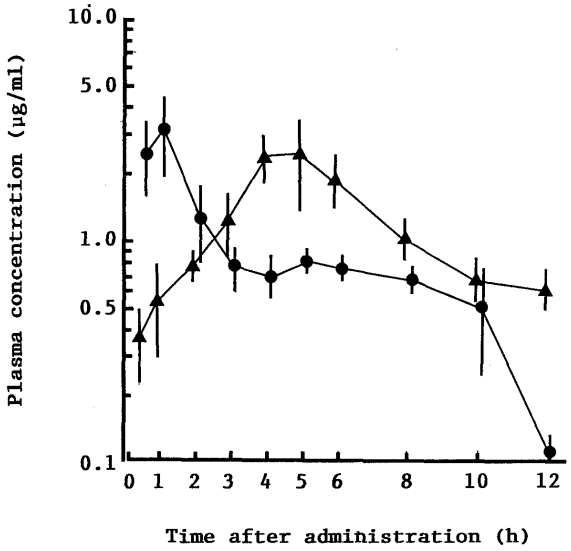


Fig. 4. Plasma Concentrations of IM after Oral Administration of Sustained-Release Formulation, Capsule B, to Gastric Acidity-Controlled Beagle Dogs

Symbols are the same as Fig. 3. Each point represents the mean of 6 dogs and the vertical bar indicates S.D.

group than in the low acidity group ($p<0.01$), although the $T_{max}$ was not different between the two groups ($p>0.05$).

Figure 6 shows the cumulative absorption profiles of IM

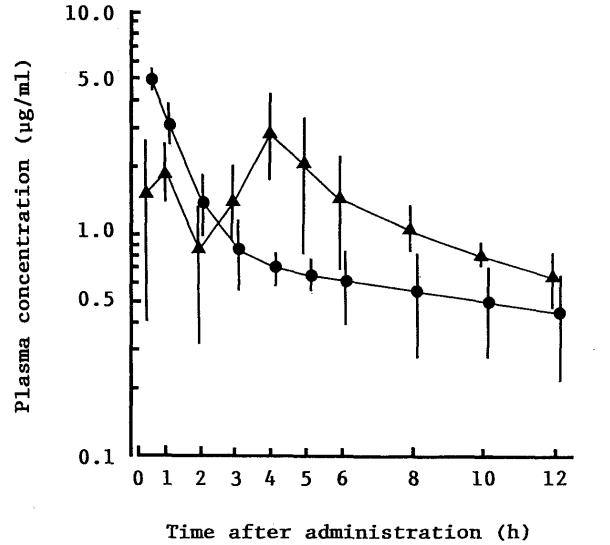


Fig. 5. Plasma Concentrations of IM after Oral Administration of Sustained-Release Formulation, Capsule C, to Gastric Acidity-Controlled Beagle Dogs

Symbols are the same as Fig. 3. Each point represents the mean of 6 dogs and the vertical bar indicates S.D.

TABLE II. Effect of Gastric Acidity on Pharmacokinetic Parameters of IM after Oral Administration of Capsule B

| Parameter | Gastric acidity | |
|---|---|---|
| | Low | High |
| $C_{max}$ (μg/ml) | 3.39±1.17 | 2.90±0.82 |
| $T_{max}$ (h) | 0.8±0.3 | 4.8±0.8[a] |
| $AUC_{0-12h}$ (μg·h/ml) | 10.80±1.58 | 14.45±2.33[a] |
| *MRT* (h) | 4.1±0.6 | 5.7±0.1[a] |

Each value represents the mean±S.D. of 6 dogs. *a*) Significance ($p<0.01$).

TABLE III. Effect of Gastric Acidity on Pharmacokinetic Parameters of IM after Oral Administration of Capsule C

| Parameter | Gastric acidity | |
|---|---|---|
| | Low | High |
| $C_{max}$ (μg/ml) | 4.91±0.47 | 3.32±0.93[b] |
| $T_{max}$ (h) | 0.5±0.0 | 2.7±1.8 |
| $AUC_{0-12h}$ (μg·h/ml) | 11.84±2.90 | 16.28±4.19[b] |
| *MRT* (h) | 3.7±0.5 | 5.2±0.5[a] |

Each value represents the mean±S.D. of 6 dogs. *a*) Significance ($p<0.05$). *b*) Significance ($p<0.01$).

after oral administration of the capsules to the gastric acidity-controlled beagle dogs. The absorption profiles of IM from the capsule A were approximately the same in the low and high gastric acidity groups. However, the absorption profiles of IM from capsules B and C were affected by gastric acidity. The rates of IM bioavailability from sustained-release formulations were faster in the low acidity group than in the high acidity group. On the other hand, the extent of IM bioavailability from the capsules was smaller in the low acidity group, compared to that in the high acidity group.

## Discussion

It is possible that the bioavailability of a drug in an oral

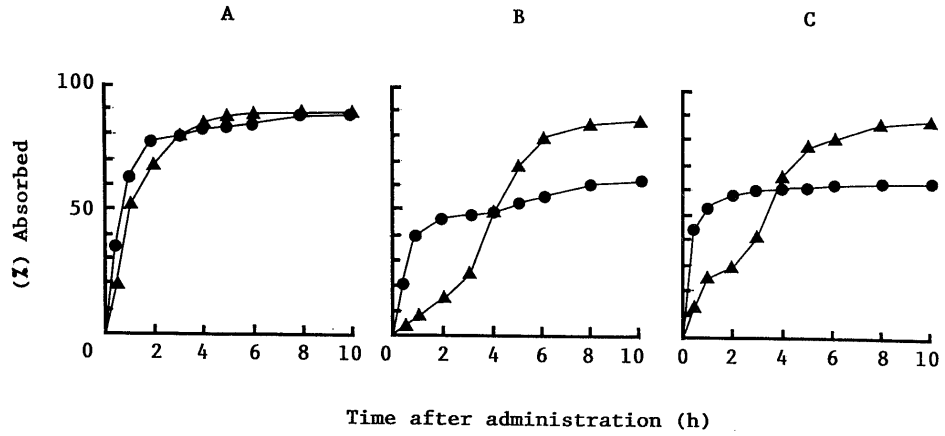


Fig. 6. Cumulative Absorption Profiles of IM after Oral Administrat of Capsules A, B and C to Gastric Acidity-Controlled Beagle Dogs
A, capsule A; B, capsule B; C, capsule C. Symbols are the same as Fig. 3. Each point represents the mean of 6 dogs.

dosage form, which exhibits *in vitro* pH-dependent dissolution characteristics, may be altered due to a change in gastric pH.[12] Clinically, such variation in drug bioavailability often causes individual differences in the efficacy and/or safety of a drug. In the present study, we examined the effect of gastric acidity on the bioavailability of three types of IM formulations. The *in vitro* dissolution studies predict that the absorption rate of IM is much faster in the low acidity group than in the high acidity one. However, the absorption profiles of IM from the rapid-release formulation, capsule A, was unaffected by gastric acidity (Fig. 6). This result might be explained as follows. The expulsion of small indigestible particles (diameter $< 1$ mm) from the stomach is not dependent on the interdigestive migratory myoelectric complex, and mean gastric emptying time of particles is about 0.5 h in the fasting beagle dogs.[13] Capsule A contained particles less than 0.1 mm diameter. In addition, it showed very rapid dissolution in simulated intestinal fluid when compared to the sustained-release formulations. In the high acidity group, IM absorption can be initiated only when the fine granules are emptied into the small intestine. Similarity of the IM bioavailability from capsule A between the two groups seems to be due to its rapid dissolution rate and to the faster gastric emptying rate in the fasting beagle dogs.

The bioavailability of IM from two sustained-release formulations was markedly influenced by gastric acidity (Tables II and III). The sustained-release formulations showed imcomplete bioavailability in the low acidity group, but not in the high acidity one. In addition, $T_{max}$ and *MRT* were approximately the same in the rapid-release and sustained-release formulations in the low acidity group. Thus, both formulations showed rapid-release under low acidity conditions. This may be caused by the pH-dependent dissolution characteristics of IM from the sustained-release dosage form. The bioavailability, however, was relatively low (Fig. 6), for which there was no reasonable explanation. It has been reported that the transition of the drug while it is present in the gastrointestinal tract in beagle dogs is considered to be fast.[14] The above results might be attributed to the shorter gastrointestinal transit time of IM.

The results suggest that these sustained-release formulations can not be expected to offer prolongation of effective plasma levels or to minimize the risk of side-effects for achlorhydric subjects. In designing sustained-release dosage forms, it is necessary to develop pharmaceutical preparations whose bioavailability is not affected by gastric acidity.

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