Gastrointestinal Physiology-Regulated Dogs for Bioavailability Evaluation of an Oral Controlled-Release Dosage Form Composed of Pulsatile Release Granules

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Gastrointestinal (GI) physiology-regulated beagle dogs (regulated dogs) were regulated by a combined treatment using intramuscular pentagastrin and intravenous atropine sulfate. In the regulated dogs, the gastric pH was shifted to around 2.0, and the GI transit time was prolonged to approximate that in humans. Pranoprofen, an acidic anti-inflammatory agent, was granulated around sucrose seeds, and then coated with low substituted hydroxypropyl cellulose used as a swelling agent to afford plain granules (A-granule). Then, A-granule was coated stepwise with ethyl cellulose used as an outer shell material to afford two kinds of pulsatile release granules (B- and C-granules). In the dissolution study using pH 1.2 and 6.8 media, A-, B- and C-granules exhibited lag times of 0, 1 and 2 h, respectively. Even in intact beagle dogs, the absorption profiles for A- and B-granules corresponded with those expected from the dissolution profiles. In contrast, the bioavailability of C-granule was only 35% in the intact dogs, but was 55% in the regulated dogs. Thus, the absorption of pranoprofen from pulsatile release granules after a longer lag time should be influenced by the location in the GI tract. Next, a controlled-release (CR) dosage form of pranoprofen was tentatively prepared by combining A-, B- and C-granules at the ratio of 3:4:3 (w/w) in contents of pranoprofen. The bioavailability of the CR dosage form was significantly diminished in the intact dogs, being about 70% as much as that in the regulated dogs. Therefore, the regulated dogs would be superior to the intact dogs in avoiding the underestimation of the bioavailability of a CR dosage form with a pulsatile release property.

Key words bioavailability; controlled-release dosage form; pulsatile release; pranoprofen; dog

A pulsatile release system, in which a drug release initiates explosively after a predesigned lag time in the gastrointestinal (GI) tract, has currently been developed as a device for chronopharmacotherapy or the site specific delivery of oral drugs.1-5) Thereby, an oral controlled-release (CR) dosage form is prepared by combining several pulsatile release components with different lag times. However, it is difficult to evaluate the bioavailability of such dosage forms. Beagle dogs are widely used as animal models in bioavailability studies, even though the GI physiology of the dogs differs significantly from that of humans. For example, the small intestinal transit time is 2.8 h in the dogs,6) but is 4.5 h in humans.7) When the lag times of pulsatile components are designed to be near 2.8 h or more, the pulsatile release would initiate in the colon or intestinal semi-solid contents of the dogs. In fact, the bioavailability for the pulsatile release tablet of diltiazem, which is absorbed from the wide range of the GI tract, has been reported to fluctuate in the dogs.8) At the moment, we have used GI physiology-regulated beagle dogs (regulated dogs) in the bioavailability study for the purpose of predicting the bioavailabilities of some dosage forms in humans. The GI physiology of the dogs is regulated by a combined treatment using intramuscular pentagastrin (10 μg/kg×2) and intravenous atropine sulfate (0.02 mg/kg×1) to show the small intestinal transit time of about 4.1 h and the gastric pH of around 2.6,8,9)

Herein, we describe the utility of the regulated dogs in the bioavailability study of a CR dosage form composed of pulsatile release granules. In this study, pranoprofen, an acidic anti-inflammatory agent, was chosen as a model drug. Pranoprofen is reported to show rapid absorption and a urinary recovery of more than 90% after oral administration of a marketed conventional tablet to humans.10)

MATERIALS AND METHODS

Materials Pranoprofen was synthesized in the Research Laboratories of Yoshitomi Pharmaceutical Industries, Ltd. The additives used were as follows: sucrose seeds (Nonparcil®, 101 grade, Freund Co., Ltd., Tokyo, Japan), hydroxypropyl cellulose (HPM-L®, Nippon Soda Co., Ltd., Tokyo, Japan), low substituted hydroxypropyl cellulose (L-HPC®, Shin-Etsu Chemical Industry Co., Ltd., Tokyo, Japan), ethyl cellulose with a viscosity of 10 cP (EC, Dow Chemical Company, Midland MI, U.S.A.), and polyethylene glycol (1540 grade, Nacalai Tesque Inc., Kyoto, Japan). Pentagastrin and atropine sulfate were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The conventional tablet (NiFran® tablet) containing 75 mg of pranoprofen was supplied by Yoshitomi Pharmaceutical Industries, Ltd. All other reagents used were of analytical grade and were available from commercial suppliers.

Formulation of Pulsatile Release Granules The pulsatile release granules were manufactured with laboratory-scale equipment in accordance with a patented method11) and the report by Ueda et al.5) Pranoprofen was granulated around sucrose seeds with hydroxypropyl cellulose (HPC-L) used as a binder, and then coated with L-HPC to afford plain granules (A-granule). Subsequently, A-granule was spray-coated with a solution of EC containing polyethylene glycol (PEG) as a plasticizer to

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Table 1. Composition of Pulsatile Release Granules

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>A-Granule</th>
<th>B-Granule</th>
<th>C-Granule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pranopronfen</td>
<td>18.0</td>
<td>18.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Sucrose seed</td>
<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
</tr>
<tr>
<td>L-HPC</td>
<td>49.0</td>
<td>49.0</td>
<td>49.0</td>
</tr>
<tr>
<td>HPC-L</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>EC</td>
<td>—</td>
<td>3.6</td>
<td>5.0</td>
</tr>
<tr>
<td>PEG</td>
<td>—</td>
<td>0.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Each value is expressed as a weight ratio.

give stepwise two kinds of pulsatile release granules (B- and C-granules). The formulae of these granules are shown in Table 1. A CR dosage form containing 75 mg of pranopronfen was prepared by combining A-, B- and C-granules (A:B:C-granules = 3 : 4 : 3, w/w in contents of pranopronfen).

**Dissolution Study** The paddle method described in the Japanese Pharmacopoeia XII (JP XII) was used in the dissolution study under the following conditions: the dissolution media, 900 ml of the first medium (pH 1.2) and the second medium (pH 6.8) for the JP XII disintegration test; temperature, 37.0 ± 0.5 °C; rotation speed, 100 rpm. The influence of agitation on the release of pranopronfen from the conventional tablet and the CR dosage form was also investigated in the second medium at 50 and 100 rpm. The amount of pranopronfen which dissolved into the test medium was spectrophotometrically monitored using a Shimadzu UV-240 spectrophotometer (Shimadzu Co., Kyoto, Japan) at 275 nm. Each experiment was carried out in triplicate to calculate an average release rate (%).

**Bioavailability Study** Healthy male beagle dogs weighing between 7.5 and 12.5 kg were used. The GI physiology of dogs was, if necessary, regulated by a combined treatment of intramuscular pentagastrin (10 μg/kg × 2) and intravenous atropine sulfate (0.02 mg/kg × 1). Each experiment described below was carried out at least a 1-week interval. Each dog was fasted overnight before and until the end of the experiment, but was allowed free access to water. The dosage forms of pranopronfen were given orally to each dog with 30 ml of water at a dose of 75 mg of pranopronfen.

In the first experiment, the bioavailabilities of A-, B- and C-granules were estimated in intact beagle dogs. Nine dogs were randomly divided into three groups. In the second experiment, the bioavailability of C-granule in the intact dogs was compared with that in the regulated dogs by means of a randomized cross-over design. Six dogs were randomly divided into two groups: 1) a group of the intact dogs; 2) a group of the dogs with regulated GI physiology. In the third experiment, the bioavailability of the CR dosage form was evaluated in the intact dogs and the regulated dogs. Six dogs were divided into two groups of three dogs. In the fourth experiment, pranopronfen (5 mg/ml) was dissolved in saline containing NaHCO₃ (1%, w/v). This solution was intravenously dosed to the six regulated dogs at a dose of 1 mg/kg of pranopronfen. Blood samples were taken with heparinized syringes 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after oral administration, and were centrifuged (1300 g for 15 min) to separate the plasma. The samples of the plasma were kept frozen until the HPLC assay of pranopronfen.

**Assay for Plasma Concentration of Pranopronfen** Plasma concentrations of pranopronfen were determined by HPLC. To 0.2 ml of the plasma were added 0.2 ml of 0.1 N citric acid solution and 1 ml of toluene. After shaking for 10 min and centrifugation at 1300 g for 5 min, the organic layer (0.8 ml) was transferred to a glass tube and evaporated to dryness under reduced pressure. The residue was dissolved in 200 μl of the mobile phase for HPLC, and this solution (40 μl) was injected onto the HPLC column. The HPLC system consisted of a Shimadzu LC-10A pump, an RF-550 fluorescence detector (excitation wavelength 298 nm, emission wavelength 370 nm) and a C-R6A integrator recorder. Separations were performed on a Shim-pack CLC-ODS (150 × 4.6 mm, 5 μm particle size, Shimadzu) with a mobile phase of 0.01 M acetic acid adjusted to pH 3.0 with phosphoric acid–MeOH (3 : 5.5, v/v) and a flow rate of 1.0 ml/min. The lower limit of the assay of pranopronfen in plasma was 100 ng/ml.

**Pharmacokinetic and Deconvolution Analysis** The Cₘₐₓ and the time required to reach the Cₘₐₓ (tₘₐₓ) were read from the individual time courses of plasma concentrations of pranopronfen. The area under the plasma concentration–time curve (AUC) was calculated using a linear trapezoidal method, and the mean residence time (MRT) was computed by moment analysis.

By the point-area deconvolution method, cumulative percentages of absorbed pranopronfen were calculated for A-, B- and C-granules from the data of intravenous and oral administration.

**Statistical Analysis** Differences in each bioavailability parameter were statistically evaluated by the paired t-test.

**RESULTS AND DISCUSSION**

**In Vitro Evaluation of the Granules of Pranopronfen** Regardless of the pH values in the dissolution media, pranopronfen initiated to dissolve immediately from A-granule, but after lag times of 1 and 2 h from B- and C-granules, respectively (Fig. 1). Here, the pulsatile property of these granules was demonstrated by the balance between the expansion of L-HPC as the swelling material and the tensile strength of EC as the outer shell material. In addition, L-HPC and EC are pH-insensitive polymers. Into both the dissolution media of pH 1.2 and 6.8, the drug dissolved rapidly from A- and B-granules after the predesigned lag times of 0 and 1 h, respectively. From C-granule, on the other hand, the drug dissolved slowly into the pH 6.8 medium within 6 h after the lag time of 2 h, and dissolved incompletely into the pH 1.2 medium. As reported by Ueda et al., these slow release rates from C-granule can be rationalized by the wider variation in the initiation of destruction of the outer shell with an increased amount of EC. In addition, the incomplete drug release from C-granule into the pH 1.2 medium would reflect an acidic property of pranopronfen, which should be partially saturated in the inside of C-granule under an acidic condition. Such a phenomenon has also been published regarding “time-controlled explosion system” of nilvadipine. In this study, all the
pulsatile release granules of pranoprofen comprised spherical particles smaller than 1 mm in diameter, which can be rapidly emptied from the stomach. Thus, the possible diminished release by gastric acidity would be negligible for C-granule.

**Absorption Properties of Pulsatile Release Granules of Pranoprofen** The drug absorption for A-, B- and C-granules was first estimated using intact beagle dogs (Fig. 2). The absorption profiles of A- and B-granules corresponded with their respective dissolution profiles in the pH 6.8 medium shown in Fig. 1. Thus, the drug absorption from the two kinds of granules was complete. In the case of C-granule, although the in vivo lag time was about 2 h, which was similar to that of the in vitro, both the rate and extent of absorption were inferior to those predicted from the dissolution profiles in the pH 6.8 medium. Because pranoprofen is well absorbed after both oral and rectal administration to humans, the so-called absorption window is likely inapplicable to this drug. The diminished bioavailability of C-granule in the intact dogs can therefore be rationalized by a change in the rate and extent of absorption between the upper and distal intestine during the GI transit of C-granule. To confirm this explanation, the drug absorption for C-granule in the intact dogs was compared with that of the regulated dogs. The cumulative percentage of absorbed pranoprofen in the intact dogs was 35%, whereas that in the regulated dogs was 55%, being about 1.5 times as much as that in the intact dogs (Fig. 3). These results suggest a superiority of the regulated dogs to the intact dogs in avoiding the underestimation of a bioavailability of a pulsatile release dosage form. In the regulated dogs, the GI transit was effectively delayed to bring about an improved bioavailability, but this is yet incomplete. The detailed cause of such a discrepancy is not sufficiently clarified in the present experiments. However, an inflection point of absorption rate was detectable at about 4 h in the graph for the intact dogs, but at about 6 h in that for the regulated dogs (Fig. 4). We have previously reported that the mean gastric emptying time and small intestinal transit time was 0.2 and 2.8 h in the intact dogs, respectively, and 0.6 and 4.1 h in the regulated dogs. This inflection behavior suggests that the drug absorption for C-granule was terminated after its arrival in the distal intestine. In the distal intestine, both the amount of water and the viscosity of semi-solid contents should be insufficient for the outer shell destruction, the drug release and the drug absorption for C-granule.

**Usefulness of the Regulated Dogs in Evaluating the Bioavailability of a CR Dosage Form with a Pulsatile**
Release Property A tentative CR dosage form of pranoprofen was prepared by combining A-, B- and C-granules at a ratio of 3:4:3 (w/w in contents of pranoprofen). In the dissolution study, the CR dosage form showed a slow release up to 6 h in the pH 6.8 medium at 50 and 100 rpm (Fig. 5). From the conventional tablet as the reference standard, however, the drug dissolved completely into the pH 6.8 medium within 15 min. The release profiles of both the dosage forms were hardly influenced by the agitation, suggesting that dose-dumping is scarcely caused by the variance in the GI contractions.

Figure 6B shows the absorption profile and pharmacokinetic parameters after administration of the conventional tablet to the regulated dogs. Here, pranoprofen was sharply absorbed from the conventional tablet, and was rapidly eliminated from the plasma. In both the intact dogs and the regulated dogs, the CR dosage form exhibited a smooth and long-lasting absorption profile up to 24 h after administration (Fig. 6A). The AUC value of the CR dosage form in the intact dogs was significantly diminished, being about 70% as much as that in the regulated dogs. This result would give an additional evidence for the possible underestimation of the bioavailability of pulsatile release granules in the intact dogs.

In the regulated dogs, the $C_{max}$ value for the CR dosage form was unexpectedly less than that for the conventional tablet (Table 2). The bioavailability of the CR dosage form was 83% of the conventional tablet after normalizing the mean AUC value on each occasion by the mean body weight. This relative bioavailability of the CR dosage form is in good agreement with the value calculated from the cumulative bioavailability on the basis of the contribution of A-, B- and C-granules under the composition ratio.

In conclusion, the regulated dogs should be superior to the intact dogs in avoiding the unfavorable under-

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Table 2. Pharmacokinetic Parameters of Pranoprofen after Oral Administration of the CR Dosage Form at a Dose of 75 mg of Pranoprofen in the Intact Dogs and the Regulated Dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>$C_{max}$ (µg/ml)</th>
<th>$t_{max}$ (h)</th>
<th>$AUC_{0-24h}$ (µg·h/ml)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact dogs</td>
<td>$15.3 \pm 1.4$</td>
<td>$2.5 \pm 1.8$</td>
<td>$166.2 \pm 19.7$</td>
<td>$9.1 \pm 0.6$</td>
</tr>
<tr>
<td>Regulated dogs</td>
<td>$18.1 \pm 1.8$</td>
<td>$2.3 \pm 0.6$</td>
<td>$228.6 \pm 9.3^a$</td>
<td>$9.7 \pm 0.5$</td>
</tr>
</tbody>
</table>

Results are expressed as the mean $\pm$ S.D. of 3 dogs. $^a$ Statistically significant ($p < 0.05$) vs. the intact dogs after normalizing the AUC value by the mean body weight.
estimation of the bioavailability of a CR dosage form with a pulsatile release property.

REFERENCES