Gastrointestinal Physiology-Regulated Dogs: Utilization of a Bioavailability Study of a New Thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]-diazepine, an Antagonist of Platelet-Activating Factor, and Its Preparations

KAZUYOSHI SAGARA,* Ichimaro YAMADA, Yasushi KAWAZOE, Hiroaki MIZUTA, and Masahiro SHIBATA

Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd., Koiwai, Yoshitomi-cho, Chikuyo-gun, Fukuoka, 871, Japan. Received May 28, 1993

The gastrointestinal (GI) physiology of beagle dogs was effectively regulated with a combined treatment using intramuscular pentagastrin (10 μg/kg × 2) and intravenous atropine sulfate (0.02 mg/kg × 1). The superiority of the GI physiology regulated-dogs over the intact dogs was confirmed by comparative bioavailability studies using two classes of preparations of poorly water-soluble 4-(2-chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-6,9-dimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine (Y-24180). Both the fine granules and the tablets of Y-24180 exhibited similar absorption profiles in the intact dogs, whereas the latter preparations revealed a delayed plasma curve of the drug in the regulated-dogs. The absorption profiles of the two classes of Y-24180 preparations in the regulated-dogs simulated those in healthy volunteers. The combined-treatment of beagle dogs with pentagastrin and atropine sulfate was suggested to supply a useful animal model for predicting the absorption characteristics of poorly water-soluble drugs and their preparations in humans.

Keywords bioavailability; poorly water-soluble drug; dog; animal model; gastrointestinal transit; gastric acidity

Beagle dogs are widely used to assess the bioavailability of drugs and pharmaceutical preparations. However, the drug absorption profiles observed in beagle dogs often differ considerably from those in humans. These phenomena bring about a critical disadvantage in the experimental evaluation of the bioavailability of slightly water-soluble drugs and sustained-release preparations. The species differences are believed to depend on a faster transit of drugs through the gastrointestinal (GI) tract, more vigorous GI-motility, and a higher gastric pH in beagle dogs than in humans. In the previous report, we described that a combined-treatment using intramuscular pentagastrin and intravenous atropine sulfate effectively regulated the GI physiology of the dogs. The gastric acidity, the gastric emptying time and the small intestinal transit time in the GI physiology regulated-dogs were, respectively, around pH 2, 0.6 h and 4 h, and these values showed good approximation to those in humans, as reported by Dressman et al., Kaniva et al., and Stanforth et al. Additionally, we have utilized the regulated dogs in a bioavailability study of a commercial sustained-release preparation of diclofenac sodium, and have discussed the utility of these animals in terms of comparability with healthy volunteers.

4-(2-Chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-6,9-dimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine (Y-24180) is a potent and specific antagonist of platelet-activating factor, and its solubility in water is less than 0.01% (w/v) at 23 °C. A difference in pharmacokinetics between 1% (w/w) fine granules and 10 mg tablets of Y-24180 was already demonstrated in the phase I study. These physico-chemical and bio-pharmaceutical characteristics of Y-24180 prompted us to evaluate the oral absorption properties of these two preparations using the regulated-dogs. The purpose of this report is to investigate the utility of the regulated-dogs for bioavailability studies employing Y-24180 preparations in comparison with the results obtained in healthy volunteers. The two classes of Y-24180 preparations used in the phase I study were again employed here.

MATERIALS AND METHODS

Materials The compound Y-24180 was synthesized in our laboratories. Pentagastrin and atropine sulfate were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents used were of analytical grade available from commercial suppliers.

Preparation Two classes of Y-24180 preparations formulated in our laboratories were used; one was 1% (w/w) fine granules and the other was 10 mg tablets.

Pharmacokinetic Studies of Y-24180 in the Intact Dogs and in the GI Physiology Regulated-Dogs Six healthy male beagle dogs weighing between 9 and 12 kg were used. Y-24180 was dissolved in propylene glycol (2 mg/ml). This solution was intravenously administered to dogs at a dose of 0.5 mg/kg. Each dog was fasted for 10 h prior to and until the finish of the experiment, but was allowed free access to water. Blood samples were taken with heparinized syringes at 0.25, 0.5, 1, 2, 3, 4, 6, and 10 h after the dosing, and were centrifuged (1300 × g for 15 min) to separate the plasma. The plasma samples were kept frozen until the HPLC assay of Y-24180. The dogs were divided into two groups of three dogs in accordance with a randomized crossed-over design as follows: 1) a group of the intact dogs; 2) a group of the dogs with GI physiology regulated by a combined-treatment of intramuscular pentagastrin (10 μg/kg) and intravenous atropine sulfate (0.02 mg/kg) at 15 min prior to the dosing, and additionally treated with intramuscular pentagastrin (10 μg/kg) at 30 min after the dosing of the preparations. Each experiment was carried out at 1-week intervals.

© 1994 Pharmaceutical Society of Japan
Dissolution Studies  The paddle method described in the Japanese Pharmacopoeia XI (JP XI) was employed in the dissolution test under the following conditions: the dissolution medium, 900 ml of the first fluid for JP XI disintegration test (pH 1.2), the second fluid for JPXI disintegration test (pH 6.8), and the second fluid containing polysorbate 80 (0.3%, w/v); temperature, 37.0 ± 0.5°C; rotation speed, 100 rpm. Five milliliters of 0.5% methylcellulose suspensions containing 5 mg of Y-24180 was dispersed in fluids with pH values of 1.2 and 6.8. The dissolution test of the two classes of Y-24180 preparations (10 mg) were carried out in pH 6.8 fluids containing polysorbate 80. Release of Y-24180 into the test medium was spectrophotometrically monitored by using a Shimadzu UV-240 spectrophotometer (Shimadzu Co., Kyoto, Japan) at 244 nm. Each experiment was carried out in triplicate to calculate an average dissolution rate (%).

Bioavailability Studies  Six healthy male beagle dogs weighing between 9 and 12 kg were used. Each dog was fasted for 24 h prior to and until the finish of the experiment, but was allowed free access to water. Blood samples were taken with heparinized syringes at 0.25, 0.5, 1, 2, 3, 4, 6, 10, and 24 h after the oral dosing of the Y-24180 preparations at a dose of 10 mg/dog, and were centrifuged (1300 × g for 15 min) to separate the plasma. The plasma samples were kept frozen until the HPLC assay of Y-24180. The dogs were divided into two groups of three dogs in accordance with a randomized cross-over design as follows: 1) a group received fine granules with 30 ml of water; 2) a group received one tablet with 30 ml of water. Each experiment was carried out at 1-week intervals. The cross-over experiments were performed on two different cases by using intact beagle dogs and GI physiology regulated-dogs.

Assay for the Plasma Concentration of Y-24180  The plasma concentration of Y-24180 was determined by HPLC. To 1 ml of plasma were added 0.5 ml of water, 0.05 ml of 5 N NaOH and 4 ml n-hexane containing 1,2-dichloroethane (20%, v/v). After 10 min of shaking and centrifugation at 1300 × g for 5 min, to the supernatant (3.6 ml) was added 2 ml of 4 N HCl containing MeOH (10%, v/v). After 10 min of shaking and centrifugation at 1300 × g for 5 min, to the aqueous phase (1.9 ml) were added 1.7 ml of 5 N NaOH and 4 ml of n-hexane containing 1,2-dichloroethane (20%, v/v). After 10 min of shaking and centrifugation at 1300 × g for 5 min, the supernatant (3.7 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 150 μl of this solution was injected onto the HPLC column. The HPLC system used consisted of a Shimadzu LC-6A, SPD-6A (wave length 264 nm) and C-R6A integrator recorder. Separations were performed on a Nucleosil 50-5 (15 cm × 4.6 mm, 5 μm particle size, M. Nagel) with a mobile phase of EtOH–NH₄OH–1,2-dichloroethane–n-hexane (14:4:0.1:5:80.5, v/v) and a flow rate of 1.0 ml/min. The lower limit of the assay of Y-24180 in plasma was 0.5 ng/ml.

Clinical Studies  The safety, pharmacological effects, and pharmacokinetics after oral dosing of Y-24180 were studied in healthy male volunteers. Six subjects received the two classes of Y-24180 preparations with at least a 1-week interval between dosage: 1) fine granules containing 10 mg Y-24180 with 150 ml of water under fasting conditions, 2) one tablet containing 10 mg Y-24180 with 150 ml of water under fasting conditions. Lunch was allowed at 4 h after the dosing. Blood samples were taken at 0.25, 0.5, 1, 2, 4, 6, 12, 24, 32, and 48 h. The detailed protocol and results were reported in Ref. 8.

Pharmacokinetic Analysis  The maximum plasma concentration (Cmax) and the time required to reach the Cmax (tmax) were read off from the individual plasma concentration–time curves of Y-24180. The area under the plasma concentration–time curves (AUC) was calculated using a linear trapezoidal method, and the mean residence time (MRT) was computed by moment analysis.9

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

RESULTS AND DISCUSSION

Pharmacokinetic Studies of Y-24180  The plasma concentration–time curves of Y-24180 after intravenous administration to the intact dogs and the GI physiology regulated-dogs declined similarly (Fig. 1). The combined-treatment of intramuscular pentagastrin and intravenous atropine sulfate was confirmed to have little influence on the pharmacokinetics of intravenous Y-24180 in dogs.

Dissolution Studies Y-24180 slightly released from 0.5% methylcellulose suspension into either the pH 1.2 or 6.8 fluids at 100 rpm (Fig. 2A). Less than 20% of the labeled amounts of Y-24180 dissolved within 1 h in 900 ml of the test solutions, probably due to the attainment of saturation. These characteristics suggested that the absorption of Y-24180 from the GI tract was dependent on bile flow. Next, dissolution studies of the Y-24180 preparations were carried out in pH 6.8 fluids containing a surfactant, polysorbate 80 (0.3%, w/v) (Fig. 2B). Y-24180 released rapidly at 100 rpm from both the fine granules and the tablets. The dissolved amounts at 0.5 and 1 h of the tablets at 50 rpm were almost similar to those of the fine granules (data not shown). The dissolution rate (%) from the two preparations decreased only slightly under a slow rotation speed. But the dissolved amounts of the tablets at 20 min decreased to 60% of the fine

---

**Fig. 1. Plasma Concentration–Time Curves of Y-24180 after Intravenous Administration to Beagle Dogs at a Dose of 0.5 mg/kg**

○ intact beagle dogs; ● GI physiology regulated-dogs. Each point represents the mean ± S.D. of 6 dogs.
Fig. 2. Dissolution Profiles of Y-24180 from (A) 0.5% Methylcellulose Suspension and (B) Preparations Using the Paddle Method at Various pH at 100 rpm

(A) ○, pH 1.2; ●, pH 6.8. (B) ○, fine granules; ●, tablets in pH 6.8 fluids containing polysorbate 80 (0.3%, w/v). Each point represents the mean in triplicate.

Fig. 3. Plasma Concentration–Time Curves of Y-24180 after Oral Administration to (A) Intact Beagle Dogs, (B) GI Physiology Regulated-Dogs, and (C) Humans.

○, fine granules; ●, tablets. Each point represents the mean ± S.D. of 6 dogs.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Cmax (ng/ml)</th>
<th>tmax (h)</th>
<th>AUC0–24h (ng·h/ml)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine granules</td>
<td>20.8 ± 9.4</td>
<td>0.6 ± 0.2</td>
<td>57.7 ± 18.8</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>Tablets</td>
<td>14.1 ± 8.6</td>
<td>1.3 ± 0.5</td>
<td>58.7 ± 24.5</td>
<td>5.1 ± 1.5</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± S.D. of 6 dogs. a) Statistically significant (p < 0.01).

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Cmax (ng/ml)</th>
<th>tmax (h)</th>
<th>AUC0–24h (ng·h/ml)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine granules</td>
<td>15.4 ± 7.4</td>
<td>0.8 ± 0.3</td>
<td>52.1 ± 17.9</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>Tablets</td>
<td>11.1 ± 4.3</td>
<td>2.5 ± 1.4</td>
<td>62.4 ± 20.2</td>
<td>6.6 ± 3.5</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± S.D. of 6 dogs. a) Statistically significant (p < 0.05).

granules. The disintegration and dispersion of the tablets seem to be affected by GI-motility.

Bioavailability Studies Both the Y-24180 tablets and the fine granules showed similar average plasma concentration–time curves in the intact dogs (Fig. 3A). Except for the tmax, there were no significant differences in the pharmacokinetic parameters between the two preparations (Table I). In humans, Y-24180 was absorbed faster from the fine granules than from the tablets (Fig. 3C), and the lag in tmax between the two preparations was approximately 2.7 h.8) The spread of this lag in humans was four times longer than in the intact dogs. For the two preparations, the GI physiology regulated-dogs shared absorption features similar to those observed in humans (Fig. 3B), demonstrating an advantage over the intact dogs. A strong destructive action in the GI tract of beagle dogs has been reported for griseofulvin tablets and cyclandelate capsules, where the bioavailability of preparations with a lower dissolution rate were higher in beagle dogs than in humans.12,10)

Comparison of Intact Dogs and GI Physiology Regulated-Dogs with Humans In order to estimate the usefulness of beagle dogs,1c,11d stomach-emptying controlled rabbits,11b or minipigs11a as animal models for bioavailability studies, a comparison between the relative values of mean pharmacokinetic parameters among test preparations in the animals with those in humans was reported. Relative values of the mean Cmax, tmax and AUC of the tablets in comparison to the fine granules were calculated in both the intact and GI physiology regulated-dogs (Fig. 4). The relative pharmacokinetic values for humans were also reckoned on the basis of the obtained data,9) and compared with the values found for the two classes of dogs (Fig. 4). The difference of tmax between the tablets and the fine granules in humans was large. The relative values for the
regulated-dogs approximated those in humans, suggesting the utility of regulated-dogs in predicting the bioavailability of the Y-24180 preparations in humans.

In the previous report, we demonstrated the superiority of GI physiology regulated-dogs over the intact dogs in a bioactivity evaluation of commercial sustained-release preparations of diclofenac sodium.\(^3\) The utility of the regulated-dogs was here and there established in terms of comparability with humans. We intend to continuously evaluate the usefulness of the regulated-dogs by employing other drugs and preparations with various absorption characteristics.

REFERENCES


