Bioavailability Study of Commercial Sustained-Release Preparations of Diclofenac Sodium in Gastrointestinal Physiology Regulated-Dogs

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The gastrointestinal (GI) physiology of beagle dogs was regulated with a combined-treatment of intramuscular pentagastrin (10 μg/kg × 2) and intravenous atropine sulfate (0.02 mg/kg × 1). Here, the gastric acidity, the gastric emptying time and the small intestinal transit time in the regulated-dogs were respectively around pH 2, 0.7 h and 4 h, approximating those in healthy humans. The superiority of the regulated-dogs over the intact dogs was confirmed in comparative bioavailability studies by using two classes of commercial preparations. Both the conventional tablet and the sustained-release capsule of diclofenac sodium exhibited simple and similar average plasma concentration–time curves of free diclofenac in the intact dogs, while the latter preparation is reported to reveal a bimodal plasma curve of the drug in healthy humans. The regulated-dogs, however, permitted a bimodal average plasma pattern of the drug for the capsules due to an approximation of the GI physiology between humans and these classes of the dogs. The combined-treatment of beagle dogs with pentagastrin and atropine sulfate seems to supply a useful animal model in predicting the absorption characteristics of the sustained-release preparations and poor water-soluble drugs.

Keywords diclofenac sodium; sustained-release preparation; bioavailability; animal model; beagle dog; gastrointestinal transit; gastric acidity

Introduction

Beagle dogs are widely used to assess the bioavailability of drugs. 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 bioavailability on slightly water-soluble drugs or sustained-release preparations.[1] The species differences are believed to depend on a faster transit of drugs through the gastrointestinal (GI) tract,[1b,e] more vigorous GI-motility[1d,e] and a higher gastric pH in beagle dogs than in humans.[2] We have already demonstrated that small intestinal transit time (SITT) is shorter in beagle dogs than in humans.[3] Intravenous atropine is well known for prolonging the SITT in humans.[4] We have utilized this agent in retarding the GI transit in beagle dogs.[3,5] Omeprazole is a potent proton-pump inhibitor,[6] and pentagastrin is a gastrosecretory hormone. Nakata *et al.*[7] used the latter compound in accelerating the secretion of gastric acid of the dogs in a bioavailability study. We also experimented with intravenous omeprazole and intramuscular pentagastrin to regulate the gastric acidity of the dogs.[8] These experiences provided us with developing a novel class of model species in which the drug bioavailabilities are comparable with those in healthy humans.

The present report describes a simultaneous regulation of the gastric emptying time (GET), the SITT and the gastric acidity in beagle dogs by a combined-treatment of intravenous atropine and intramuscular pentagastrin. Two kinds of commercially available diclofenac sodium preparations, a conventional tablet and a sustained-release capsule, were employed for the evaluation of usefulness of the dogs with regulated GI physiology. Furuya *et al.*[9] have recently announced the prolonging of SITT in beagle dogs by oral loperamide hydrochloride. In this report, we tentatively discussed their method in comparison with ours.

Experimental

Materials Sustained-release preparations containing 37.5 mg of diclofenac sodium (Voltaren® SR-capsule) and conventional preparations containing 25 mg of the drug (Voltaren® tab.) were purchased from Japan Ciba-Geigy Pharmaceuticals, Co. (Hyogo, Japan). Pentagastrin, atropine sulfate and loperamide hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO., U.S.A.). All other reagents used were of analytical grade available from commercial suppliers.

Dissolution Studies The paddle method of the Japanese Pharmacopoeia XI (JP XI) was employed in the dissolution test of diclofenac sodium preparations under the following conditions: the dissolution medium, 900 ml of the first fluid for JP XI disintegration test (pH 1.2), 0.05 M citric acid buffer (pH 4.0) and the second fluid for JP XI disintegration test (pH 6.8); temperature, 37.0±0.5 °C; rotation speed, 100 rpm for all of the test medium and 50 rpm for the 2nd fluid. Release of diclofenac into the test medium was spectrophotometrically monitored by using a Shimadzu UV-240 spectrophotometer (Shimadzu Co., Kyoto, Japan) at 275 nm. Each experiment was carried out in triplicate to calculate an average dissolution rate (%).

Measurement of the Gastric pH and the GI Transit Time The gastric pH was measured with a KR-500 pH/pCO_2 monitor (Kuraray Co., Japan) connected with ion-selective field transistor pH sensor as reported previously by us.[8]

The GET and the SITT were measured by a double-marker method[3] that employed acetaminophen (AAP) and salicylazosulfapyridine (SASP) as marker compounds. A hard gelatin capsule containing 200 mg AAP and 250 mg SASP was given to the dogs, and the mean absorption time of AAP and the time for the first appearance of sulfapyridine (SP: a bacterial metabolite of SASP in colon) in plasma were used for the indexes for GET and SITT, respectively. The detailed procedure is described in our previous paper.[3]

Effect of Atropine Sulfate on the GI Transit Time in Beagle Dogs Six healthy male beagle dogs weighing between 8 and 15 kg were used. Each dog was fasted for 24 h prior to an until the finish of the experiment, but was allowed free access to water. Each dog received an intravenous administration of atropine sulfate at a dose of 0.01, 0.02 and 0.1 mg/kg and the GI transit time was monitored by the double-marker method.

Effect of Pentagastrin on the Gastric pH and the GI Transit Time in Beagle Dogs with and without Combined-Treatment of Atropin Sulfate Six healthy male beagle dogs weighing between 10 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. The dogs were divided into three groups of two dogs in accordance with a randamized crossed-over design as follows: 1) a group received two treatments with intramuscular pentagastrin (10 μg/kg) with a 45 min interval; 2) a group received a combined-treatment with intramuscular pentagastrin (10 μg/kg) and intravenous atropine sulfate (0.02 mg/kg) and an additional treatment with intramuscular pentagastrin (10 μg/kg) with a 45 min interval; 3) a group received no treatment with the agents. Each experiment was carried out with 1-week intervals. A set of the crossed-over experiments was performed to monitor the effects of the agents on the gastric pH, and the other set on the GI transit time. The gastric pH was measured prior to the treatment and at

0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h after the start of the experiment. The GI transit time was assessed by the double-marker method. In each treatment, at the time corresponding to 15 min after the first treatment with pentagastrin, a hard gelatin capsule containing 200 mg AAP and 250 mg SASP was given to each dog and blood samples were collected at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 h after dosing of the capsule. The plasma concentrations of AAP and SP were determined simultaneously by high performance liquid chromatography (HPLC) under the condition described previously.[3]

Assay for the Plasma Concentration of Diclofenac The plasma concentration of diclofenac was determined by HPLC. To 250 μl of plasma were added 100 μl of 0.1 M citric acid buffer (pH 4.0) and 1 ml toluene containing 1 μg of diazepam as the internal standard. After 10 min of shaking and centrifugation at 1300 *g* for 5 min, the supernatant (0.8 ml) was transferred to a glass tube and evaporated to dryness under reduced pressure. The residue was dissolved in 100 μl of the mobile phase for HPLC and 20 μl of this solution was injected onto the HPLC column. The HPLC system used consisted of a Shimadzu LC-6A, SPD-6A (wave length 282 nm) and C-R3A integrator recorder. Separations were performed on a Shim pack CLC-ODS (15 cm × 4.6 mm, 5 μm particle size, Shimadzu) with a mobile phase of 0.1 M ammonium acetate (pH 4.5)–acetonitrile (2 : 3, v/v) and a flow rate of 1.0 ml/min. The lower limit of the assay of diclofenac in plasma was 0.04 μg/ml.

Bioavailability Studies Six healthy male beagle dogs weighing between 8 and 15 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 0.5, 1, 2, 3, 4, 6, 8 and 12 h after the oral dosing of diclofenac sodium preparations, and were centrifuged (1300 *g* for 15 min) for the separation of plasma. The plasma samples were kept frozen until the HPLC assay of diclofenac. The dogs were divided into two groups of three dogs in accordance with a randamized crossed-over design as follows: 1) a group received two conventional tablets of diclofenac sodium with 30 ml of water; 2) a group received one sustained-release capsule. Each experiment was carried out with 1-week intervals. The crossed-over experiments were performed on three different cases by using intact beagle dogs, by using the GI physiology regulated-dogs and by using the beagle dogs treated with loperamide hydrochloride. In the second case, each dog was treated with intramuscular pentagastrin (10 μg/kg) and intravenous atropine sulfate (0.02 mg/kg) at 15 min prior to the dosing of the diclofenac sodium preparations, and additionally treated with intramuscular pentagastrin (10 μg/kg) at 30 min after the dosing of the preparations. In the last case, each dog was treated with oral loperamide hydrochloride (0.12 mg/kg) in accordance with Furuya's method.[9]

Pharmacokinetic Analysis The maximum plasma concentration (C_{max}) and the time to reach the C_{max} (t_{max}) were read off from individual plasma concentration–time curves of diclofenac. The area under the plasma concentration–time curves (*AUC*) was calculated by the linear trapezoidal method and the mean residence time (*MRT*) was computed by moment analysis.[10]

Statistical Analysis The GET and the SITT were subjected to the analysis of variance in accordance with the randamized block design, and subjected to Tukey's test. Differences in each bioavailability parameter or transit time were statistically evaluated by the paired *t*-test.

Results and Discussion

Atropine sulfate prolonged the GET by increasing the dose, but such an effect of the agent reached a plateau at around 5 h for the SITT at a dose of 0.02 mg/kg (Fig. 1). The dose of intravenous atropine sulfate was therefore established as 0.02 mg/kg.

Notable intra- and inter-individual variations of the gastric pH were observed in the intact dogs, while the mean pH value was fluctuated around 5.5 (Fig. 2). Pentagastrin lowered the gastric pH to settle down around 2 within 45 min after the first treatment with the agent and the acidification was slightly delayed by the combination with atropine sulfate. Pentagastrin hardly influenced the SITT, but markedly prolonged the GET (Dozois *et al.*[11] and Cooke *et al.*[12] already reported such a phenomenon.) (Table I). On the other hand, combined pentagastrin and atropine sulfate prolonged both the GET and the SITT.

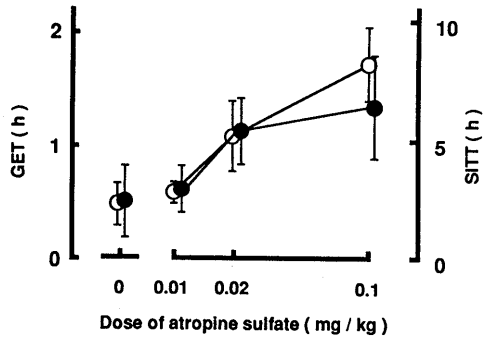

Fig. 1. Effect of Atropine Sulfate on the Gastric Emptying Time (GET) and the Small Intestinal Transit Time (SITT) in Beagle Dogs

○, GET; ●, SITT. Each point represents the mean ± S.D. of 6 dogs.

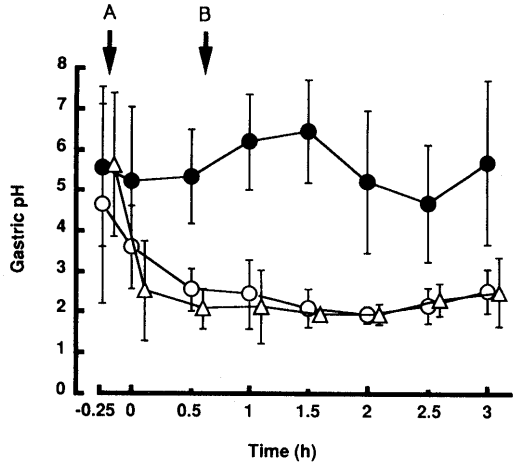

Fig. 2. Gastric pH–Time Profiles in Beagle Dogs

A, pentagastrin 10 μg/kg i.m. with or without combined-treatment of atropine sulfate 0.02 mg/kg i.v.; B, pentagastrin 10 μg/kg i.m.; ●, intact; △, pentagastrin (10 μg/kg × 2 i.m.); ○, pentagastrin (10 μg/kg × 2 i.m.) with combined-treatment of atropine sulfate (0.02 mg/kg i.v.). Each point represents the mean ± S.D. of 6 dogs.

TABLE I. Gastrointestinal Transit Time in Beagle Dogs Assessed Using Acetaminophen (AAP) and Salicylazosulfapyridine (SASP) as Markers

	GET (h)	SITT (h)
Intact	0.22 ± 0.17	2.83 ± 0.93
Pentagastrin[a]	0.67 ± 0.26[c]	2.67 ± 0.88
Pentagastrin[a] + atropine[b]	0.62 ± 0.22[c]	4.08 ± 1.11[d]

Results are expressed with the mean ± S.D. of 6 dogs. *a*) 10 μg/kg × 2 i.m. with a 45 min interval, *b*) 0.02 mg/kg i.v. *c*) Statistically significant ($p < 0.01$) *v.s.* intact, *d*) $p < 0.05$ *v.s.* pentagastrin.

A combined-treatment of intramuscular pentagastrin and intravenous atropine sulfate effectively regulated the GI physiology of the dogs. Here, the gastric acidity, the GET and the SITT in the regulated-dogs were respectively around pH 2, 0.7 h and 4 h, and these values showed good approximations to those in humans reported by Dressman *et al.*,[13] Kaniwa *et al.*[14] and Staniforth *et al.*[15] In accordance with these results, our first aim was presumed to be achieved. We began therefore to evaluate the utility of our model animals by actual bioavailability studies using commercially available preparations of diclofenac sodium. Tsunoo *et al.*[16] have reported that oral Voltaren® SR-capsule (sustained-release capsule of diclofenac sodium)

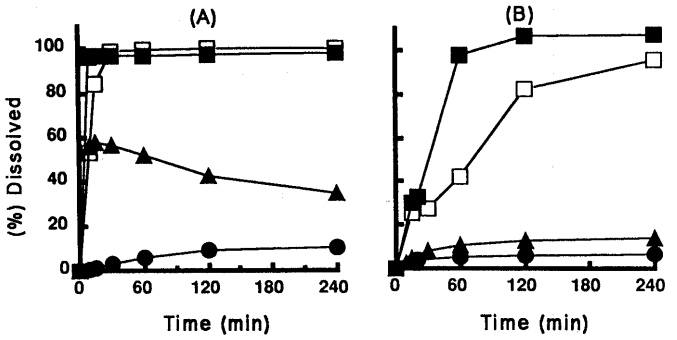

Fig. 3. Dissolution Profiles of Diclofenac from (A) Voltaren® Tab. and (B) Voltaren® SR-Capsule Using the Paddle Method at Various pH

(●, pH 1.2; ▲, pH 4.0; ■, pH 6.8 at 100 rpm; □, pH 6.8 at 50 rpm). Each point represents the mean in triplicate.

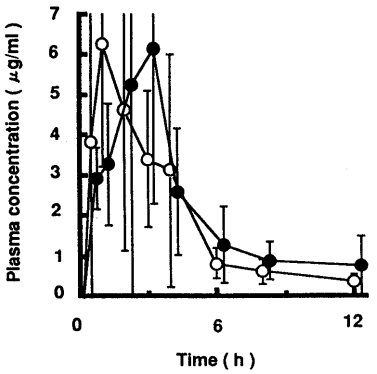

Fig. 4. Plasma Concentration–Time Curves of Diclofenac after Oral Administration to Intact Beagle Dogs

○, Voltaren® tab.; ●, Voltaren® SR-capsule. Results are expressed as a dose of 37.5 mg/dog. Each point represents the mean ± S.D. of 6 dogs.

TABLE II. Pharmacokinetic Parameters after Oral Administration of Diclofenac Preparations to Intact Beagle Dogs

	C_{max} (μg/ml)	t_{max} (h)	AUC_{0-12h} (μg·h/ml)	MRT (h)
Tablet	8.1 ± 3.4	1.6 ± 1.3	23.6 ± 7.7	3.2 ± 0.4
SR-capsule	8.3 ± 5.7	2.1 ± 1.1	25.9 ± 11.1	4.1 ± 1.1[a]

Data are estimated as a dose of 37.5 mg/dog. Results are expressed as the mean ± S.D. of 6 dogs. *a*) Statistically significant ($p < 0.05$).

exhibited a bimodal average drug profile in the plasma of healthy humans. Such a specific property of the preparations appeared to supply us with a tool for the above mentioned purpose.

The dissolution studies were carried out for the conventional tablets and the sustained-release capsules of diclofenac sodium (Fig. 3). From the tablet, diclofenac scarcely released into pH 1.2 fluid, but rapidly and independently of rotation-speed dissolved into the pH 6.8 fluid. On the other hand, about half of the drug content in a tablet rapidly released into the pH 4.0 fluid, but the dissolution rate (%) decreased with time (Fig. 3A). Such a pH dependent dissolution profile of the conventional tablets seems to reflect the physico-chemical property of diclofenac sodium itself. From the capsules, diclofenac hardly released into both the pH 1.2 and 4.0 fluids, but rapidly and dependently of rotation-speed dissolved into the pH 6.8 fluid (Fig. 3B). The capsules thus behaved more or less as

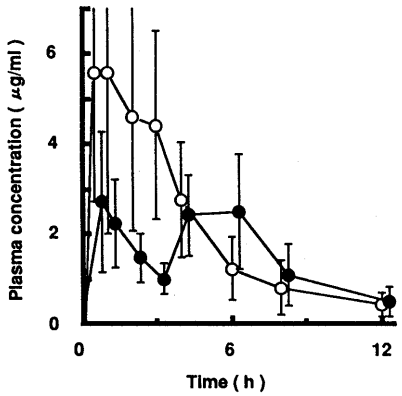

Fig. 5. Plasma Concentration–Time Curves of Diclofenac after Oral Administration to GI Physiology Regulated-Dogs

○, Voltaren® tab.; ●, Voltaren® SR-capsule. Results are expressed as a dose of 37.5 mg/dog. Each point represents the mean ± S.D. of 6 dogs.

TABLE III. Pharmacokinetic Parameters after Oral Administration of Diclofenac Preparations to GI Physiology Regulated-Dogs

	C_{max} (μg/ml)		t_{max} (h)		AUC_{0-12h} (μg·h/ml)	MRT (h)
	1st	2nd	1st	2nd		
Tablet	6.8	—	1.7	—	25.9	3.4
	3.0	—	1.5	—	12.5	0.5
SR-capsule	3.0	2.8	0.7	5.0	18.4	4.8[a]
	1.3	1.0	0.3	1.1	5.2	1.0

Data are estimated as a dose of 37.5 mg/dog. Results are expressed as the mean ± S.D. of 6 dogs. *a*) Statistically significant ($p < 0.05$).

enteric features, and exhibited a two-phase dissolution pattern in the pH 6.8 fluid at 50 rpm, suggesting a possibility of gut motility dependency on their absorption.

The conventional tablets and the sustained-release capsules both showed simple average plasma concentration–time curves of diclofenac in the intact dogs (Fig. 4). There were no significant differences in the pharmacokinetic parameters except for the MRT between the two preparations (Table II). These results for the capsules were in contrast to the bimodal drug profile in plasma reported on the healthy humans.[16] On the other hand, the GI physiology regulated-dogs demonstrated an advantage in terms of comparability with humans. The sustained-release capsules thus showed a bimodal profile of diclofenac absorption in the regulated-dogs with an almost complete similarity to that in humans, while the conventional tablets exhibited a unimodal pattern in the same animals (Fig. 5). From the viewpoint of gut motility in the regulated-dogs, such a unique absorption property of the capsules may be predictable from the two-phase dissolution profile in the pH 6.8 fluid at 50 rpm rather than 100 rpm of the rotation speed. Both the first and second peak plasma concentrations in the capsules were half of the C_{max} in the tablets. This result seemed to be caused from the regulation of gastric pH around 2, the prolongation of SITT and the distinction between first and slow release of the drug under a regulated GI motility. There was a significant difference in the MRT between the capsules and the tablets (Table III). In the GI physiology regulated-dogs, the AUC_{0-12h} for the capsules was estimated to be about 70% of that for the tablets (Table III), while the bioavailability of the tablets in the

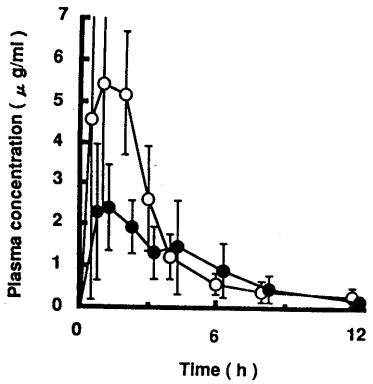

Fig. 6. Plasma Concentration–Time Curves of Diclofenac after Oral Administration to Beagle Dogs Treated with Loperamide Hydrochloride

○, Voltaren® tab.; ●, Voltaren® SR-capsule. Results are expressed as a dose of 37.5 mg/dog. Each point represents the mean ± S.D. of 6 dogs.

TABLE IV. Pharmacokinetic Parameters after Oral Administration of Diclofenac Preparations to Beagle Dogs Treated with Loperamide Hydrochloride

	C_{max} (μg/ml)		t_{max} (h)		AUC_{0-12h} (μg·h/ml)	MRT (h)
	1st	2nd[a)]	1st	2nd[a)]		
Tablet	7.8	—	1.4	—	18.9	2.9
	2.4	—	0.7	—	2.3	0.9
SR-capsule	2.9	1.9	1.2	4.5	12.0[b)]	4.5[b)]
	1.1	1.3	0.7	1.0	4.3	1.3

Data are estimated as a dose of 37.5 mg/dog. Results are expressed as the mean ± S.D. of 6 dogs. *a*) Data are calculated for 4 among 6 dogs since no bimodal plasma pattern of drug absorption was observed in the remaining. *b*) Statistically significant ($p<0.05$).

regulated-dogs was approximately equal to that of the tablets and the capsules in the intact dogs (Tables II and III). It was reported that the bioavailability for diclofenac sodium preparations were linearly related to doses over the range of 25 to 50 mg of the drug after oral administration to dogs, suggesting no dose-dependent metabolic processes in this experiment.[16] These absorption characteristics in the regulated-dogs would be destined by slow and incomplete drug release from the capsules in the pH 6.8 medium under a slow rotation-speed (50 rpm: Fig. 3) or in the intestinal juice under a depressed GI motility.

In the next stage, Furuya's method[9] was compared with our procedure by actual bioavailability studies. The sustained-release capsules also exhibited a bimodal drug absorption pattern of diclofenac absorption in the dogs treated with oral loperamide hydrochloride (Fig. 6). In this case, unimodal plasma patterns were observed, however, in 2 among 6 dogs to result in a marked lowering of the second peak height of the average bimodal curve. The AUC_{0-12h} for the capsules was estimated to be about 60% of that for the conventional tablets (Table IV). Additionally, the AUC_{0-12h} value for the tablets determined by the loperamide method was approximately equivalent to that for the capsules by the combined pentagastrin and atropine method (Tables III and IV). The use of loperamide hydrochloride thus showed a propensity to result in an underestimation of the drug absorption for both the conventional and sustained-release diclofenac sodium preparations. The decrease of AUC by the loperamide method may result in the inhibition of absorption of diclofenac by loperamide in the GI track since loperamide hydrochloride is orally pre-administrated. Our observations in the loperamide method were similar to those lectured by Furuya *et al.*[9] on the bioavailability of sustained-release acetaminophen granules. The combined use of pentagastrin and atropine sulfate was tentatively concluded to be superior to loperamide hydrochloride.

Utilization of the GI physiology regulated-dogs was thus confirmed in terms of comparability with healthy humans. But there may be some limitations, since the bioavailability of the sustained-release preparations was underestimated by use of these animal models. Utilization of the gastric acidity regulation was not yet ascertained in the bioavailability study of diclofenac sodium preparations. We intend to evaluate the usefulness of the GI physiology regulated-dogs by employing other drugs and preparations of various absorption characteristics.

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