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Evaluation of gastrointestinal transit controlled-beagle dog as a suitable animal model for bioavailability testing of sustained-release acetaminophen dosage form

Kenji Yamada [a,*], Atsushi Furuya [a], Masayuki Akimoto [a], Tohru Maki [a], Toshio Suwa [a], Hiroyasu Ogata [b]

[a] *Research Center, Taisho Pharmaceutical Co., Ltd, 1-403 Yoshino-cho, Ohmiya, Saitama 330, Japan*
[b] *Department of Pharmaceutics, Meiji College of Pharmacy, 1-22-1 Yato-cho, Tanashi, Tokyo 188, Japan*

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Abstract

In order to develop a suitable beagle dog model for evaluating the bioavailability of a sustained-release dosage form, we tried to control the gastrointestinal (GI) transit time in beagle dogs so as to make it similar to that in humans by means of pharmacological modification of GI motility using loperamide hydrochloride, an antidiarrheal drug. Acetaminophen showed greater absorbability from jejunum and ileum than that from colon in rats. Relative bioavailability of acetaminophen from a sustained-release dosage form after oral administration (31%) in beagle dogs, compared with a solution as reference dosage form, was obviously lower than that (90%) in humans, probably because of the shorter GI transit time. The bioavailability was significantly increased (2-fold) in beagle dogs treated with loperamide hydrochloride (0.12 mg/kg). Treatment with loperamide did not exert any significant effects on the clearance of acetaminophen, but did increase the GI transit time from 3.5 to 5.0 h. In this study, it was suggested that GI transit controlled-beagle dog would be effective enough to avoid the underestimation of the bioavailability of a sustained-release dosage form containing a drug with site-specific GI absorption, such as acetaminophen.

Keywords: Acetaminophen; Sustained-release dosage form; Bioavailability; Human; Beagle dog; Animal model; Gastrointestinal transit

1. Introduction

Recently, numerous sustained-release dosage forms have been developed in order to reduce dosing frequency, maintain stable therapeutic effects and decrease adverse effects. Beagle dogs are generally used as an animal model for evaluating the bioavailability of drugs in place of humans. However, the bioavailabilities in beagle dogs do not always show a high correlation with those in humans (Barr, 1972). It has been demonstrated that some discrepancies in drug bioavailability after oral administration between humans and beagle dogs are mainly ascribed to differences in GI physiology, such as gastric acidity

* Corresponding author.

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(Aoyagi et al., 1985; Ogata et al., 1985, 1986), and/or agitating ability in the gastrointestinal tract (Aoyagi et al., 1982; Ogata et al., 1984) and/or GI transit time (Aoyagi et al., 1982; Ogata et al., 1982) between the two species. In particular, GI transit is thought to be the most important factor determining the bioavailability of a sustained-release dosage form, since a drug is designed to be released from the dosage form during the period of GI transit. Dogs often showed lower bioavailability in comparison with humans, such as in the case of aminorex fumarate (Cressman and Sumner, 1971), valproic acid (Bialer et al., 1984a,b, 1985, 1986), molsidomine (Yashiki et al., 1985; Morimoto et al., 1986) and ampicillin (Uchida et al., 1986), which seemed to be ascribed to a shorter GI transit time in dogs compared with humans. On the other hand, a comparatively good correlation of bioavailability between the two species was recognized with chlorphenesin carbamate (Akimoto et al., 1993), phenylpropanolamine hydrochloride (Dressman and Yamada, 1991) and theophylline (Hussein et al., 1987), which are sufficiently absorbed from an entire region of the GI tract, including the colon. Acetaminophen showed a poor correlation between the two species probably because of the shorter GI transit time in beagle dogs (Dressman and Yamada, 1991). The purpose of this study was to develop a suitable beagle dog model for evaluating the bioavailability of a sustained-release dosage form of acetaminophen by means of prolonging the GI transit time to a duration similar to that in humans.

2. Materials and methods

2.1. Materials

Acetaminophen (APAP) was obtained from Iwaki Pharmaceutical Co., Ltd (Japan). Loperamide hydrochloride, salicylazosulfapyridine (SASP), sulfapyridine (SP), β-glucuronidase and sulfatase were purchased from Sigma Chemical Co. (USA). All other reagents used were of analytical grade.

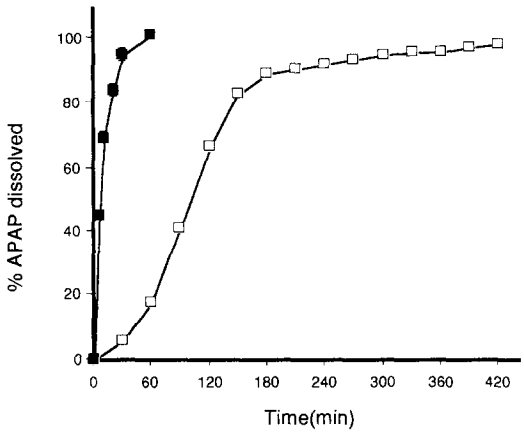

Fig. 1. Dissolution profiles of APAP from the sustained-release granules and immediate-release granules using the paddle method in JP XI 2nd fluid (pH 6.8). (□) Sustained-release granules; (■) immediate-release granules. Each point represents the mean ± S.E.

2.2. Dosage forms

The immediate-release spherical granules containing 90% by weight of APAP were prepared by the wet granulation method. To obtain sustained-release granules, the granules were placed in the prewarmed chamber of a centrifugal granulator. A coating solution of Eudragit RS® (Roehm Pharma, Germany) was sprayed onto the granules and coated to a 15% weight increase. The granules were dried at 70°C for 1 h using a fluidized bed drying machine. The dissolution rates of APAP from these dosage forms were determined at 37 ± 1°C using the paddle method (JP XI) in pH 6.8 phosphate buffer. The dissolution profile of sustained-release granules was apparently slower than that from immediate-release granules (Fig. 1).

2.3. Bioavailability studies in humans

20 male adult volunteers, aged between 20 and 24 years and weighing 48.0–79.5 kg, participated in the present study. The volunteers were confirmed in advance to be normal regarding blood pressure, pulse rate, electrocardiogram, profiles of hematology and serum chemistry and urinalysis. Written informed consent was obtained from

each volunteer. Subjects were randomly divided into two groups of 10 subjects. Subjects in both groups were orally administered the solution (30 ml) containing 300 mg of APAP or the sustained-release granules containing 450 mg of APAP with 150 ml of water. Solution instead of immediate-release granules was used in consideration of coherence with dog study I carried out before the human study. The subjects were fasted from at least 12 h before and until 4 h after taking the drug. Blood samples were collected from the subjects at the designated time intervals after administration, and plasma samples were stored in a freezer (−20°C) until assay. Plasma concentrations of APAP were analysed using high-performance liquid chromatography (HPLC).

2.4. Bioavailability studies in beagle dogs

13 male beagle dogs, weighing 9.4–13.8 kg, were used. The beagle dogs were randomly divided into three groups for the following four studies. All studies except study IV were carried out after overnight fasting according to a cross-over design with a period of at least 1 week. Blood samples were taken at the designated time intervals after the administration of the drug. Plasma samples obtained were stored in a freezer (−20°C) until assay.

Study I: The same solution and sustained-release granules as used in the human study were orally administered with 20 ml of water to four beagle dogs at a dose of 37.5 mg/kg of APAP. Plasma concentrations of APAP were determined using HPLC.

Study II: 30 min after the oral administration of loperamide hydrochloride as an aqueous suspension at different doses of 0, 0.03, 0.12 or 0.3 mg/kg (loperamide treatment), 25 mg/kg of SASP powder was orally administered with 20 ml of water to four beagle dogs. The oral-caecal transit time was determined as the time taken for the first detection of SP in plasma after the oral administration of SASP (SASP method; Kennedy et al., 1979). Plasma concentrations of SP were analyzed using HPLC.

Study III: Sustained-release granules of 37.5 mg/kg as APAP were administered with 20 ml of water to five beagle dogs with or without loperamide treatment (0.12 mg/kg oral dosing 30 min before administration of the granules). Plasma concentrations of APAP and total APAP (unchanged APAP plus its glucuronide and sulfate conjugate forms) were evaluated using HPLC.

Study IV: A solution of 37.5 mg/kg as APAP was orally administered to the same beagle dogs as used in study I after loperamide treatment (0.12 mg/kg oral dosing 30 min before administration of the solution). Plasma concentrations of APAP were analyzed using HPLC.

2.5. GI absorption of APAP in rats

The regional absorbability of APAP from the GI tract was investigated in three male Wistar rats, weighing 200–250 g. Rats fasted overnight were anesthetized with urethane (i.p., 1.5 g/kg). After abdominal incision, both ends of a chosen GI part, viz., stomach, jejunum, ileum or colon (each 5 cm in length), were ligated to make a loop. The solution (0.25 ml) containing 2.5 mg of APAP was introduced into the loop. Blood samples were collected from the jugular vein at the designated time intervals to determine the plasma concentrations of APAP.

2.6. Assay of APAP

The plasma concentration of APAP was determined by HPLC according to the method reported by Black et al. (1978). To 0.5 ml of plasma were added 0.5 ml of β-hydroxytheophylline (40 μg/ml) aqueous solution as an internal standard, 5.0 ml of ethyl acetate and about 0.5 g of anhydrous sodium sulfate. The mixture was vigorously shaken for 10 min and centrifuged for 10 min at 3000 rpm (2000 × *g*). 4 ml of the organic layer was evaporated to dryness under reduced pressure at 40°C. The residue was dissolved with 0.5 ml of the mobile phase. After centrifugation for 5 min at 12000 rpm (15000 × *g*), 20 μl of the supernatant was analyzed by HPLC. The HPLC system consisted of a TSK Gel LS-410 (ODS) column (5 μm, 4 mm × 150 mm; Toyo Soda), pump (Shimadzu LC-6A), variable UV detector

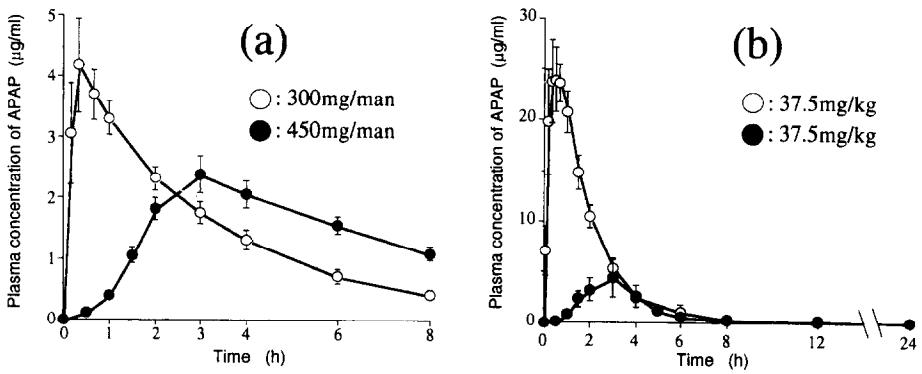

Fig. 2. Plasma concentrations of APAP after oral administration of solution (○) and sustained-release granules (●) in humans (a) and dogs (b). Each point represents the mean ± S.E.

(Shimadzu SPD-6AV) and integrator recorder (Shimadzu C-R4AX). The mobile phase, a mixture of 10 mM phosphate buffer (pH 4.0) and tetrahydrofuran (100:1, v/v), was pumped at 1.0 ml/min and monitored at 245 nm. The lower limit of the assay was 0.05 μg/ml of APAP in plasma. To determine the plasma concentration of total APAP, 0.1 ml of enzyme solution containing β-glucuronidase (10000 U/ml) and sulfatase (10 U/ml) was added to 0.5 ml of plasma. After incubation at 37°C for 2 h, APAP in the plasma was analyzed under the same condition as described above.

2.7. Assay of SP in plasma

The plasma concentration of SP was determined by HPLC according to the method of Mizuta et al. (1989). 1 ml of 0.5 M phosphate (Na_2HPO_4-KH_2PO_4) buffer (pH 7.4) and 2 ml of ethyl acetate were added to 0.1 ml of plasma. The mixture was vigorously shaken for 10 min and centrifuged at 3000 rpm for 10 min. 1 ml of organic layer was evaporated to dryness under reduced pressure at 40°C. The residue was dissolved with 0.2 ml of mobile phase containing 0.4 μg of 8-chlorotheophylline as an internal standard, and 20 μl of the solution was analyzed by

Table 1
Pharmacokinetic parameters of APAP following oral administration in humans and dogs

Species	Dosage form	Dose	Number of animals	C_{max} (μg/ml)	T_{max} (h)	AUC (μg h ml^{-1})	Relative BA (%)
Human	solution	300 mg	10	5.43 ± 0.48 [a,d]	0.7 ± 0.2 [e]	14.54 ± 1.41 [N.S.]	(100) [a,b]
	sustained-release granules	450 mg	10	2.41 ± 0.30 [a,d]	3.3 ± 0.3 [e]	19.64 ± 1.59 [N.S.]	90.0
Dog	solution	37.5 mg/kg	4	26.60 ± 3.40 [e]	0.5 ± 0.2 [d]	54.22 ± 2.21 [c,e]	(100)
	sustained-release granules	37.5 mg/kg	4	5.42 ± 2.23 [e]	2.6 ± 0.4 [d]	16.89 ± 5.16 [c,e]	31.1

[a] Statistical analysis was performed for C_{max} and AUC normalized for dosing; [b] $0 \rightarrow \infty$; [c] $0 \rightarrow 24$ h.
[d,e] Difference in the pharmacokinetic parameters of APAP after administration of solution and sustained-release granules is significant ([d] $p < 0.05$, [e] $p < 0.01$). N.S., difference is not significant ($p > 0.05$). Each data represents the mean ± S.E.

HPLC. The HPLC system consisted of the same equipment as used in the assay of APAP, except that the column was Nucleosil C_{18} (ODS, 4 mm × 250 mm; M. Nagel). The mobile phase, a mixture of water, acetonitrile and acetic acid (89:10:1, v/v), was pumped at 1.5 ml/min and monitored at 270 nm. The lower limit of the assay was 0.1 μg/ml of SP in plasma.

2.8. Pharmacokinetic and statistical analyses

The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were observed values. The area under the plasma concentration-time curve from 0 to 24 h (AUC_{0-24}) was calculated based on the trapezoidal rule. The percent absorbed-time profile of APAP was calculated according to a previously reported method (Wagner and Nelson, 1964). The pharmacokinetic parameters were statistically analyzed by analysis of variance (ANOVA), and Dunnet's multiple range test was carried out where necessary.

3. Results

3.1. Comparative bioavailability of APAP between humans and beagle dogs

The mean plasma concentrations of APAP after oral administration of solution and sustained-release granules to humans and beagle dogs are shown in Fig. 2. Table 1 summarizes the mean pharmacokinetic parameters. In humans, the expected sustained-release characteristics were observed, as indicated by the considerably lower C_{max} and longer Tmax compared with those of the solution. Its relative bioavailability to the solution, calculated from the ratio of the dose-normalized AUC, was sufficient, i.e., 90%. On the other hand, as shown in Fig. 2b, the plasma concentration of sustained-release granules in beagle dogs was apparently lower than that of the solution, and its relative bioavaliability was significantly lower in comparison with that in humans, being only 31%.

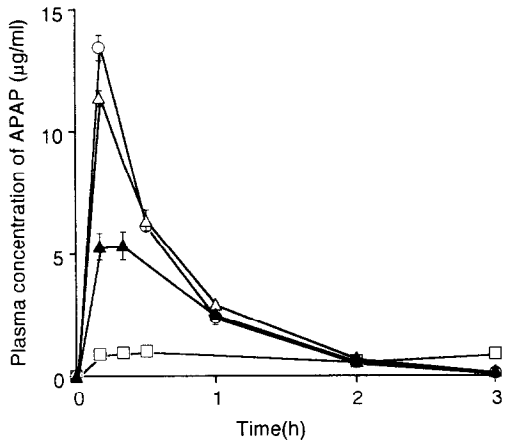

Fig. 3. Absorption of APAP from four parts of the gastrointestinal tract in rats (dose: 10 mg/kg). (□) Stomach; (○) jejunum; (△) ileum; (▲) colon. Each point represents the mean ± S.E.

3.2. GI absorption of APAP

The regional absorbability of APAP was evaluated in rats. Fig. 3 displays the plasma concentrations of APAP obtained after introduction into the stomach, jejunum, ileum and colon. Although the absorbabilities of APAP from the jejunum and ileum were rapid and sufficient, that from the colon was relatively low and was very poor from the stomach.

3.3. Effects of loperamide on GI transit

Fig. 4 shows the relationship between dose of loperamide hydrochloride and the resulting oral-caecal transit time in beagle dogs. The oral-caecal transit time was 3.5 h without drug treatment, and was dose-dependently delayed to 5.0 h at more than 0.12 mg/kg of loperamide hydrochloride.

3.4. Effect of loperamide on bioavailability of APAP from sustained-release granules

Fig. 5 demonstrates the mean plasma concentrations of APAP and total APAP after oral administration of sustained-release granules of 37.5 mg/kg as APAP to beagle dogs with and without treatment of loperamide (0.12 mg/kg).

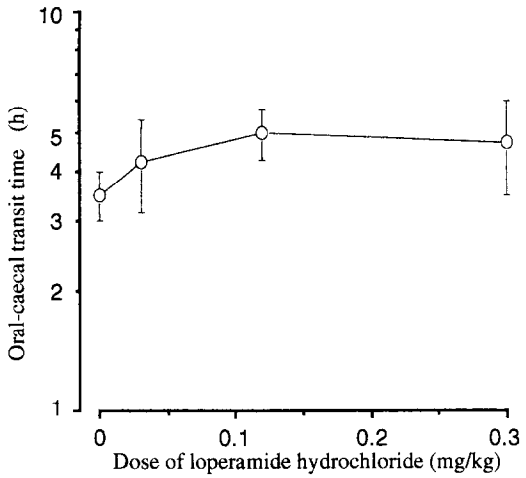

Fig. 4. Oral-caecal transit time in dogs. Each point represents the mean ± S.E.

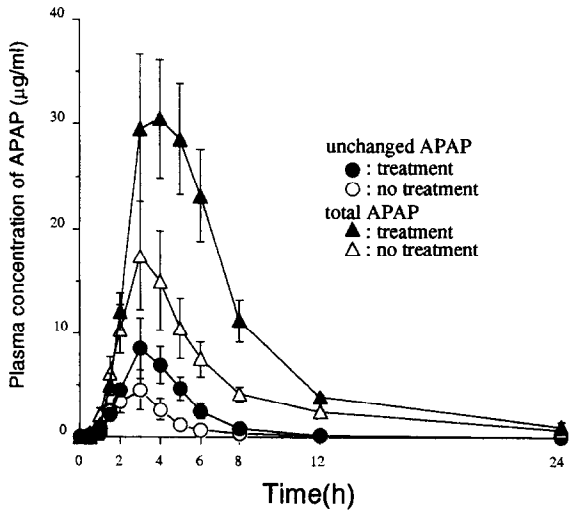

Fig. 5. Plasma concentrations of APAP and total APAP after oral administration (dose: APAP, 37.5 mg/kg) of sustained-release formulation with and without treatment with loperamide hydrochloride (0.12 mg/kg, p.o.). Each point represents the mean ± S.E. of five dogs.

The mean pharmacokinetic parameters for APAP are listed in Table 2. When loperamide was administered, the mean plasma concentrations of both APAP and total APAP were obviously higher in comparison with those for no treatment. The AUC_{0-24} of APAP and total APAP with loperamide treatment were significantly increased, being about 2-fold as much as those without treatment.

3.5. Effect of loperamide on clearance of APAP

Fig. 6 shows the plasma concentrations of APAP after oral administration of APAP solution with and without loperamide treatment (0.12 mg/kg oral dosing). The plasma concentration-time curves were almost the same, regardless of the treatment. There were no significant differences in the pharmacokinetic parameters between loperamide treatment and no treatment ($p > 0.05$).

4. Discussion

Since the GI transit time in beagle dogs is shorter than that in humans (mean small intestinal transit time of 111 ± 17 min in dogs, and

Table 2
Pharmacokinetic parameters of APAP following oral administration (37.5 mg/kg) to dogs

	Treatment [a]	C_{max} (μg/ml)	T_{max} (h)	$AUC_{0-24\,h}$ (μg h ml^{-1})
APAP	no treatment	5.33 ± 1.73	2.4 ± 0.4	16.27 ± 4.05
		N.S.	N.S.	b
	treatment	9.85 ± 2.55	3.4 ± 0.5	32.78 ± 4.81
Total APAP	no treatment	17.88 ± 5.16	2.6 ± 0.2	101.16 ± 19.10
		b	b	b
	treatment	35.23 ± 6.31	4.2 ± 0.6	205.47 ± 20.90

[a] Treatment with loperamide hydrochloride (0.12 mg/kg).
[b] Difference in the pharmacokinetic parameters of APAP after administration of sustained-release granules is significant ([b] $p < 0.05$). N.S., difference is not significant ($p > 0.05$). Each data represents the mean ± S.E. of five dogs.

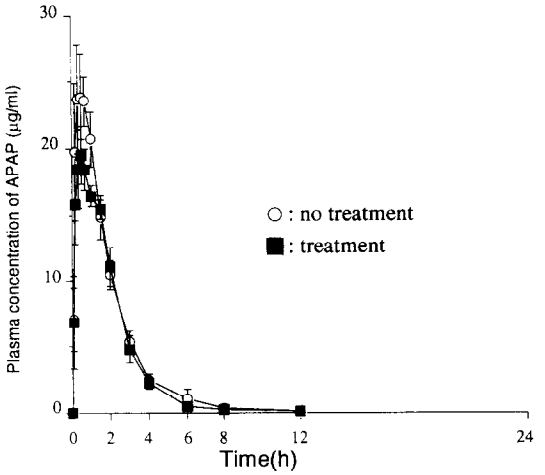

Fig. 6. Plasma concentrations of APAP after oral administration of solution (dose: 37.5 mg/kg) in dogs. (○) No treatment; (■) treatment with loperamide hydrochloride (0.12 mg/kg, p.o.). Each point represents the mean ± S.E. in four dogs.

234 ± 14 min in humans (Dressman, 1986)), a sustained-release dosage form orally administered may pass through the absorption site of the GI tract with incomplete drug release, and consequently with relative poor bioavailability in beagle dogs as compared with humans (Bialer et al., 1988). This phenomenon may be dependent on the absorption characteristics and site specificity of the drugs administered. Chlorphenesin carbamate is well absorbed from the entire intestine, including the colon, and showed similar relative bioavailability from a sustained-release dosage form compared with an immediate-release dosage form in both species, humans and beagle dogs (Akimoto et al., 1993). On the other hand, reduction of the bioavailability from a sustained-release dosage form in dogs has been reported with aminorex fumarate (Cressman and Sumner, 1971), valproic acid (Bialer et al., 1984a,b, 1985, 1986), molsidomine (Yashiki et al., 1985; Morimoto et al., 1986) and ampicillin (Uchida et al., 1986). In APAP studied in this report, the relative bioavailability from sustained-release granules was 31% in beagle dogs as compared with solution, which was obviously lower than in humans (90%). The regional absorbability estimated using the loop method in rats indicated that the most efficient absorption of APAP occurred in the entire small intestine, in accordance with the report of Bagnall et al. (1979). From these observations, the lower relative bioavailability in beagle dogs might be ascribable to a suggested shorter transit time in the small intestine as compared with humans. Therefore, the prolongation of the GI transit time of beagle dogs so as to make it comparable to that of humans may improve relative bioavailability in beagle dogs. Atropine has been used as a potent inhibitor of GI motility (Mizuta et al., 1990; Sagara et al., 1992), however, delayed gastric emptying has been reported by Nimmo (1976) and Gamble et al. (1976). Loperamide is an orally active antidiarrheal drug, the action of which results from a direct effect on the nerve endings and/or intramural ganglia of the intestinal wall (Niemegeers et al., 1974; Heel et al., 1978). Loperamide significantly delayed small intestinal transit, but had no significant effect on gastric emptying in contrast with atropine (Sninski et al., 1986). From these findings, it was confirmed that loperamide would be a suitable drug for delaying the GI transit time.

In order to investigate the effects of loperamide on GI transit, SASP was used for estimating the oral-caecal transit time (Kellow et al., 1986; Mizuta et al., 1989). The optimum dose of loperamide hydrochloride for delaying the GI transit time in beagle dogs was established as being 0.12 mg/kg. The mean oral-caecal transit time of SASP in beagle dogs at 0.12 mg/kg oral dose of loperamide hydrochloride was favourable at 5.0 h, which is almost the same as the period of 4.5 h for humans (Kennedy et al., 1979).

When treated with loperamide hydrochloride (0.12 mg/kg), the bioavailability of APAP calculated from the AUC_{0-24} was significantly increased, being about 2-fold greater than that without treatment. As shown in Fig. 7, the absorption profile, calculated by the Wagner-Nelson method, was suspended only for 3 h and was incomplete in beagle dogs without treatment, whereas in beagle dogs treated with loperamide, absorption was prolonged up to 5 h and showed a similar rate and extent of bioavailability as compared with humans. The AUC_{0-24} of the total APAP was also comparably increased. Since

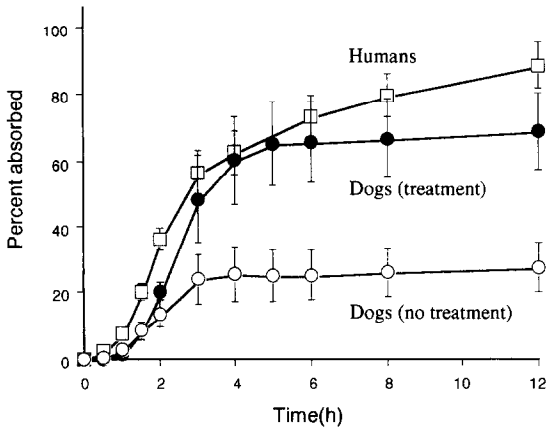

Fig. 7. Percent absorbed-time plot (mean ± S.E.) of APAP in humans and dogs after the administration of sustained-release granules.

APAP absorbed orally exists in dog plasma as unchanged APAP or its conjugate forms (Sample et al., 1968), total APAP would indicate the extent of absorption. Moreover, loperamide treatment did not exert any significant effects on the clearance of APAP. This result does not support the possibility of avoidance of the first-pass effect of APAP by loperamide and suggests an increase in absorption. The delay of small intestinal transit time due to loperamide treatment probably provided sufficient time for release and absorption of APAP from the sustained-release granules. Although the relative bioavailability (about 60% from Tables 1 and 2) of APAP from sustained-release granules in gastrointestinal transit controlled-beagle dogs did not necessarily reach that (90%) in humans, this difference might be related to the antisecretory effect of loperamide (Sandhu et al., 1981) which would reduce the intestinal fluid and consequently be disadvantageous to absorption of APAP. With regard to Fig. 7, the rate of absorption of the absorbed-time plot in 2 or 3 h after dosing in dog without treatment was more than 2-fold lower than that in humans. This difference in absorption rate between dogs and humans might be caused by the dosing amount of APAP and the volume of GI fluid. The dosing amount (37.5 mg/kg) in dogs was 5-fold more than that (7.5 mg/kg = 450 mg/man) in humans. The volume of GI fluid in dogs would be less than that in humans. These findings would be disadvantageous for dogs to the dissolution of APAP from sustained-release granules in GI tract. Consequently, it is thought that the absorption rate in dogs would be limited by a slower dissolution rate. Furthermore, Fig. 7 also shows that the absorption rate for 2–3 h in dogs would be increased by loperamide treatment vs the result in Fig. 6. This fact suggests that loperamide treatment may have some effect on GI permeability and the improvement of bioavailability by loperamide treatment cannot be completely explained only by the prolongation of GI transit time.

From the results obtained in the present study, we concluded that the reduction of bioavailability of APAP from sustained-release granules in beagle dog studies would result from the shorter transit time in the small intestine compared with human, and treatment with loperamide hydrochloride (0.12 mg/kg, oral dosing) improves the bioavailability mainly as a result of the prolongation of GI transit time. The gastrointestinal transit controlled-beagle dog developed in this study may be a useful animal model for predicting the human bioavailability of a sustained-release dosage form containing a drug such as APAP of which the absorption is restricted mostly from the small intestine. In addition, it is believed that this beagle dog model may also be applicable for evaluating the bioavailability of dosage forms containing a drug with poor solubility and poor absorption from the colon which may lead to the possibility of underestimation of the bioavailability in beagle dogs as compared with humans, as has been observed in sustained-release dosage forms.

References

Akimoto, M., Furuya, A., Maki, T., Yamada, K., Suwa, T. and Ogata, H., Evaluation of sustained-release granules of chlorphenesin carbamate in dogs and humans. *Int. J. Pharm.*, 100 (1993) 133–142.

Aoyagi, N., Ogata, H., Kaniwa, N., Ejima, A., Nakata, H. Tsutsumi, J., Fujita, T. and Amada, I., Bioavailability of indomethacin capsules in humans: III. Correlation with bioavailability in beagle dogs. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 23 (1985) 578–584.

Aoyagi, N., Ogata, H., Kaniwa, N., Koibuchi, M., Shibasaki, T., Ejima, A., Tamaki, N., Kamimura, H., Katougi, Y. and Omi, Y., Bioavailability of griseofulvin from tablets in beagle dogs and correlation with dissolution rate and bioavailability in humans. *J. Pharm. Sci.*, 71 (1982) 1169–1172.

Bagnall, W.E., Kelleher, J., Walker, B.E. and Losowsky, M.S., The gastrointestinal absorption of paracetamol in the rat. *J. Pharm. Pharmacol.*, 31 (1979) 157–160.

Barr, W.H., The use of physical and animal models to assess bioavailability. *Pharmacology*, 8 (1972) 55–101.

Bialer, M., Friedman, M. and Dobrovsky, J., Comparative pharmacokinetic analysis of a novel sustained-release dosage form of valproic acid in dogs. *Biopharm. Drug Dispos.*, 5 (1984a) 1–10.

Bialer, M., Friedman, M. and Dobrovsky, J., Effect of sustained-release on the pharmacokinetics of valproic acid in dogs. *Int. J. Pharm.*, 20 (1984b) 53–63.

Bialer, M., Friedman, M. and Dobrovsky, J., Pharmacokinetic evaluation of novel sustained-release dosage forms of valproic acid in humans. *Biopharm. Drug Dispos.*, 6 (1985) 401–411.

Bialer, M., Friedman, M. and Dobrovsky, J., Relation between absorption half-life values of four novel sustained-release dosage forms of valproic acid in dogs and humans. *Biopharm. Drug Dispos.*, 7 (1986) 495–500.

Bialer, M., Kaplan, S.A. and Yacobi, A., Animal models in the primary screening of controlled release formulations. In *Oral Sustained Release Formulation: Design and Evaluation*, 1st Edn, Pergamon, New York, 1988, pp. 183–193.

Black, M., Sprague, K., Rapid micromethod for acetaminophen determination in serum. *Clin. Chem.*, 24 (1978) 1288–1289.

Cressman, W.A. and Sumner, D., The dog as a quantitative model for evaluation of nondisintegrating sustained-release tablets. *J. Pharm. Sci.*, 60 (1971) 132–134.

Dressman, J.B., Comparison of canine and human gastrointestinal physiology. *Pharm. Res.*, 3 (1986) 123–131.

Dressman, J.B. and Yamada, K., Animal models for oral drug absorption. In *Pharmaceutical Bioequivalence*, 1st Edn, Dekker, New York, 1991, pp. 235–266.

Gamble, J.A.S., Gaston, J.H., Nair, S.G. and Dundee, J.W., Some pharmacological factors influencing the absorption of diazepam following oral administration. *Br. J. Anaesth.*, 48 (1976) 1181–1185.

Heel, R.C., Brogden, R.N., Speight, T.M. and Avery, G.S., Loperamide: a review of its pharmacological properties and therapeutic efficacy in diarrhoea. *Drugs*, 15 (1978) 33–52.

Hussein, Z., Bialer, M., Friedman, M. and Raz, I., Comparative pharmacokinetic evaluation of sustained-release theophylline formulations in dogs and humans. *Int. J. Pharm.*, 37 (1987) 97–102.

Kellow, J.E., Borody, T.J., Phillips, S.F., Haddad, A.C. and Brown, M.L., Sulfapyridine appearance in plasma after salicylazosulfapyridine: Another simple measure of intestinal transit. *Gastroenterology*, 91 (1986) 396–400.

Kennedy, M., Chinwah, P. and Wade, D.N., A pharmacological method of measuring mouth caecal transit time in man. *Br. J. Clin. Pharmacol.*, 8 (1979) 372–373.

Mizuta, H., Kawazoe, Y. and Ogawa, K., Gastrointestinal absorption of chlorothiazide: Evaluation of a method using salicylazosulfapyridine and acetaminophen as the marker compounds for detection of gastrointestinal transit time in dog. *Chem. Pharm. Bull.*, 38 (1990) 2810–2813.

Mizuta, H., Kawazoe, Y., Haga, K., Ogawa, K. and Yokobe, T., Determination of small intestinal transit time in beagle dogs using salicylazosulfapyridine. *Yakugaku Zasshi*, 109 (1989) 760–765.

Morimoto, M., Yamashita, K., Koyama, H., Fujimoto, H. Toguchi, H. and Kitamori, N., Evaluation of sustained-release capsules of molsidomine (SIN-10) in dogs and monkeys. *Chem. Pharm. Bull.*, 34 (1986) 1257–1263.

Niemegeers, C.J.E., Lenaerts, F.M., Janssen, P.A.J., Van Nueten, J.M., Fontaine, J., Marsboom, R., Herin, V., Verstrasten, A., Vandesteene, R., Fransen, J., Heykants, J., Michiels, M., Knaeps, A., Brugmans, J., Schuermans, V., Van Lommel, R., Dom, J., De Cree, J. and Layman, R., Loperamide (R18553), a novel type of antidiarrheal agent: 1–8. *Arzneim-Forsch (Drug Res.)*, 24 (1974) 1633–1665.

Nimmo, W.S., Drugs decrease and altered gastric emptying. *Clin. Pharmacokinet.*, 1 (1976) 189–198.

Ogata, H., Aoyagi, N., Kaniwa, N., Ejima, A., Kitaura, T., Ohki, T. and Kitamura, K., Evaluation of beagle dogs as an animal model for bioavailability testing of cinnarizine capsules. *Int. J. Pharm.*, 29 (1986) 121–126.

Ogata, H., Aoyagi, N., Kaniwa, N., Koibuchi, M., Shibasaki, T., Ejima, A., Shimamoto, T., Yashiki, T., Ogawa, Y., Uda, Y. and Nishida, Y., Correlation of the bioavailability of diazepam from uncoated tablets in beagle dogs with its dissolution rate and bioavailability in humans. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 20 (1982) 576–581.

Ogata, H., Aoyagi, N., Kaniwa. N., Shibazaki, T., Ejima, A., Takagishi, Y., Ogura, T., Tomita, K., Inoue, S. and Zaizen, M., Bioavailability of metronidazole from sugar-coated tablets in humans: II. Evaluation of beagle dogs as an animal model. *Int. J. Pharm.*, 23 (1985) 289–298.

Ogata, H., Aoyagi, N., Kaniwa, N., Shibazaki, T., Ejima, A., Takasugi, N., Mafune, E., Hayashi, T. and Suwa, K., Bioavailability of nalidixic acid from uncoated tablets in humans: II. Bioavailability in beagle dogs and its correlation with bioavailability in humans and in vitro dissolution rates. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 22 (1984) 240–245.

Sagara, K., Nagamatsu, Y., Yamada, I., Kawata, M., Mizuta, H. and Ogawa, K., Bioavailability study of commercial sustained-release preparations of diclofenac sodium in gastro-intestinal physiology regulated-dogs. *Chem. Pharm. Bull.*, 40 (1992) 3303–3306.

Sample, R.G., Rossi, G.V. and Packman, E.W., Thiry-Vella dog as a biologic model for drug absorption from the intestinal mucosa. *J. Pharm. Sci.*, 57 (1968) 795–798.

Sandhu, B.K., Tripp, J.H., Candy, D.C.A. and Harries, J.T., Loperamide: studies on its mechanism of action. *Gut*, 22 (1981) 658–662.

Sninski, C.A., Davis, R.H., Clench, M.H., Thomas, K.D. and Mathias, J.R., Effect of lidamidine hydrochloride and loperamide on gastric emptying and transit of the small intestine. *Gastroenterology*, 90 (1986) 68–73.

Uchida, T., Kawata, M. and Goto, S., In vivo evaluation of ethyl cellulose microcapsules containing ampicillin using rabbits, beagle dogs and humans. *J. Pharmacobio-Dyn.*, 9 (1986) 631–637.

Wagner, J.G. and Nelson, E., Kinetic analysis of blood levels and urinary excretion in the absorption phase after single dose of drug. *J. Pharm. Sci.*, 83 (1964) 1392–1403.

Yashiki, T., Kitamori, N. and Inoue, H., Dosage form design of a sustained release preparation of molsidomine. *J. Takeda Res. Lab.*, 44 (1985) 156–170.