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Animal models for predicting potency of oral sustained-release adhesive microspheres in humans

Naoki Nagahara [a,b], Yohko Akiyama [a], Kazutaka Higaki [b], Toshikiro Kimura [b,*]

[a] *Pharmaceutical Technology Research & Development Laboratories, Pharmaceutical Production Division, Takeda Pharmaceutical Company Limited, 17-85, Jusohonmachi 2-Chome, Yodogawa-Ku, Osaka 532-8686, Japan*

[b] *Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-Naka 1-1-1, Okayama 700-8530, Japan*

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Abstract

The sustained-release (SR) adhesive microspheres successfully improved the absorption of furosemide, of which the absorption is limited to the upper small intestine, after oral administration to humans based on the adhesion to the gastric mucosa in our previous study. To develop a new drug using SR-adhesive microspheres, however, some adequate animal models should be needed to predict the potency of the formulation in humans. To find out an adequate animal model, the effect of the SR-adhesive microspheres on furosemide absorption was investigated in rats, dogs and monkeys and the release kinetics of furosemide from SR-adhesive microspheres was also studied. SR-adhesive and SR-non-adhesive microspheres showed very similar characteristics of drug release. The rotation speed did not affect the release kinetics, but higher pH increased the drug release from both microspheres. The absorption of furosemide after SR-adhesive microspheres administration to rats and dogs was significantly higher than that after SR-non-adhesive microspheres administration, which was very similar to the results obtained in humans. On the other hand, in monkeys, SR-adhesive microspheres were not able to improve the absorption of furosemide at all. These findings indicated that rats and dogs were in vivo animal models suitable for predicting the potency of SR-adhesive microspheres in humans.
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1. Introduction

In the process of developing a new drug, animal data are traditionally used to estimate the first dose to human and to assess safety and efficacy of a newly discovered compound. More generally, one of the objectives of animal studies is to predict pharmacokinetics (PK) and pharmacodynamics (PD) of the compound in humans. However, there are, of course, many differences in anatomy and physiology among animal species including humans, which could have influences on PK/PD of the compound. These differences are assumed to be the main factors that could cause discrepancy in PK/PD including absorption kinetics between humans and animals. No single animal can completely mimic the characteristics of human gastrointestinal (GI) tract, that is the reason why it is very difficult to completely predict the absorption kinetics in humans based on animal studies (Kararli, 1995). In the process of developing drug delivery systems, several animal models also have been used to select appropriate dosage forms and to predict their performances in humans (Hildebrand et al., 1991; Goto et al., 2006; Dali et al., 2006). Considering the differences between animals and humans, it is necessary to select the adequate animal model that is suitable for estimating the function of the drug delivery systems.

It was reported that humans, dogs, pigs and monkeys were fairly similar in anatomy of the stomach, which has influences on drug absorption process (Kararli, 1995). Dogs are frequently used for testing oral dosage form since physiology of the stomach in the fasted state such as motility patterns and gastric emptying of indigestible solids and liquids, is similar to those of humans and dogs. Furthermore, the dimensions of canine GI tract permit the administration of dosage forms intended for subsequent testing in humans (Dressman, 1986), and it is easier to handle dogs than other species of similar size such as pigs and monkeys (Dressman, 1986). Cynomolgus monkeys are non-human primates often used for safety studies of new drug

* Corresponding author. Tel.: +81 86 251 7948; fax: +81 86 251 7926.
E-mail address: kimura@pharm.okayama-u.ac.jp (T. Kimura).

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candidates. However, the use of monkeys in bioavailability studies has been limited because of insufficient information of GI physiology and GI transit, although it was reported that under fasted condition, cynomolgus monkeys and humans were similar in gastric emptying rate for liquids and gastric pH, while small-intestinal transit time was about 1.5 h shorter in cynomolgus monkeys than that in humans (Kondo et al., 2003a,b).

There is a lot of interest in developing oral mucoadhesive drug delivery systems that can extend the residence time of drugs in particular section of the GI tract. The goals of mucoadhesive drug delivery systems are to increase the absorption of drugs into the circulation and to prolong direct pharmacological action for mucin and/or epithelial cell membranes (Lehr, 1994; Takeuchi et al., 2001; Chowdary and Rao, 2004; Peppas, 2004). We have developed a novel mucoadhesive drug delivery system, sustained-release (SR) adhesive microspheres, consisting of drugs and adhesive polymers such as a cross-linked polyacrylic acid derivative (carboxyvinyl polymer) dispersed in a matrix of polyglycerol esters of fatty acids, and confirmed that the system adhered to the rat GI mucosa and prolonged the residence time in GI tract by direct observation of the inner GI tract and evaluation of GI transit patterns of the SR-adhesive microspheres after oral administration (Akiyama et al., 1995). Furthermore, we have reported that the absorption of furosemide and riboflavin, of which the absorption is limited to the upper part of the GI tract, was significantly enhanced by the SR-adhesive microspheres due to the prolonged residence time of drugs in GI tract, especially in the stomach (Akiyama et al., 1998). The SR-adhesive microspheres also successfully improved the direct action of amoxicillin to clear *Helicobacter pylori* from the stomach (Nagahara et al., 1998).

The objective of this study was to evaluate the usefulness of animal models for predicting the potency of the SR-adhesive microspheres in humans. We evaluated the release kinetics of furosemide from the SR-adhesive microspheres and performed the absorption studies by utilizing rats, dogs and monkeys as model animals to select the most suitable animal model.

2. Materials and methods

2.1. Materials

Tetraglycerol hexabehenate and tetraglycerol monostearate, polyglycerol esters of fatty acids, were purchased from Sakamoto Yakuhin Kogyo (Osaka, Japan). Hiviswako 104 (carboxyvinyl polymer) was purchased from Wako Pure Chemicals (Osaka) and furosemide was purchased from Hoechst Japan (Tokyo, Japan). Lactose was of Japanese Pharmacopoeia (JP) grade. Other chemicals were of analytical grade.

2.2. Animals

Male Sprague–Dawley rats from Clea Japan Inc. (Shizuoka, Japan), maintained at 23 °C and 55% humidity, were allowed free access to standard laboratory food (Clea Japan Inc.) and water prior to the experiments. Rats weighing between 250 and 350 g, 8–11 weeks old, were randomly assigned to each experimental group. Male beagle dogs from Kitayama Labes (Nagano, Japan), maintained at 23 °C and 55% humidity, were given standard laboratory food (Japan SLC Inc.) and allowed free access to water prior to the experiments. Dogs weighing between 12 and 14 kg, 1.5–2 years old, were randomly assigned to each experimental group. Male cynomolgus monkeys from Keari (Wakayama, Japan), maintained at 23 °C and 55% humidity, were given standard laboratory food (Japan SLC Inc.) and allowed free access to water prior to the experiments. Monkeys weighing between 4 and 5 kg, 2–3 years old, were randomly assigned to each experimental group. Our investigations were performed after approval by our local ethical committee at Takeda Pharmaceutical Company and Okayama University in accordance with "Principles of Laboratory Animal Care" (NIH publication #85–23).

2.3. Preparation of microspheres

SR-non-adhesive and SR-adhesive microspheres, which release furosemide at a similar rate (Akiyama et al., 1998), were prepared by mixing waxy bases that have different hydrophile/lipophile balance values. Tetraglycerol hexabehenate (hydrophile/lipophile-balance value: 1.8) and tetraglycerol monostearate (hydrophile/lipophile-balance value: 8.4) were used as waxy bases.

2.3.1. SR-non-adhesive microspheres

Furosemide (10%, w/w) and lactose (30%, w/w) were dispersed in a molten mixture of 2:1 tetraglycerol hexabehenate–tetraglycerol monostearate (60%, w/w) at 90 °C. Spray-chilling-molten non-adhesive microspheres were prepared by dropping the molten mixture onto a 15 cm diameter aluminum disc rotating at 2000–3000 rpm. The molten mixture spread on the disc and was sprayed from the periphery of the disc, the microspheres being formed on cooling (Akiyama et al., 1993a).

2.3.2. SR-adhesive microspheres

Furosemide (10%, w/w) and carboxyvinyl polymer (15%, w/w) were dispersed in a molten mixture of 65:10 tetraglycerol hexabehenate–tetraglycerol monostearate (75%, w/w) at 90 °C. SR-adhesive microspheres were prepared by the same method used for SR-non-adhesive microspheres.

Both SR-non-adhesive and SR-adhesive microspheres in the particle size range 177–500 μm, obtained by sieving, were used for in vitro dissolution test and in vivo experiments in rats, and gelatin capsules (#3) filled with 100 mg of microspheres containing 10 mg furosemide were used for in vivo experiments in dogs and monkeys.

2.4. In vitro release test

The in vitro release of furosemide from the SR-adhesive microspheres and/or SR-non-adhesive microspheres was studied using the USP apparatus 2 paddle method (paddle rotation speeds; 25, 50, 100 and 150 rpm) at 37 °C in 900 mL. The

dissolution media used were JP first fluid (pH 1.2) containing 5% sodium dodecylsulfate and buffer solution (pH 6.0), which was prepared using 0.05 M KH_2PO_4 and K_2HPO, containing 5% sodium dodecylsulfate. The amount of furosemide released was determined by high-performance liquid chromatography (HPLC) with fluorometric detection (ex. 250 nm and em. 389 nm) using YMC-Pack ODS-A column (YMC Co., Ltd., Kyoto) with pH 7.2 phosphate buffer (0.08 M): CH_3CN (65:35, v/v) as mobile phase (flow rate 1.0 mL/min). The squared correlation coefficient (r^2) for the standard curve was over 0.997.

2.5. In situ absorption study in rats

Male Sprague–Dawley rats were fasted overnight, and anaesthetized by an i.p. injection of sodium pentobarbital. The rats were restrained in a supine position on the thermostatically controlled board at 37 °C and kept body temperature. The GI tract was exposed following small midline incision carefully made in the abdomen, and the loop of stomach and 10 cm segments of duodenum (upper small intestine), ileum (lower small intestine) or colon were prepared. Furosemide (10 mg/kg) was administered as a 0.5% (w/v) methylcellulose suspension to each of the loop using gastric sonde from one side of the loop. After administration, the loops were securely ligated to prevent fluids loss, and carefully returned to their original location inside the peritoneal cavity. The plasma samples were collected from the jugular vein at 0.5, 1, 2 and 3 h after administration and stored at −20 °C until analysis for furosemide. CH_3CN (400 μL) were added to the plasma samples (200 μL) and the mixture was agitated vigorously for 1 min and centrifuged at 3000 rpm for 15 min. The concentration of furosemide in the supernatant was determined by the HPLC method described in the section of in vitro release test. The value of r^2 for the standard curve was over 0.997. The area under the plasma concentration–time curve up to 3 h after administration, $AUC_{0–3\,h}$, was calculated by the trapezoidal method from the individual plasma furosemide concentration curves.

2.6. In vivo absorption study in animal models

Male Sprague–Dawley rats were fasted overnight. SR-adhesive and SR-non-adhesive microspheres containing furosemide (10 mg/kg) were placed in a polyethylene tube (PF260; Nippon Becton Dickinson, Tokyo) with one end covered with hydroxypropylcellulose film. The tube was put into the stomach by a gastric sonde, and the microspheres were pushed through the tube and administered. Then, immediately 1.0 mL of water was dosed by a syringe attached to a gastric sonde to conscious rats. Male beagle dogs and male cynomolgus monkeys were fasted overnight and #3 gelatin capsules filled with 100 mg of the SR-adhesive or SR-non-adhesive microspheres containing 10 mg furosemide were orally administered with 100 mL water for dogs and 50 mL water for monkeys.

In rats, the plasma samples were collected at 0.5, 1, 2, 4, 6, 8 and 24 h after oral administration. In dogs, the plasma samples were collected at 0.5, 1, 2, 4, 6, and 8 h after oral administration. In monkeys, the plasma and urine samples were collected at 1, 2, 4, 6, 8 and 10 h after oral administration. Obtained plasma and urine samples were stored at −20 °C until analysis.

Plasma levels of furosemide were determined by the analytical procedure described in the section of in situ absorption study in rats. The maximum plasma concentration, C_{max}, and the time required to reach C_{max}, T_{max}, were obtained from the individual plasma concentration–time curves of furosemide. The AUC up to the final sampling time point after administration, $AUC_{0–24\,h}$ for rats, $AUC_{0–8\,h}$ for dogs and $AUC_{0–10\,h}$ for monkeys, were calculated by the trapezoidal method. Urinary concentrations of furosemide were determined by the HPLC method described in the section of in vitro release test, after appropriate dilution. Urinary recovery of furosemide in 10 h was determined in monkeys.

2.7. Assessment of extending effect

The extending effect on gastric residence time of adhesive microspheres based on the adhesion to the gastric mucosa has been assessed by calculating an extending factor (ET factor), the ratio of AUC values obtained by oral administration of SR-adhesive microspheres to those of SR-non-adhesive microspheres (Eq. (1)):

$$\text{ET factor} = \frac{\text{AUC}_{\text{SR-adhesive microspheres}}}{\text{AUC}_{\text{SR-non-adhesive microspheres}}} \quad (1)$$

2.8. Statistical analysis

The results were expressed as the mean ± S.E. of at least three experiments. Significant differences in mean values were evaluated by the Student's *t*-test. A difference was considered to be statistically significant when *p*-value was less than 0.05.

3. Results and discussion

3.1. Mechanism of drug release from adhesive microspheres

The release kinetics of furosemide from SR-adhesive microspheres was investigated in terms of rotation speed (Figs. 1 and 2) and pH (Fig. 3). First of all, release profiles of furosemide from SR-adhesive microspheres were measured at various paddle rotation speeds (25, 50, 100 and 150 rpm) using JP 1st medium (pH 1.2) in the dissolution test (Fig. 1). The obtained results showed that the release profiles of furosemide were almost superimposed at all the conditions, indicating that the paddle speed, namely the external agitation, did not affect the release profile of furosemide. This phenomenon suggests that the internal diffusion of drugs in the matrices is one of the main processes regulating the drug release from the matrix-type spherical microspheres. Generally, the release rates of drugs from matrices are controlled by diffusion or erosion (Nambu, 1986; Hui and Robinson, 1987). As the particle size of SR-adhesive microspheres did not change during the dissolution test by a visual

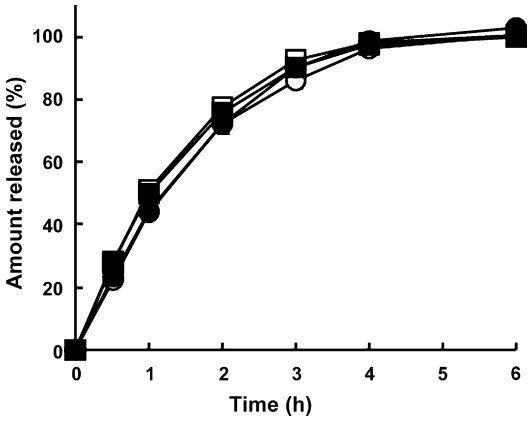

Fig. 1. Release profiles of furosemide from SR-adhesive microspheres at various paddle speeds. Each point expresses the mean ± S.E. ($n = 3$). Keys: ◯, 25 rpm; ●, 50 rpm; □, 100 rpm; ■, 150 rpm.

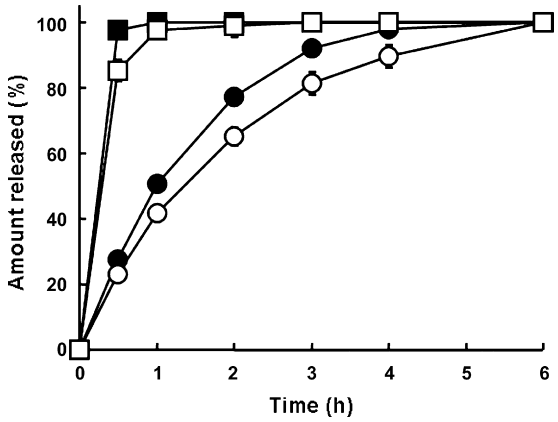

Fig. 3. Effect of medium pH on release profile of furosemide from adhesive microspheres. Each point expresses the mean ± S.E. ($n = 3$). Keys: ◯, SR-non-adhesive (pH 1.2); ●, SR-adhesive (pH 1.2); □, SR-non-adhesive (pH 6.0); ■, SR-adhesive (pH 6.0).

observation, the release cannot be explained by the erosion of the microspheres.

On the other hand, it was found that the release of drug from matrix-type spherical microspheres was regulated by the diffusion of drugs within the microspheres, since the release profile was described by Jander's equation (Akiyama et al., 1993b). Jander's equation, which considers the change in the interfacial area where the release of a solid drug actually occurred, is expressed by the following equation:

$$1 - (1 - \chi)^{1/3} = \frac{K_2}{r} t^{1/2} = K t^{1/2} \quad (2)$$

where χ is the fraction of drug released at time t, and r is the radius of microspheres. K_2 and K are the rate constants ($K_2/r = K$). Therefore, the value of $1 - (1 - \chi)^{1/3}$ was plotted as a function of square root of time ($t^{1/2}$) (Fig. 2), showing an excellent linearity for all paddle rotation speeds. The obtained values of release rate constant, K, were 0.52, 0.52, 0.51 and 0.51 for 25, 50, 100 and 150 rpm, respectively, which also indicates that the external agitation does not change the release characteristics of the microspheres. On the other hand, the linear relation was not established when the fraction of released furosemide was plotted against the square root of time ($t^{1/2}$) according to Higuchi's equation (Higuchi, 1961) (data not shown). These results mean that the release of furosemide from SR-adhesive microspheres is regulated by the diffusion of furosemide within the core of the microspheres where furosemide remains to be released. The diffusion through the outer layer where furosemide was already released would not be a rate-limiting process because furosemide should be diffused through the pores in the layer (Akiyama et al., 1993b). As reported previously (Akiyama et al.,

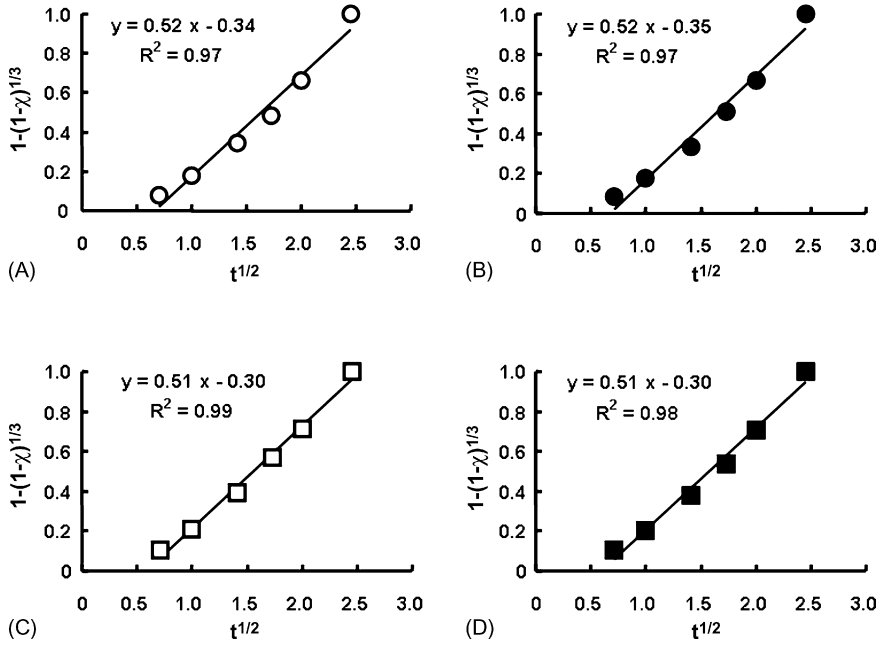

Fig. 2. Jander's plot of furosemide release from SR-adhesive microspheres. Effect of paddle rotation speed. Each point expresses the mean ± S.E. ($n = 3$). Keys: (A) ◯, 25 rpm; (B) ●, 50 rpm; (C) □, 100 rpm; (D) ■, 150 rpm.

1998), the release profile of furosemide from SR-non-adhesive microspheres was almost the same as that from SR-adhesive microspheres in the medium of pH 1.2. In the present study, the release kinetics from SR-non-adhesive microspheres was not affected by the external agitation, either (data not shown).

Fig. 3 shows the effect of pH on the release profile of furosemide from SR-adhesive microspheres as well as SR-non-adhesive microspheres. Furosemide was released pH-dependently from both microspheres and the release rates at pH 6.0 were much faster than those at pH 1.2. The difference in release rates between SR-adhesive microspheres and SR-non-adhesive microspheres was very small. The release rate of the drug could be affected by its solubility, and the solubility of furosemide, an acidic drug, changes drastically as pH changes. That is to say, the solubility of furosemide with a pK_a value of 3.9 becomes higher at higher pH because of larger fraction of ionized form (Avdeef et al., 2000). Present study also revealed the higher release rate at higher pH where ionized form of furosemide was larger, indicating that the release of furosemide from both microspheres is regulated the solubility as well.

3.2. Predictability of animal models

In our previous study, the in vivo performance of SR-adhesive microspheres containing carboxyvinyl polymer as an adhesive polymer and a model drug, furosemide, which is suggested to have a narrow absorption window in the upper part of small intestine, was examined in humans and compared with SR-non-adhesive microspheres providing release rates similar to SR-adhesive microspheres. As a result, SR-adhesive microspheres successfully improved the absorption of furosemide in comparison with SR-non-adhesive microspheres (Akiyama et al., 1998). Considering the prolongation of GI-transit time by SR-adhesive microspheres (Akiyama et al., 1995), it was concluded that SR-adhesive microspheres could adhere to and retain on the gastric mucosa and/or the upper small intestine, which are close to the absorption window, resulting in the absorption improvement of furosemide in humans (Akiyama et al., 1998). On the other hand, SR-non-adhesive microspheres could pass through the absorption window before releasing the entire drug contained in the dosage form, resulting in the decrease in the absorption of furosemide in humans (Akiyama et al., 1998). Therefore, the increase in bioavailability could reflect the prolonged residence of the dosage form around the absorption window for an SR-adhesive dosage form containing a drug with a narrow absorption window.

In the present study, SR-adhesive and SR-non-adhesive microspheres containing furosemide examined in human studies (Akiyama et al., 1998) were studied to select the suitable animal model for predicting the potency of the adhesive microspheres in humans by utilizing rats, dogs and monkeys. Since the gastric residence time of drugs depends on some factors such as administration forms (liquids or solids), particle size of solid dosage form, administration volume and timing of meal (Dressman, 1986; Itoh et al., 1986; Aoyagi et al., 1992; Kondo et al., 2003a), the SR-adhesive and SR-non-adhesive microspheres with the same particle size and the same amount were administered for all the experiments under the same conditions.

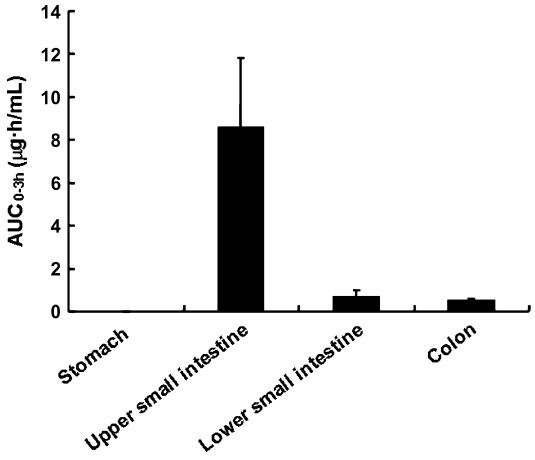

Fig. 4. Segmental absorption of furosemide in in situ rat closed loop study. The area under the plasma concentration–time curve up to 3 h ($AUC_{0–3\,h}$) was obtained after administration of furosemide solution into the four different GI-segments. Results are expressed as the mean with the bar showing S.E. ($n = 4$).

In the case of rats, the absorbability of furosemide in the four different segments of GI-tract was evaluated by the in situ loop study. Fig. 4 clearly shows that the absorption from the upper segment of small intestine was extremely high as compared with other segments, confirming that furosemide has an absorption window in the upper small intestine. The mean plasma concentrations versus time profiles of furosemide following oral administration of SR-adhesive microspheres or SR-non-adhesive microspheres with 1.0 mL water, a maximum volume that does not induce a vomiting in rats, to fasted rats are shown in Fig. 5, and relevant PK parameters are listed in Table 1. $AUC_{0–24\,h}$ of furosemide was 12.95 ± 1.25 μg h/mL for SR-adhesive microspheres while that was 6.57 ± 0.52 μg h/mL for SR-non-adhesive microspheres, indicating that SR-adhesive microspheres significantly increased the absorption of furosemide. Table 1 also shows the results obtained after dosing with 0.2 mL water (Akiyama et al., 1998), but any significant difference from the results for 1.0 mL water was not found for SR-adhesive

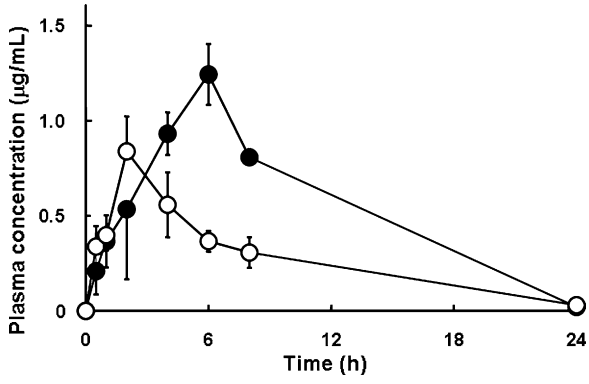

Fig. 5. Plasma levels of furosemide after oral administration of SR-adhesive and SR-non-adhesive microspheres to fasted rats. Results are expressed as the mean with the bar showing S.E. ($n = 4$). Keys: ●, SR-adhesive microspheres; ○, SR-non-adhesive microspheres.

Table 1
Pharmacokinetic parameters of furosemide after oral administration of SR-adhesive and SR-non-adhesive microspheres to fasted rats

Parameters	With 0.2 mL water[a]		With 1.0 mL water	
	Adhesive microspheres	Non-adhesive microspheres	Adhesive microspheres	Non-adhesive microspheres
$AUC_{0-24\,h}$[b] (μg h/mL)	11.57 ± 1.84*	6.56 ± 0.93	12.95 ± 1.25*	6.57 ± 0.52
C_{max}[b] (μg/mL)	1.5 ± 0.1*	0.9 ± 0.1	1.4 ± 0.1	0.9 ± 0.4
T_{max}[b] (h)	4.0 ± 1.0	5.0 ± 1.3	5.5 ± 0.5	2.6 ± 1.2
ET factor[c]	1.76	–	1.97	–

[a] Data were quoted from Akiyama et al. (1998).
[b] Results are expressed as the mean ± S.E. ($n = 4$).
[c] Extending factor was calculated by Eq. (1).
* $p < 0.05$, compared with non-adhesive microspheres.

Table 2
Pharmacokinetic parameters of furosemide after oral administration of SR-adhesive and SR-non-adhesive microspheres to fasted dogs, monkeys and humans

Parameters	Dogs[a]		Monkeys[a]		Humans[b]	
	Adhesive microspheres	Non-adhesive microspheres	Adhesive microspheres	Non-adhesive microspheres	Adhesive microspheres	Non-adhesive microspheres
AUC[c] (μg h/mL)	0.76 ± 0.06*	0.35 ± 0.04	0.45 ± 0.04	0.47 ± 0.05	0.59 ± 0.03*	0.33 ± 0.04
C_{max} (μg/mL)	0.15 ± 0.02*	0.06 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.20 ± 0.04*	0.08 ± 0.01
T_{max} (h)	2.5 ± 0.5	4.0 ± 0.8	7.5 ± 1.0	8.5 ± 1.0	2.5 ± 0.5	3.3 ± 0.4
ET factor[d]	2.17	–	0.96	–	1.79	–

[a] Results are expressed as the mean ± S.E. ($n = 4$).
[b] Data were quoted from Akiyama et al. (1998). Results are expressed as the mean ± S.E. ($n = 10$).
[c] Dogs, $AUC_{0-8\,h}$; monkeys, $AUC_{0-10\,h}$; humans, $AUC_{0-24\,h}$.
[d] Extending factor was calculated by Eq. (1).
* $p < 0.05$, compared with non-adhesive microspheres.

microspheres, indicating that the volume of water administered together with SR-adhesive microspheres does not affect the performance of the formulation, namely mucoadhesive and SR properties in the stomach and/or the upper segment of small intestine. ET factor in rats was 1.97 (Table 1), which was very similar to that in humans (1.79) shown in Table 2, where PK parameters describing the absorption kinetics of furosemide in humans in the previous study (Akiyama et al., 1998) were cited. These results suggest that rats would be a useful animal model for assessing the performance of SR-adhesive microspheres.

SR-adhesive and SR-non-adhesive microspheres were administered to fasted dogs and monkeys as a capsule formulation, which was used in human study in the previous study (Akiyama et al., 1998). In dogs, SR-adhesive microspheres significantly enhanced the absorption of furosemide by comparison with SR-non-adhesive microspheres (Fig. 6). $AUC_{0-8\,h}$ values were 0.76 ± 0.06 and 0.35 ± 0.04 μg h/mL for SR-adhesive and SR-non-adhesive microspheres, respectively (Table 2). As well as in rats, the ET factor in dogs (2.17) was similar to that in humans. On the other hand, in monkeys, the plasma concentration–time profiles of furosemide were almost superimposed for SR-adhesive and SR-non-adhesive microspheres (Fig. 7). Therefore, there was no significant difference in PK parameters between the two microspheres and the ET factor was calculated as 0.96 (Table 2).

The dogs are frequently adopted as an animal model for oral dosage form testing since the dimensions of the GI tract are similar enough to permit the administration of dosage forms intended for subsequent testing in humans. Furthermore, under fasted condition, GI motility patterns in dogs are very much similar to that in humans and the two species are also very much similar in gastric residence times of indigestible monolithic dosage forms (Dressman, 1986).

There are, however, some differences that could affect the drug absorption kinetics, such as the length of small intestine and pH in the stomach (Lui et al., 1986). It is well known that the length of small intestine, a major site of absorption, of dogs is relatively short compared to human, therefore, the total GI

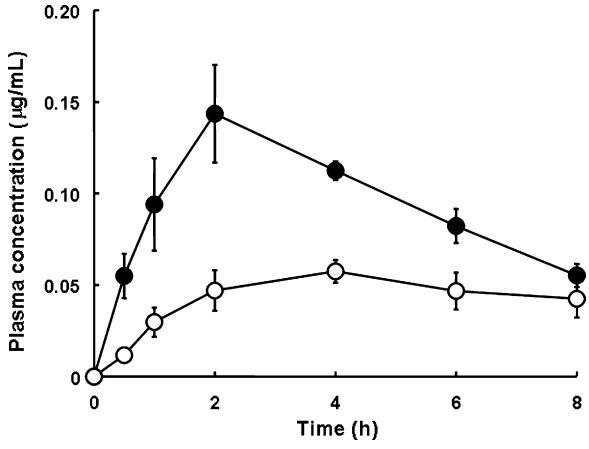

Fig. 6. Plasma levels of furosemide after oral administration of SR-adhesive and SR-non-adhesive microspheres to fasted dogs. Results are expressed as the mean with the bar showing S.E. ($n = 4$). Keys: ●, SR-adhesive microspheres; ○, SR-non-adhesive microspheres.

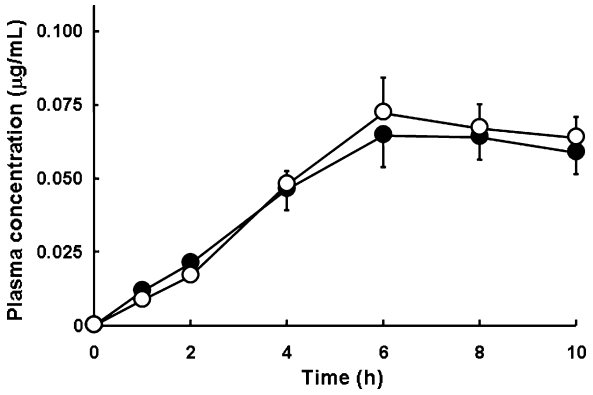

Fig. 7. Plasma levels of furosemide after oral administration of SR-adhesive and SR-non-adhesive microspheres to fasted monkeys. Results are expressed as the mean with the bar showing S.E. ($n = 4$). Keys: ●, SR-adhesive microspheres; ○, SR-non-adhesive microspheres.

transit time including colon in dogs is much shorter (6–8 h) than that in human (20–30 h) (Gruber et al., 1987). As the difference in the intestinal transit time can affect the oral bioavailability of drugs, especially poorly absorbable drugs so much (Kimura and Higaki, 2002), dogs are not suitable for evaluation of conventional (non-adhesive) SR-dosage forms, which usually release drugs at controlled and relatively low rates mainly in the small and large intestines (Sagara et al., 1992). However, in the case of our SR-adhesive microspheres, as the microspheres adsorb and are retained in the stomach and/or the upper small intestine (Akiyama et al., 1995, 1998) and then furosemide is released at adequate rates around the main absorption sites, the absorption kinetics would not be influenced by short GI transit time in dogs and was very similar to that in humans in the present study. As for gastric pH, basal secretary rates of gastric acid are lower in dogs than in humans and the pH in dogs is more variable in the fasted state (Itoh et al., 1986; Ogata et al., 1986; Yamada and Haga, 1990). Kararli reported that the pH values in canine stomach were around pH 5.5 at anterior portion and pH 3.4 at posterior portion (Kararli, 1995). However, the value of pH in canine stomach is still controversial, as Dressman reported (1986) that gastric pH in the fasted state in dogs was quite acidic with a range of 0.9–2.5 as well as in humans with a range of 1–3.2. As shown in Fig. 3, the release of furosemide from SR-adhesive microspheres was much faster at pH 6.0 than that at pH 1.2. If gastric pH in dogs is as high as Kararli reported (1995), furosemide would not be absorbed efficiently as shown in Fig. 6, because the fast release from the microspheres would cancel out the advantage of prolonged retention on gastric mucosa. Our present results clearly showed that SR-adhesive microspheres successfully improved the absorption of furosemide in dogs, which was similar to human study (Table 2, Akiyama et al., 1998). These findings suggest that the value of canine gastric pH is also similar to human gastric pH and that the performance of SR-adhesive microspheres in human would be predictable based on the results obtained in dogs.

It was suggested that monkeys offered some advantages over beagle dogs as an animal model and might be more useful for bioavailability studies (Chiou et al., 2000; Chiou and Buechler, 2002), and it was also reported that gastric emptying rates for liquids in unfed cynomolgus monkeys were similar to those in fasted humans (Kondo et al., 2003a). In the present study, however, the plasma profiles of furosemide (Fig. 7) and the ET factor (Table 2) clearly indicated that SR-adhesive microspheres was not able to improve the bioavailability of furosemide, meaning that monkeys are not suitable for assessing the potency of SR-adhesive microspheres in humans. Although the reason why SR-adhesive microspheres did not improve the absorption of furosemide in monkeys has not been clarified yet, the low absorbability of furosemide in monkeys might be responsible for it. The urinary recovery of furosemide after oral administration of SR-adhesive microspheres to monkeys was about 3%, approximately one eleventh of that in humans (about 35%) (Akiyama et al., 1998), suggesting that monkeys might lack the absorption window in GI tract.

4. Conclusions

In order to evaluate the usefulness of animal models for predicting the potency of SR-adhesive microspheres after oral administration in humans, SR-adhesive microspheres containing furosemide, of which the absorption is limited in the upper small intestine, were orally administered to rats, dogs and monkeys. The absorption of furosemide in rats and dogs was significantly higher than that after oral administration of non-adhesive microspheres, which was similar to the results in humans. On the other hand, in monkeys, SR-adhesive microspheres did not improve the absorption of furosemide at all. These findings indicated that rats and dogs were suitable in vivo animal models for predicting the potency of SR-adhesive microspheres in humans. In addition, rats would be more suitable animal model for formulation screenings of SR-adhesive microspheres at the early stage of drug development. On the other hand, dogs would be more suitable for estimating the dosage form intended for subsequent testing in humans.

References

Akiyama, Y., Horibe, H., Yoshioka, M., Hirai, S., Kitamori, N., Toguchi, H., 1993a. Novel oral controlled-release microspheres using polyglycerol esters of fatty acids. J. Control. Release 26, 1–10.

Akiyama, Y., Yoshioka, M., Horibe, H., Hirai, S., Kitamori, N., Toguchi, H., 1993b. Mechanism of drug release from polyglycerol ester of fatty acid-based microspheres. J. Control. Release 27, 37–45.

Akiyama, Y., Nagahara, N., Kashihara, T., Hirai, S., Toguchi, H., 1995. In vitro and in vivo evaluation of mucoadhesive microspheres prepared for the gastrointestinal tract using polyglycerol esters of fatty acids and a poly (acrylic acid) derivative. Pharm. Res. 12, 397–405.

Akiyama, Y., Nagahara, N., Nara, E., Kitano, M., Iwasa, S., Yamamoto, I., Azuma, J., Ogawa, Y., 1998. Evaluation of oral mucoadhesive microspheres in man on the basis of pharmacokinetics of furosemide and riboflavin, compounds with limited gastrointestinal absorption sites. J. Pharm. Pharmacol. 50, 159–166.

Aoyagi, N., Ogata, H., Kaniwa, N., Uchiyama, M., Yasuda, Y., Tanioka, Y., 1992. Gastric empting of tablets and granules in humans, dogs, pigs, and stomach-emptying-controlled rabbits. J. Pharm. Sci. 81, 1170–1174.

Avdeef, A., Berger, C.M., Brownell, C., 2000. pH-metric solubility. 2. Correlation between the acid–base titration and the saturation shake-flask solubility-pH methods. Pharm. Res. 17, 85–89.

Chiou, W.L., Jeong, H.Y., Chung, S.M., Wu, T.C., 2000. Evaluation of using dog as an animal model to study the fraction of oral dose absorbed of 43 drugs in humans. Pharm. Res. 17, 135–140.

Chiou, W.L., Buechler, P.W., 2002. Comparison of oral absorption and bioavailability of drugs between monkeys and human. Pharm. Res. 19, 868–874.

Chowdary, K.P., Rao, Y.S., 2004. Mucoadhesive microspheres for controlled drug delivery. Biol. Pharm. Bull. 27, 1717–1724.

Dali, M.M., Moench, P.A., Mathias, N.R., Stetsko, P.I., Heran, C.L., Smith, R.L., 2006. A rabbit model for sublingual drug delivery: comparison with human pharmacokinetic studies of propranolol, verapamil and captopril. J. Pharm. Sci. 95, 37–44.

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

Goto, T., Morishita, M., Kavimandan, N.J., Takayama, K., Peppas, N., 2006. Gastrointestinal transit and mucoadhesive characteristics of complexation hydrogels in rats. J. Pharm. Sci. 95, 462–469.

Gruber, P., Longer, M.A., Robinson, J.R., 1987. Some biological issues in oral, controlled drug delivery. Adv. Drug Delivery Rev. 1, 1–18.

Higuchi, T., 1961. Rate of release of medicaments from ointment containing drug in suspension. J. Pharm. Sci. 50, 874–875.

Hildebrand, H., McDonald, F.M., Windt-Hanke, F., 1991. Characterization of oral sustained release preparations of iloprost in a pig model by plasma level monitoring. Prostaglandins 41, 473–486.

Hui, H., Robinson, J.R., 1987. Design and fabrication of oral controlled release drug delivery systems. In: Robinson, J.R., Lee, V.H.L. (Eds.), Controlled Drug Delivery. Marcel Dekker Inc., New York, pp. 373–432.

Itoh, T., Higuchi, T., Gardner, C.R., Caldwell, L., 1986. Effect of particle size and food on gastric residence time of non-disintegrating solids in beagle dogs. J. Pharm. Pharmacol. 38, 801–806.

Kararli, T.T., 1995. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm. Drug Dispos. 16, 351–380.

Kimura, T., Higaki, K., 2002. Gastrointestinal transit and drug absorption. Biol. Pharm. Bull. 25, 149–164.

Kondo, H., Takahashi, Y., Watanabe, T., Yokohama, S., Watanabe, J., 2003a. Gastrointestinal transit of liquids in unfed cynomolgus monkeys. Biopharm. Drug Dispos. 24, 131–140.

Kondo, H., Shinoda, T., Nakashima, H., Watanabe, T., Yokohama, S., 2003b. Characteristics of the gastric pH profiles of unfed and fed cynomolgus monkeys as pharmaceutical product development subjects. Biopharm. Drug Dispos. 24, 45–51.

Lehr, C.M., 1994. Bioadhesion technologies for the delivery of peptide and protein drugs to the gastrointestinal tract. Crit. Rev. Ther. Drug Carrier Syst. 11, 119–160.

Lui, C.Y., Amidon, G.L., Berardi, R.R., Fleisher, D., Youngberg, C., Dressman, J.B., 1986. Comparison of gastrointestinal pH in dogs and humans: implication on the use of the beagle dog as a model for oral absorption in humans. J. Pharm. Sci. 75, 271–274.

Nagahara, N., Akiyama, Y., Nakao, M., Tada, M., Kitano, M., Ogawa, Y., 1998. Mucoadhesive microspheres containing amoxicillin for clearance of *Helicobacter pylori*. Antimicrob. Agents Chemother. 42, 2492–2494.

Nambu, N., 1986. Controlled-release dosage form. In: Sezaki, H. (Ed.), Drug Delivery System, Nankodo, Tokyo, pp. 109–135.

Ogata, H., Aoyagi, N., Kaniwa, N., 1986. Evaluation of beagle dogs as an animal model for bioavailability testing of cinnarizine capsules. Int. J. Pharm. 29, 121–126.

Peppas, N.A., 2004. Devices based on intelligent biopolymers for oral protein delivery. Int. J. Pharm. 277, 11–17.

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

Takeuchi, H., Yamamoto, H., Kawashima, Y., 2001. Mucoadhesive nanoparticulate systems for peptide drug delivery. Adv. Drug Deliv. Rev. 47, 39–54.

Yamada, I., Haga, K., 1990. Measurement of gastric pH during digestion of a solid meal in dogs. Chem. Pharm. Bull. 38, 1755–1756.