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

Yohko Akiyama,[1,4] Naoki Nagahara,[1] Toshio Kashihara,[2] Shinichiro Hirai,[2] and Hajime Toguchi[3]

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Two types of polyglycerol ester of fatty acid (PGEF)-based microspheres were prepared: Carbopol 934P (CP)-coated microspheres (CPC-microspheres) and CP-dispersion microspheres (CPD-microspheres). Comparative studies on mucoadhesion were done with these microspheres and PGEF-based microspheres without CP (PGEF-microspheres). In an in vitro adhesion test, the CPD-microspheres adhered strongly to mucosa prepared from rat stomach and small intestine because each CP particle in the CPD-microsphere was hydrated and swelled with part of it remaining within the microsphere and part extending to the surface serving to anchor the microsphere to the mucus layer. The gastrointestinal transit patterns after administration of the CPD-microspheres and PGEF-microspheres to fasted rats were fitted to a model in which the microspheres are emptied from the stomach monoexponentially with a lag time and then transit through the small intestine at zero-order. Parameters obtained by curve fitting confirmed that the gastrointestinal transit time of the CPD-microspheres was prolonged compared with that of the PGEF-microspheres. MRT in the gastrointestinal tract was also prolonged after administration of the CPD-microspheres compared with that following the administration of the PGEF-microspheres.

KEY WORDS: mucoadhesive microsphere; gastric emptying; gastrointestinal transit; carbopol 934P (CP); polyglycerol ester of fatty acid (PGEF); mean residence time (MRT).

INTRODUCTION

Oral drug delivery is a common route of administration for the systemic delivery of drugs. An extended-release drug delivery system based on osmotic pressure-control has received regulatory approval for marketing, and the pharmaceutical superiority and clinical benefits have been widely recognized (1, 2). However, most drugs delivered via this system, for example phenylpropanolamine HCl, metoprolol, oxoprolol and nifedipine, can be absorbed from both the large and small intestines. Davis *et al.* (3) reported that in the fasted conditions, the gastric emptying time is less than 2 hours regardless of whether a multiple-unit or single-unit dosage form was used; in the fed conditions, it was 4 hours or more. The small intestine transit time was 3~4 hours irrespective of the feeding conditions. This means that a solid dosage form will reach the large intestine in 5~8 hours. Therefore, a sustained-release system does not necessarily maximize the bioavailability of a drug nor optimize clinical treatment if the absorption site is limited to a specific segment of the small intestine. It has been reported that the absorption of chlorothiazide (4), riboflavin (5), and furosemide (6) is site-specific. It is necessary to keep such a drug in the vicinity of the absorption site for a longer period of time by modulating the transit time of the dosage form. Approaches used to prolong gastrointestinal transit time include mucoadhesive gastrointestinal drug delivery systems (4, 7) and intragastric floating gastrointestinal drug delivery systems (8).

Using polyglycerol ester of fatty acid (PGEF), we previously developed oral controlled-release microspheres from which the drug release could be regulated by selecting an appropriate hydrophile-lipophile-balance (HLB)(9). In general, the microspheres offer obvious advantages over a single-unit dosage form; They spread over a large area of the gastrointestinal tract avoiding exposure of the mucosa to high concentrations of the drug and have a lower risk of dose dumping. Feeding conditions have little effect on gastric transit time of the microspheres, while single-unit dosage forms remain in the stomach until the phase III activity of myoelectric migrating contractions (MMC) (10). This means that sustained-release microspheres of a drug with the narrow absorption window cannot be therapeutically beneficial due to their rather short residence time.

The purpose of this study is to add mucoadhesive properties to the PGEF-based microspheres (PGEF-microspheres) to prolong the gastrointestinal transit time and to evaluate their mucoadhesiveness in vitro and in vivo.

MATERIALS AND METHODS

Materials

Tetraglycerol pentastearate (TGPS) and tetraglycerol monostearate (TGMS) were purchased from Sakamoto Yakuhin Kogyo Co. Ltd. (Osaka). Carbopol 934P (CP) was obtained from The BF Goodrich Company (USA, Cleveland). Other chemicals were of reagent grade.

Preparation of Microspheres

PGEF-microspheres: a mixture of TGPS and TGMS was melted at 85 °C and microspheres were prepared by spraying and chilling the melted mixture (9). CP-coated microspheres (CPC-microspheres): PGEF-microspheres (50g) were placed in a centrifugal fluidizer (CF, Freund Industrial Co. Ltd., Japan) and coated by spraying a CP suspension (5w/v %) in methanol from a spray nozzle of 2 mm in diameter at a constant rate of 1 ml/min under the following conditions : a rotating speed , 60 rpm; a spray air rate , 120 l/min;

[1] DDS Research Laboratories, Takeda Chemical Industries, Ltd., Japan.
[2] Pharmaceutical Development Laboratories, Takeda Chemical Industries, Ltd., Japan.
[3] Research on Research, Takeda Chemical Industries, Ltd., Japan.
[4] To whom correspondence should be addressed at DDS Research Laboratories, Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan.

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a spray air pressure, 2.0 kg/cm^2; a slit air rate, 400l/min. A slit air temperature of 46 °C was used in order to achieve a microsphere bed temperature of 32~36 °C. CP-dispersion microspheres (CPD-microspheres): CP was dispersed in the melted TGPS and TGMS in a ratio of 160:5 and microspheres were prepared by spraying and chilling the melted mixture.

These 3 types of microspheres (177-500 μm in diameter) were used for the experiments. The formulations of PGEF-microspheres, CPC-microspheres and CPD-microspheres are shown in Table I.

Testing of Swelling Properties

A drop of water was placed on the CPC-microspheres or CPD-microspheres spread on a slide glass. Five minutes later, the process of swelling and gel-forming of CP was examined with a microscope.

In Vivo Evaluation of Mucoadhesiveness

Male Sprague-Dawley rats (300-400 g) were fasted overnight. PGEF-microspheres, CPC-microspheres or CPD-microspheres were placed in a polyethylene tube (PE260, Nippon Becton Dickinson Co. Ltd.) having one end covered with hydroxypropyl cellulose film. Approximately 50 mg of each type of microspheres was orally adminstered through the polyethylene tube attached to a gastric sonde with 0.2 ml water, i.e., the polyethylene tube containing 50 mg of each type of microspheres was attached to the gastric sonde. The gastric sonde was attached to a microsyringe containing 0.2 ml water. The microspheres and water were pushed out through the polyethylene tube and orally administered to conscious rats (11). After 2.5 hours, each rat was killed by ether, the stomach was opened and the extent of adhesion of the microspheres to the stomach wall was evaluated visually.

In Vitro Evaluation of Mucoadhesiveness

PGEF-microspheres, CPC-microspheres and CPD-microspheres were tested for mucoadhesion using the method designed by Ranga Rao & Buri (12). Briefly, the stomach obtained from male Sprague-Dawley rats (300-400 g) fasted overnight and dissected under ether anesthesia was opened along the great curvature and rinsed in the 10 ml of the 1st fluid specified in JP (Japanese Pharmacopoeia) XII. One thousand ml of 1st fluid contains 2 g of NaCl and 7.0 ml of HCl (pH 1.2). The small intestine (jejunum) obtained from the same rats was cut longitudinally and rinsed in 10 ml of physiological saline. The experiment to evaluate the adhesive properties were started within 2 hours after dissection. A sheet of stomach tissue (2 cm x 1 cm) or jejunum tissue of 4 cm in length was prepared.

Table I. Formulation of PGEF-Microspheres, CPC-Microspheres, and CPD-Microspheres

	Microspheres		
	PGEF-microsphere (g)	CPC-microsphere (g)	CPD-microsphere (g)
TGPS	160.0	160.0	160.0
TGMS	5.0	5.0	5.0
CP	—	50.0	18.0

One hundred PGEF-microspheres, CPC-microspheres or CPD-microspheres were placed uniformly on the mucosa of the stomach or the small intestine which was fixed on a polyethylene support. The tissues with the microspheres were placed in a chamber maintained at 93 % relative humidity and room temperature to allow CP to hydrate and to prevent drying of the mucus. After 20 min, the polyethylene support was adjusted to the inclined position (45 °). The stomach and intestine tissues were rinsed with the 1st fluid and physiological saline for 5 min respectively, at a rate of 22 ml/min. The number of remaining microspheres was then counted.

Measurement of Microspheres Remaining in the Gastrointestinal Tract

Male Sprague-Dawley rats weighing 300-400 g were fasted with free access to water for 24 hours prior to the experiment. One hundred of PGEF-microspheres or CPD-microspheres were administered to each rat (n = 3). At specified time intervals, rats were sacrificed with ether, and the stomach and small intestine were removed. The small intestine was further cut into 3 equal segments and opened longitudinally. The microspheres in the stomach and each intestinal segment were counted.

Model of Gastrointestinal Transit of the Microspheres

Transit of the microspheres through the gastrointestinal tract was evaluated according to a proposed model in which gastric emptying and intestinal transit patterns are expressed with relations containing a lag time as shown in Fig. 1(a).

It was assumed that microspheres administered to a rat were emptied from the stomach monoexponentially after a lag time, Ts, and that transit of the microspheres through each segment of the small intestine was at zero-order. The microspheres proceeded through the upper, middle and lower segment of the small intestine in Tu, Tm and Tl hours, respectively and then reached the colon. Rs, Rsu, Rsm and Rsl represent the % of the microspheres remaining in the stomach, the stomach and the upper intestine (Region I), the stomach and upper and middle intestine (Region II) and the stomach and the entire intestine (Region III), respectively as described in Fig. 1(b). The time at which 50 % of microspheres has been emptied from the stomach is expressed as Ts50. The time at which 50 % of microspheres has reached the colon is expressed as T50. The % of the microspheres remaining in the stomach, Rs at time t is written as

$$Rs = 10^2 \quad (t \leq Ts) \quad \text{Eqn(1)}$$

$$Rs = 10^{2-Ks(t-Ts)} \quad (t > Ts) \quad \text{Eqn(2)}$$

where Ks is defined as the first-order gastric emptying rate constant.

The pattern of gastrointestinal emptying is expressed as follows, assuming that the stomach and the upper segment of the small intestine belong to one compartment defined as Region I and that a microsphere passes through the upper segment of the small intestine in time Tu,

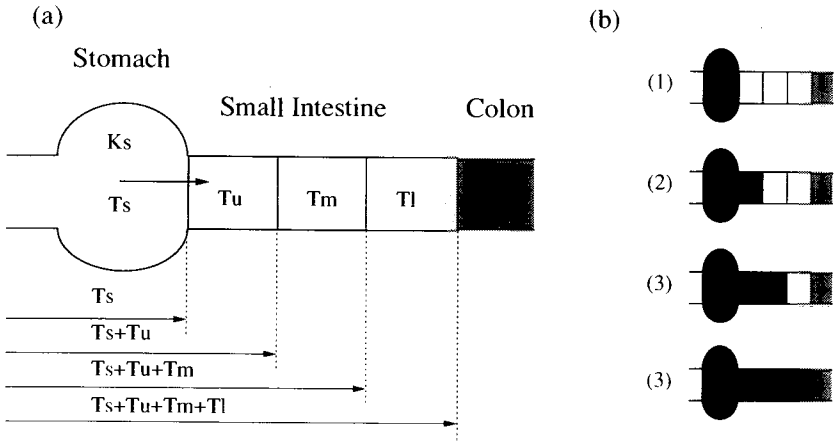

Fig. 1. Gastrointestinal emptying model. (a); Ks, first-order gastric emptying rate constant; Ts, lag time; Tu, Tm and Tl, transit time through upper, middle and lower small intestine. (b); (1), stomach; (2), Region I; (3), Region II, (4), Region III.

$$Rsu = 10^{2} \quad (t \leq Ts + Tu) \quad Eqn(3)$$
$$Rsu = 10^{2-Ks(t-Ts-Tu)} \quad (t > Ts + Tu) \quad Eqn(4)$$

where Rsu is the % of the microspheres remaining in Region I, i.e., the gastrointestinal pattern is expressed as Eqns (3) and (4) which are obtained by shifting the gastric emptying pattern by time Tu, because each microsphere emptying from the stomach passes through the upper segment of the small intestine in time Tu. Similarly, assuming that the stomach and the upper and middle segments of the small intestine belong to one compartment defined as Region II and that a microsphere passes through the middle part of the small intestine in time Tm, the following relations are obtained.

$$Rsm = 10^{2} \quad (t \leq Ts + Tu + Tm) \quad Eqn(5)$$
$$Rsm = 10^{2-Ks(t-Ts-Tu-Tm)} \quad (t > Ts + Tu + Tm) \quad Eqn(6)$$

where Rsm is the % of the microspheres remaining in the Region II. Assuming that the stomach and the entire small intestine are regarded as one compartment (Region III) and that a microsphere passes through the lower part of the small intestine in time Tl, the following relations are obtained.

$$Rsl = 10^{2} \quad (t \leq Ts + Tu + Tm + Tl) \quad Eqn(7)$$
$$Rsl = 10^{2-Ks(t-Ts-Tu-Tm-Tl)} \quad (t > Ts + Tu + Tm + Tl) \quad Eqn(8)$$

where Rsl is the % of the microspheres remaining in the Region III.

The small intestine transit time, Tsi, the time required for 50% of the microspheres to be emptied from the stomach (the mean gastric emptying time), Ts50 and the time required for 50% of the microspheres to arrive at the colon (cecum), T50 are written as

$$Tsi = Tu + Tm + Tl \quad Eqn(9)$$
$$Ts50 = Ts + (2 - \log 50)/Ks \quad Eqn(10)$$

and

$$T50 = Ts + Tsi + (2 - \log o 50)/Ks \quad Eqn(11)$$

respectively.

RESULTS AND DISCUSSION

Swelling and Gel-Forming Properties of the Microspheres

In order to design mucoadhesive microspheres, we tried adding mucoadhesive properties to PGEF-microspheres previously developed in our laboratory and from which drug release can be regulated by selecting an appropriate HLB value of the PGEF (9). CP, a water soluble polymer of acrylic acid and loosely crosslinked with allyl sucrose, was chosen as the adhesive polymer, because it has been reported to have good mucosa-adhesive properties (13, 14) and is listed in National Formulary XVII.

When a drop of water was placed on the surface of the CPC-microspheres, a gel-layer was formed around the microspheres and the gel-layer then swelled and expanded (Fig. 2(a)). On the other hand, when the CPD-microspheres came in contact with a drop of water, the polymer particles dispersed inside the microspheres swelled with part reaching the surface of the microspheres and the remaining part staying within the microspheres as shown in Fig. 2(b). In order to swell as shown in Fig. 2(b), the mean particle size of CP particles need to be small enough (9.43 μm) to be dispersed uniformly in the microspheres in diameter of 177~500 μm. The PGEF-microspheres did not show any change in appearance even when they came in contact with water, i.e., they looked just like the CPD-microspheres shown in Fig. 2(b) "before" (photographs of PGEF-microspheres are not shown).

Mucoadhesion, defined as an adhesion phenomenon occurring between a mucosal membrane covered with mucus and non biological materials consists of a two-step process. The first step is considered to be an interfacial phenomenon influenced by the surface energy effects and spreading of both the mucus and the mucoadhesive hydrogels. The second step involves interdiffusion or interpenetration of polymer chains of both phases. This step (interdiffusion or interpenetration), which requires hydration of the polymers, is influenced by the molecular weight, molecular mobility, viscosity of the adhesive and swelling (and gel-forming) properties of both the adhesive and the mucus (15).

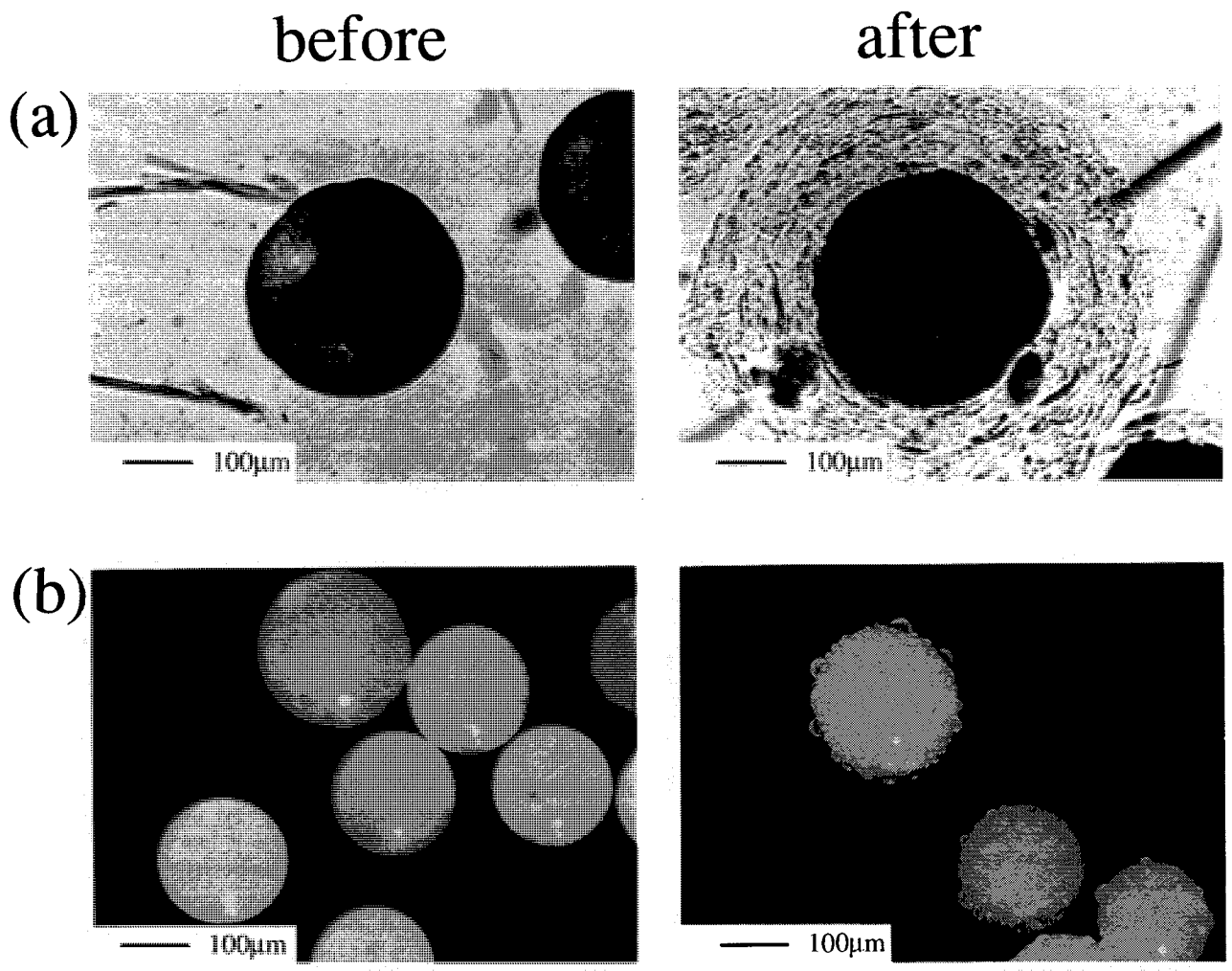

Fig. 2. Micrographs of microspheres 5min before and after contact with water. (a), CPC-microspheres; (b), CPD-microspheres.

In vitro methods for measuring the mucoadhesive strength of adhesive polymers using a modified tensiometer have been reported (7, 15). Park & Robinson (16) showed that the tensile strength of adhesive polymers in the rabbit stomach correlates with their degree of hydration. The degree of hydration is related to the expanded nature of the polymer network. Therefore, swelling is a prerequisite for mucoadhesive drug delivery systems.

Since the CPC-microspheres and CPD-microspheres showed swelling properties, these two types of microspheres were thought to be candidates for a mucoadhesive drug delivery system.

In Vitro Evaluation of Mucoadhesiveness

Table II shows the results of the in vitro adhesive test conducted according to the method which Ranga Rao & Buri (12) reported in order to test the mucoadhesiveness of glass spheres or drug particles coated with an adhesive polymer.

More than 90 % of the CPD-microspheres remained on the gastric mucosa and small intestinal mucosa. The percentage of PGEF-microspheres or CPC-microspheres remaining on the gastric mucosa was less than 10 % after rinsing with the 1st fluid. Neither the PGEF-microspheres nor the CPC-microspheres were retained on the jejunum after rinsing with saline. The mucoadhesion of CP was expected to be directly related to the pH of the medium as was shown in the case of polycarbophil, polyacrylic acid loosely crosslinked with divinylglycol (16). They reported a higher binding ability at pH lower than the pKa of polyacrylic acid (4.75) and suggested that strong mucoadhesion occurs only when the carboxylic groups are in their acid forms. Our results from the in vitro testing of the mucoadhesion of the CPC-microspheres are in agreement with this. The fact that a percentage of the CPD-

Table II. Percent of Microspheres Remaining After Rinsing Mucosa of the Stomach and Small Intestine with 1st Fluid (pH 1.2) and Saline, Respectively

	% of microspheres remaining	
	Stomach 1st fluid (pH 1.2)	Small intestine Saline
PGEF-microspheres	5.5	0.0
CPC-microspheres	7.0	0.0
CPD-microspheres	92.0	94.5

Each value is the mean of two measurements.

microspheres remained on the intestinal mucosa even after rinsing with a physiological saline suggests that the CPD-microspheres adhered to the mucus more strongly than the PGEF-microspheres or the CPC-microspheres.

In Vivo Evaluation of Mucoadhesiveness

The three types of microspheres (PGEF-microspheres, CPC-microspheres and CPD-microspheres) were administered orally to rats fasted for 24 hours. After 2.5 hours, the extent of the adhesion of these microspheres to the gastric mucosa was evaluated visually as shown in Fig. 3. Most of the CPD-microspheres were found in the stomach while the PGEF-microspheres or the CPC-microspheres were hardly found (the photo with PGEF-microspheres is not shown). The adhesion of the CPD-microspheres was so strong that they could not be removed from the stomach even by rinsing the gastric mucosa with water. Taking the results of the swelling test and adhesive properties into consideration, the process of in vivo adhesion is considered to occur as follows. As soon as the CPC-microspheres came in contact with water, CP particles formed a gel-layer around the microspheres. The gel-layer soon separated from the core of the microspheres because the hydrophilic gel lacked affinity for the core. On the other hand, CP particles swelled from within the microsphere to the surface when the CPD-microspheres came in contact with water as shown schematically in Fig. 4. The swollen CP particles were strongly associated with the microsphere, unlike the case of CPC-microspheres, since the CPD-microspheres adhered to the mucosa leaving part of the swollen CP particles behind within the microsphere. Therefore, the microspheres did not detach from the stomach wall. This type of dosage form which consists of CPD-microspheres is referred to as an Adhesive Micromatrix System (AdMMS).

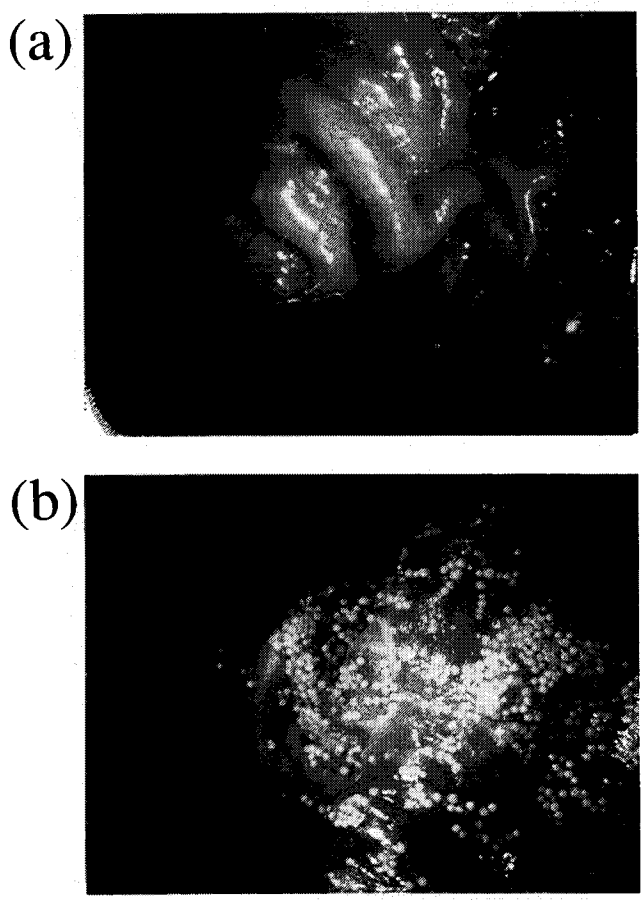

Fig. 3. Microspheres remaining in the stomach 2.5 hours after administration to a rat. (a), CPC-microspheres; (b), CPD-microspheres.

Junginger (15) speculated that differences in crosslinking co-monomer might be responsible for difference in mucoadhesive potential, because there is an optimum chain mobility necessary for the interpenetration with the mucus. When the mobility becomes too high due to excessive swelling (hydration), the adhesive strength decreases. Longer *et al.* (17) who examined a broad range of polymers regarding as to their binding affinity to mucus suggested that water-insoluble polymers such as polycarbophil would offer advantages over water-soluble polymers (18). CP, which was incorporated into our system, is a water-soluble polymer. Therefore, there is a possibility that the mucoadhesive potential of polycarbophil-dispersed microspheres is much stronger than that of CPD-microspheres.

The mucoadhesive drug delivery systems for the gastrointestinal tract require a stronger adhesive potential than a topical application system, such as those for the oral mucosal tissues (buccal, sublingal or gingival), nasal cavity, rectum or skin, because the system has to adhere to the mucosa under dynamic conditions. Therefore, the weight and volume of a mucoadhesive drug delivery system for the gastrointestinal tract and the area for adhesion are important factors. A heavy and bulky dosage form such as a tablet is apt to detach from the mucosa while traveling through the gastrointestinal tract even if the dosage form has attached to the mucosa. Since the CPD-microspheres whose appearance is a powder which is light and small, they are expected to fill the requirements for a mucoadhesive drug delivery system for the gastrointestinal tract.

In Vivo Gastrointestinal Transit Studies

The gastrointestinal transit of the CPD-microspheres in rats was evaluated and compared with that of the PGEF-microspheres as a control. One hour after administering microspheres to conscious rats through the gastric sonde connected polyethylene tube, the percentage of PGEF-microspheres remaining in the stomach was only 20.7 %, and 5 hours later, few PGEF-microspheres were found (Fig. 5). On the other hand, the percentage for CPD-microspheres remaining in the stomach was 78.3 % one hour after administration. Even at 5 hours, 5.3 % could be found in the stomach. The gastric emptying time of the CPD-microspheres was greatly prolonged compared with that for the PGEF-microspheres as shown in Fig. 5. In addition, 89.6 % of the CPD-microspheres were still in the stomach or the small intestine 5 hours after administration, while only 43.0 % of the PGEF-microspheres were in the same region i.e., only 10.4 % of the CPD-microspheres had reached the colon, while more than 57.0 % of the PGEF-microspheres had reached the colon, assuming that the number of the microspheres that reached the colon (cecum) is the difference be-

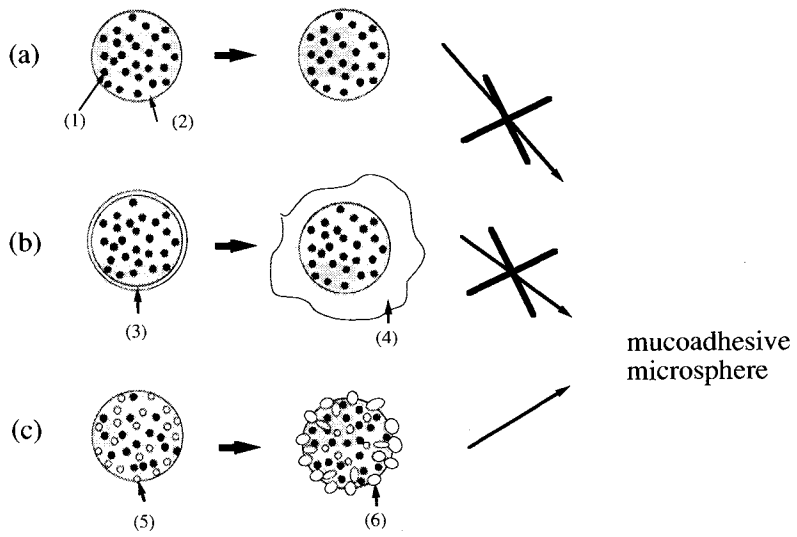

Fig. 4. Schematic description of the mucoadhesion process with microspheres. (a), PGEF-microsphere; (b), CPC-microsphere; (c), CPD-microsphere; (1), drug; (2), PGEF; (3), coated CP layer; (4), swollen CP layer; (5), dispersed CP; (6), swollen CP.

tween the number of microspheres administered and the number of microspheres still in the stomach and the small intestine. The results indicate that the transit of the CPD-microspheres to the colon was prolonged, indicating that the CPD-microspheres hydrated rapidly in vivo, adhered to the mucus-covered mucosa of the stomach or small intestine and remained there for extended periods.

To quantify the rate of the gastric emptying and the small intestine transit, the time-course of distribution of the microspheres (PGEF-microspheres and CPD-microspheres) in the stomach and different segments of the small intestine was calculated using the above mentioned model. Simultaneous fitting the % of the microspheres remaining vs time curves to the equations using a nonlinear least square program (MULTI)(19) gave the parameters listed in Table III. The percentage of microspheres remaining in the stomach and each region were plotted on the computer-generated profiles in Fig. 6. Since the percentage generated by the computer agreed well with the percentage of the CPD-microspheres and PGEF-microspheres remaining in the stomach and small intestine, the model seems to be appropriate for predicting the in vivo distribution of the microspheres.

The gastric emptying rate constant, Ks, after administering the CPD-microspheres to fasted rats was about one-third of the value after administration of PGEF-microspheres. When the CPD-microspheres were administered to rats, the lag time, Ts, was 0.5 hours, while it was nearly zero when the PGEF-microspheres were administered. This means that the PGEF-microspheres were emptied from the stomach exponentially without a lag time as reported by Hunt *et al.* (20) who examined the gastric emptying time of the human stomach and concluded that the gastric emptying of a standard pectin meal (750 ml) was exponential. On the other hand, the large positive Ts value of 0.5, when the CPD-microspheres were administered indicated that they adhered strongly to the gastric mucosa and remained in the stomach for a long period of time.

The calculated transit times of the CPD-microspheres in the upper, middle and lower segment of the small intestine, Tu, Tm and Tl, were longer than those of the PGEF-

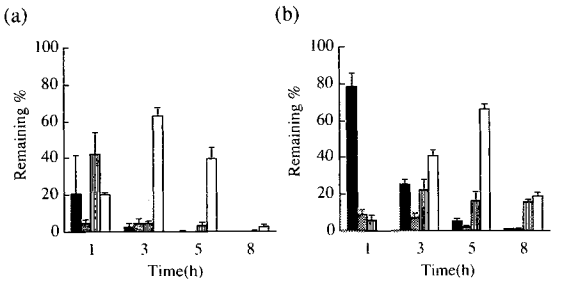

Fig. 5. Distribution of (a) PGEF-microspheres and (b) CPD-microspheres in the stomach and upper, middle and lower segment of the small intestine in rats. (■), stomach; (▨), upper segment; (□), middle segment; (□), lower segment. Data are shown as mean ± SEM (n = 3).

Table III. Rate and Time Constants for Gastrointestinal Transit of PGEF-Microspheres and CPD-Microspheres

	PGEF-microspheres	CPD-microspheres
Ks	0.61/h	0.21/h
Ts	0.0 h	0.5 h
Tu	0.1	0.3
Tm	0.7	1.2
Tl	3.7	4.1
Tu + Tm + Tl	4.5	5.6
Ts50	0.5	1.8
T50	4.9	7.3

Ks, first-order gastric emptying rate constant; Ts, lag time; Tu, Tm or Tl, transit time through upper, middle or lower small intestine; Ts50, time required for 50% of microspheres to leave stomach; T50, time required for 50% of microspheres to reach colon.

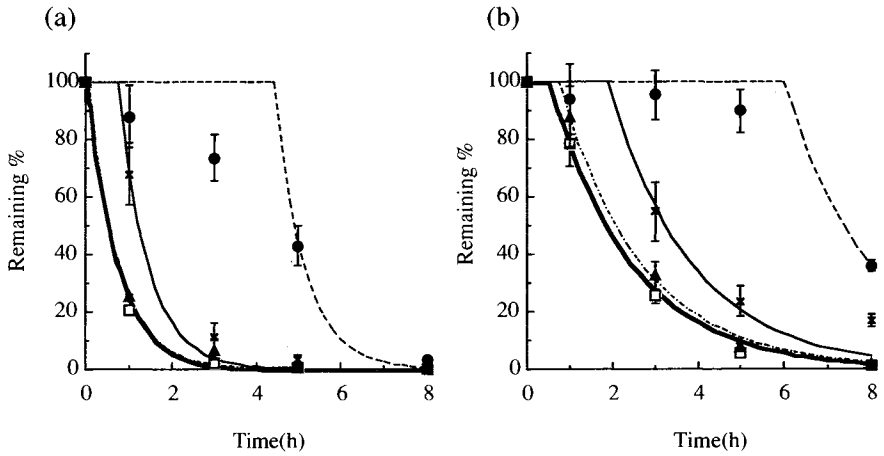

Fig. 6. Percentage of (a) PGEF-microspheres and (b) CPD-microspheres remaining in the stomach, Region I (stomach and upper small intestine), Region II (stomach and upper and middle small intestine) and Region III (stomach and upper, middle and lower small intestine) with computer-generated gastrointestinal transit profiles. (□), stomach; (×), Region I; (▲), Region II; (●), Region III. computer-generated profiles. (_____), stomach; (_._._.), Region I; (_____), Region II; (_ _ _ _), Region III. (n = 3)

microspheres, i.e., the transit of the CPD-microspheres in the small intestine was prolonged by the incorporation of the CP particles.

The mean gastric emptying time for the CPD-microspheres, Ts50 was longer than that for the PGEF-microspheres by 1.3 hours. In conclusion, the gastrointestinal transit time was prolonged by 2.4 hours. In this way, the CPD-microspheres could provide a localized platform in the gastrointestinal tract for drug release and prolong the gastrointestinal transit time.

The model-independent approach based on the center of gravity of the area under the remaining % vs time curve, i.e., the evaluation of the mean residence time (MRT) is useful to characterize the residence time of mucoadhesive microspheres in the gastrointestinal tract. Lehr *et al.* (7) reported that MRT for polycarbophil-coated microspheres resided longer by 20-30 min in the rat intestinal loop compared to the non-coated microspheres. Average values of MRT for PGEF- and CPD-microspheres in stomach, Region I, II and III are shown in Table IV. In each region, CPD-microspheres resided longer compared to PGEF-microspheres. In Region III, i.e., the stomach and the entire small intestine, MRT for CPD-microspheres was higher by 3 hours than that for PGEF-microspheres.

Longer *et al.* (17) reported that a capsule containing polycarbophil and Amberlite 200 resin beads showed a substantially longer gastrointestinal transit time due to mucoadhesion of polymers than the control, a capsule containing Amberlite 200 resin beads, in rat gastrointestinal transit studies using a cross-linked polymer (polycarbophil). They observed bioadhesion of the polymer to the mucin-epithelial surface on animal biopsy and concluded that it caused a delay in transit time. In their experiment, however, each animal was anesthetized. A #3 gelatin capsule containing the test materials was inserted into the stomach through an opening with 4 ml of saline. The opening was then tied with a loop of surgical silk thread. Even if the time interval for surgery was short (~2 min), the effect of anesthesia and surgery on the transit time cannot be neglected. In addition, the volume of test material (150 mg of swellable polymer and 4 ml of saline) is rather large for rats and will give a slower gastric emptying time.

Table IV. MRT of PGEF-Microspheres and CPD-Microspheres in the Stomach, Region I, Region II, and Region III (n = 3)

	MRT $(h)^{0-\infty}$	
	PGEF-microspheres	CPD-microspheres
Stomach	0.57 ± 0.03	1.52 ± 0.05
Region I	0.80 ± 0.13	1.72 ± 0.06
Region II	1.16 ± 0.16	3.75 ± 0.04
Region III	2.61 ± 0.08	5.58 ± 0.07

Data are expressed as mean ± SEM.

The CPD-microspheres which contain only 5~6 mg of CP are not expected to elicit MMC of the fed stomach because the effect of the volume of CP is negligible. Inhibition of the movement of the gastrointestinal tract due to anesthesia is also not an issue because the CPD-microspheres were administered to conscious animals. Therefore it was suggested that the CPD-microspheres adhere to the gastric or intestinal mucosa in the rat with strong affinity due to the swollen CP.

What remains to be understood about the CPD-microspheres is why small intestine transit was delayed only slightly compared with the delay in the gastric emptying rate. Overhydration and overswelling of CP at higher (5~6) pH levels might have resulted in decreased mucoadhesion. Lehr *et al.* (21) reported that a cationic polymer, chitosan, showed the same degree of mucoadhesiveness as polycarbophil when measuring the force of detachment for swollen polymer film from pig mucosa in a saline medium. The mucoadhesion obtained with cationic polymers should be investigated in order to increase the mucoadhesion potential in the small intestine. Irrespective of the intrinsic performance of the polymers used for these dosage forms, the maximal res-

idence time is probably limited by the renewal of mucus gel layers. Turnover of mucin should also be investigated because mucin is always being replaced and this is expected to affect the duration of mucoadhesion. Mucoadhesive drug delivery systems are not expected to adhere for more than 4~5 hours, considering the mucin turnover time (50~270 min) calculated by Lehr *et al.* (22). The effects of the thickness and structure of mucin which differ from animal to animal cannot be neglected (23-25), as is also the case with the characteristics of mucin in disease states and the coadministration of other drugs (26).

Khosla & Davis (27) and Harris *et al.* (28) reported that polyacrylic acid derivative did not retard the human gastric emptying of the pellets in their study using γ-scintigraphy and Russel & Bass (29) reported that the large amount of polycarbophil may have elicited MMC of the fed stomach which would result in a slower rate of gastric emptying. The formulation used by Khosla & Davis (27) was prepared by mixing polycarbophil (in a diameter of 0.5-1.0 mm) and pellets (Amberlite IRA410 anionic resin), and placing the mixture in a #0 hard gelatin capsule. The lack of retardation of the gastric empyting of the pellets may be due to the low affinity between the Amberlite (in a diameter of 0.5-1.0 mm) and polycarbophil. The mucoadhesion of CPD-microspheres to human mucosa should be confirmed, although CPD-microspheres differ from the pellets used by other researchers with regard to affinity for the adhesive polymer.

Considering the strong adhesion of the gastric mucosa, the absorption of the drug which has an absorption window in the upper segment of the small intestine could be enhanced and prolonged by being released slowly. Especially, when being applied to the drug having lower solubility in the stomach, the CPD-microspheres could serve as a reservoir from which the drug release would occur. Effects of the prolongation of the CPD-microspheres through the gastrointestinal tract on the bioavailability of drugs should be investigated in the future.

CONCLUSIONS

Two types of PGEF-based microspheres were prepared: CPC-microspheres and CPD-microspheres. The CPD-microspheres adhered to the mucosa of the rat stomach and small intestine in vitro. The CPD-microspheres adhered more strongly to the mucosa of the rat stomach remained than PGEF-microspheres (without CP) or CPC-microspheres and were still present 2.5 hours after oral administration. The strong adhesion might have resulted from the strong affinity between CP and the microsphere because only part of the swollen CP was pushed out of the microsphere with part remaining behind within the microsphere when a CPD-microsphere came in contact with water whereas the swollen layer of CP separated from the core microsphere entirely due to overhydration when a CPC-microsphere came in contact with water.

The gastrointestinal transit patterns of the microspheres after oral administration to rats fitted the model in which the microspheres were emptied from the stomach monoexponentially with a lag time and traveled through the small intestine linearly. The CPD-microspheres showed a longer gastric emptying time and slower transit in the small intestine than the PGEF-microspheres. In addition, MRT as a model-independent evaluation also showed that the CPD-microspheres resided longer than the PGEF-microspheres in the gastrointestinal tract. The CPD-microspheres should be especially suitable for drugs which act locally in the stomach or those with an absorption window in the upper segment of the small intestine.

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