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Comparison of gastroretentive microspheres and sustained-release preparations using theophylline pharmacokinetics

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Abstract

The objective of this study was to use the pharmacokinetics of theophylline to compare various gastroretentive microspheres. Three types of theophylline microspheres prepared from a hydrophobic dextran derivative were characterized in terms of drug release in-vitro and floating and mucoadhesive properties. Theophylline pharmacokinetic studies were conducted in Beagle dogs, comparing bulk powder, commercial sustained-release granules (Theodur™), sustained-release microspheres, floatable microspheres and mucoadhesive microspheres. Theodur and sustained-release microspheres resulted in a lower maximum concentration (C_{max}) ($P < 0.01$) and larger values for mean residence time (MRT) ($P < 0.05$) than bulk powder, whereas area under the concentraion–time curve (AUC) were lower. The floatable microspheres showed a larger value for MRT than bulk powder ($P < 0.01$), and a larger AUC than Theodur ($P < 0.05$). The pharmacokinetic parameters of the mucoadhesive microspheres indicated an increase in AUC without decreasing the rate of bioavailability. Overall, the gastroretentive microspheres improved the extent of bioavailability of theophylline, which is absorbable from the entire gastrointestinal tract. The mucoadhesive microsphere showed a prolonged serum drug level, indicating a superior sustained-release delivery system for theophylline.

Introduction

In oral controlled-release systems, gastrointestinal (GI) transit time is extremely important for extended drug absorption. Having passed through the absorption site, the released drug is not absorbed any more. A traditional sustained-release formulation releases most of the drug in the colon. If the drug is not absorbable in the colon or throughout the GI tract, drug absorption is unsatisfactory despite excellent in-vitro release profiles (Welling, 1993). Therefore, both prolongation of GI transit time and controlled drug release are required for such systems.

Several approaches have been proposed to retain oral dosage forms in the stomach. These methods include bioadhesive systems, swelling and expanding systems, and floating systems (Bardonnet et al 2006). The mucoadhesive system is one such approach, which involves adherence to the mucin epithelial surface of the GI tract, thus providing a longer transit time. Another approach to increase gastroretention has been to use floating dosage forms. These forms are expected to remain buoyant on the gastric contents because their bulk density is lower than that of the gastric contents.

We have developed mucoadhesive microspheres and floatable microspheres, prepared using an emulsion–solvent evaporation method. Mucoadhesive microspheres prepared using a mixture of dextran sulfate and 2-diethylaminoethyl dextran as mucoadhesive polymers were retained in the stomach for a few hours after oral administration to rats, improving the bioavailability of thiamine disulfide (Miyazaki et al 2003a). The floatable microspheres were prepared from a hydrophobic dextran derivative using a poor solvent addition method (Miyazaki et al 2007). These microspheres were expected to float on the gastric fluid on the basis of results of an in-vitro floating study (Miyazaki et al 2008). In the current study, model microspheres, including mucoadhesive microspheres (MS-adhesive), floatable microsphere (MS-float) and sustained-release microsphere (MS-sustain), were prepared from theophylline and a hydrophobic dextran derivative. Theophylline was used as

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a model drug because it is absorbed from the entire GI tract without undergoing first-pass elimination (Ogiso et al 1993). Hydrophobically modified dextran (used in contact lenses) was selected as a water-insoluble polymer.

The aim of the present study was to evaluate the in-vivo performance of MS-adhesive and MS-float for gastroretentive drug delivery systems. To provide a comparison, Theodur G was used as a reference because it is a clinically acceptable sustained-release dosage form of theophylline. This study also compared the extended drug release characteristics of MS-float and MS-adhesive.

Materials and Methods

Materials

Theophylline was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) and was used after sieving through a 100-mesh sieve. Hydrophobic dextran derivate (PDME) and dextran sulfate (DS, MW 500000) were kindly donated by Meito Sangyo Co., Ltd (Nagoya, Japan). PDME is prepared from dextran (MW 40000) by substitution of 0.6 mol acetyl, 0.8 mol propyl, 1.4 mol butyl and 0.16 mol methacrylate per anhydroglucose unit. [2-(Diethylamino) ethyl] dextran (EA, MW 500000) was purchased from Sigma Co. (St Louis, MI, USA). Acetone, polyoxyethylene (20) sorbitan monooleate (Tween 80) and polyoxyethylene (20) sorbitan monolaurate (Tween 20) were purchased from Wako Pure Chemical Industry Co., Ltd (Osaka, Japan). Liquid paraffin conforming to the Japanese Pharmacopoeia was obtained from Iwaki Seiyaku Co., Ltd (Tokyo, Japan). Sucrose-ester (DKF-10) was generously supplied by Dai-ichi Kogyo Seiyaku Co., Ltd (Kyoto, Japan) and was used as an emulsifier. Ultra-purified water (Direct-Q, Millipore Co., Tokyo, Japan) was used for preparation of the dispersed phase. Theodur G™ (Theodur), in the form of granules containing 20% theophylline, were obtained from Mitsubishi Pharm Co. (Osaka, Japan). 7-(2-Hydroxyethyl)-theophylline (HETH, Tokyo Kasei Kogyo, Tokyo, Japan) was used as an internal standard for the theophylline assay. All other chemicals were of special reagent grade and were used as received.

Preparation of microspheres

Microspheres were prepared by an emulsion–solvent evaporation method using acetone and liquid paraffin as the dispersed phase and dispersion medium, respectively. The formulation and preparative conditions are summarized in Table 1. PDME was dissolved in 15 mL acetone solution. Theophylline was then dispersed in the PDME solution under stirrer agitation. In the case of MS-adhesive, a 3:7 mixture of DS and EA was additionally dispersed. The dispersion was poured into agitated liquid paraffin (150 mL) containing DKF-10 (0.75 g) in a vessel settled into a water bath. Following emulsification for 30 min at 20°C in the water bath, the system was heated to 50°C at a rate of 0.5°C min^{-1}. After allowing the microspheres to settle, the liquid paraffin was decanted off. The microspheres were washed three times with n-hexane and then dried under reduced pressure at room temperature overnight.

Evaluation of microspheres

Product recovery was determined from the weight ratio of dried microspheres to the loaded drug and polymer.

Theophylline content was determined as follows. Approximately 50 mg microspheres were weighed accurately and dissolved completely in methylene chloride. The solution was then filtered (Millex-LG, Millipore Co.) and the theophylline concentration was measured spectrophotometrically at 274 nm. The polymers did not interfere with analysis at this wavelength.

Particle-size distribution was determined by sieving through a set of standard sieves (Martin 1993). The fraction containing the mean diameter was used for further studies.

In-vitro release studies

In-vitro release of theophylline from preparations was investigated using a standard JPXIV dissolution apparatus (Toyama Sangyo Co. Ltd, Osaka, Japan) with a paddle speed of 100 revmin^{-1} in 900 mL of JPXIV first fluid (pH 1.2, HCl/NaCl solution) containing 0.1% (w/v) Tween 80 at 37 ± 0.5°C. Tween 80 was added to each dissolution medium to improve the wetting of the preparations. In the case of Theodur, JPXIV second fluid (pH 6.8, phosphate buffer solution) was used as the dissolution medium because drug release from Theodur was pH-dependent, with neglible release in first fluid. Samples (5 mL) of the solution were withdrawn from the dissolution apparatus at appropriate time intervals, and the volumes were replaced with fresh dissolution medium. The samples were filtered through a membrane filter (pore size 0.45 μm) and the absorbance of the filtrate determined

Table 1 Formulations and preparative conditions for microspheres (MS) prepared by an emulsion–solvent evaporation method

Preparation	Formulation			Condition			
	TH (g)	PDME (g)	DS/EA (g)	Drug loading (%)	Acetone (mL)	Water (mL)	Stirring rate (rpm)
MS-sustain	2.25	6.75	–	25	15.0	–	400
MS-float	2.25	6.75	–	25	13.5	1.5	400
MS-adhesive	1.0	2.25	3.0	16	15.0	–	600

TH, theophylline; PDME, hydrophobic dextran derivate; DS, dextran sulfate; EA, [2-(diethylamino) ethyl] dextran.

spectrophotometrically at 274 nm for theophylline content. The experiments were repeated three times.

In-vitro floating test

The floating behaviours of the preparations were studied using a digital magnetic stirrer (DUS-13, Isuzu Seisakusho Co. Ltd, Tokyo, Japan) at a rotation speed of 200 rev min^{-1} at 37 ± 1°C, soaking 50 particles in 150 mL JPXIV first fluid containing 0.02% (w/v) Tween 20 (Kawashima et al 1992; Umamaheshwari et al 2003). The number of particles remaining buoyant on the test fluid was counted at 0.5, 1, 2, 3, 4 and 6 h. Stirring was stopped during counting and resumed immediately. The experiments were performed in triplicate.

In-vitro mucoadhesive test

Animal experiments complied with the regulations of the Committee on Ethics in the Care and Use of Laboratory Animals at Hoshi University.

An in-vitro mucoadhesive test was carried out, as described by Miyazaki et al (2003b). Briefly, fasted male Sprague–Dawley rats (300–350 g, Sankyo Labo, Co. Ltd, Tokyo, Japan) were killed and the small intestinal tissue was excised and flushed with saline. Five-centimetre segments of jejunum were everted using a glass rod. Ligatures were placed at each end of the segment. One hundred microspheres were scattered uniformly on the everted sac and the sac was suspended by a wire in a 10 mL tube containing 8 mL saline, so that it was completely immersed. The sacs were incubated at 37°C and agitated horizontally. The sacs were taken out of the medium after immersion for 0.5, 1, 2, 3, 4 and 5 h, immediately repositioned as before in a similar tube containing 8 mL fresh saline and unbound microspheres were counted. The percentage adhering was calculated from the equation: % adherence = $(100 - \Sigma Nt)/100 \times 100$, where Nt is the number of unbound microspheres at time t after incubation.

Pharmacokinetic study

Experiments complied with the regulations of the Committee on Ethics in the Care and Use of Laboratory Animals at Hoshi University. Three male Beagle dogs weighing 10.0–11.0 kg were fasted for 18 h before administration. A single dose of each preparation containing 100 mg theophylline in a hard gelatin capsule was administered orally with 50 mL of water. The dogs were kept in the fasting state for 6 h. Blood samples were taken from the forefoot vein at predetermined intervals up to 12 h after dosing. Blank blood samples were collected a few minutes before administering the preparation. Plasma was obtained by centrifugation of the blood at 3000 rev min^{-1} for 3 min, and was stored at −20°C until assay. A crossover design was employed. A minimum interval of 1 week was allowed between trials.

Analysis of theophylline levels in plasma

Plasma concentrations of theophylline were measured by reverse-phase HPLC using HETH as the internal standard (Miyazaki et al 2003a). The apparatus consisted of an LC-10AS pump connected to a SPD-10AV UV/VIS detector and a SIL-10A auto injector (Shimadzu Co., Ltd, Kyoto, Japan). A reverse-phase HPLC column (Shim-pack ODS, 4.6 × 150 mm, Shimadzu) was used at room temperature. The mobile phase was a 1:9 mixture of acetonitrile and 0.01 M acetate buffer (pH 4.0) delivered at a flow rate of 2 mL min^{-1}. The wavelength for determination was 274 nm.

Before analysis, frozen samples were thawed at room temperature and any precipitants removed. A 100 μL aliquot was placed in a centrifuge tube and spiked with 200 μL methanol containing HETH (10 μg mL^{-1}). The mixture was mixed vigorously for 30 s and then centrifuged at 12000 rev min^{-1} for 5 min to remove the protein precipitation. A 100 μL aliquot of supernatant was injected onto the HPLC system.

Pharmacokinetic analysis

The maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}) were determined from the plasma–concentration time curves. The area under the plasma concentration–time curve (AUC) was calculated by the linear trapezoidal method. Mean residence time (MRT) was computed by moment analysis (Yamaoka et al 1978).

Statistical analysis

The percentage of buoyancy and adhering, and pharmacokinetic parameters were compared by analysis of variance followed by Tukey's multiple range test. A difference was considered to be significant when the P value was less than 0.05.

Results

Microsphere characters

All the microspheres, including MS-sustain, MS-float and MS-adhesive, were made of PDME according to previous studies (Miyazaki et al 2003b, 2006, 2007). Table 2 summarizes their characteristics – product recovery, drug content and mean diameter. Product recovery was good, ranging from 81.4 to 88.5%. Drug content of all microspheres tended to be slightly higher than the theoretical drug loading. These results indicate that the reduction in yield was derived from loss of PDME. Concerning mean diameter, MS-adhesive was 1.5-fold larger than the others. MS-adhesive contained DS and EA as mucoadhesive polymers, which did not dissolve in the dispersed phase.

Table 2 Product recovery and particle characters of microspheres

Preparation	Yield (%)	Drug content (%)[a]	Mean diameter (μm)
MS-sustain	88.5	26.9 ± 0.8	853
MS-float	81.4	29.0 ± 0.6	824
MS-adhesive	85.3	17.9 ± 0.1	1233

[a]Mean ± s.d. (n = 3)

These hydrophilic dextrans would have a dominant effect on mean diameter of the microspheres, owing to the large loading amount and their bulky nature. The particle sizes, which ranged from 0.7 to 6.5 mm, did not show any influence on the rate of gastric emptying (Davis et al 1986). Overall, adequate preparations for this study were yielded.

Drug release behaviour

In-vitro release profiles of theophylline from the preparations are shown in Figure 1. The cumulative percentage released in 8 h ranged from 37% to 98%. A wide deviation in the release profiles of the three kinds of microspheres was observed. This indicated that it was difficult to provide the microspheres with same release ability and formulation. As a result, the drug release rate of MS-sustain became quite slow. Theodur was therefore used as a standard sustained-release formulation for further studies. Release data were analysed by fitting to Higuchi's equation (Higuchi 1963) because the preparations had a non-disintegrated matrix. The kinetic parameters of release are listed in Table 3, showing a good fit. Time required for 50% release (T50%) was also provided for comparison. MS-sustain showed the slowest release rate, resulting from their rigid matrix (Miyazaki et al 2006). MS-float released theophylline twice as fast as MS-sustain, indicating a similar rate as Theodur. MS-adhesive showed the fastest drug release, owing to swelling of DS and EA.

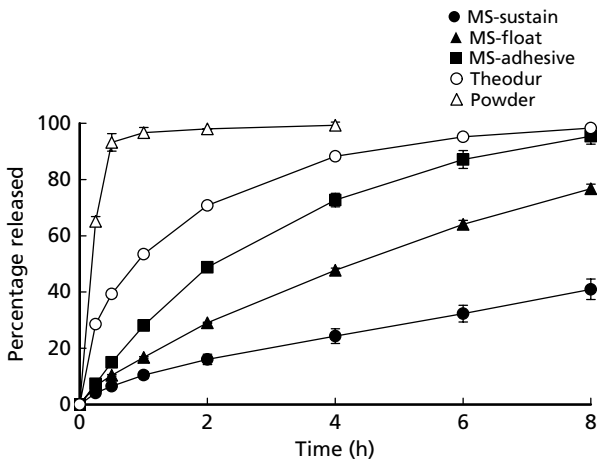

Figure 1 Release profiles of theophylline from various preparations in JPXIV first fluid containing 0.1% (w/v) Tween 80. In the case of Theodur, the release medium was JPXIV second fluid containing 0.1% (w/v) Tween 80. Data are mean ± s.d. (n = 3).

Table 3 Release kinetic parameters for microsphere (MS) formulations and Theodur according to Higuchi's equation

Preparation	Release rate constant (h^{-1})	Correlation coefficient (r^2)	T50% (h)[a]
Theodur	30.4	0.950	0.953
MS-sustain	15.4	0.991	12.6
MS-float	30.7	0.994	4.07
MS-adhesive	39.4	0.990	2.37

[a]Time required for 50% drug release.

In-vitro floating behaviour

The in-vitro floating profiles of the microspheres with Theodur are shown in Figure 2. All preparations except MS-float settled down within 30 min. In contrast, MS-float showed a significant buoyancy at all observed points ($P<0.01$), providing 80% of buoyancy at 5 h. These microspheres are thought to float because of their low density (Miyazaki et al 2008). These results suggested that only MS-float had floatability on the gastric fluid.

In-vitro mucoadhesion

The results of mucoadhesive experiments, presented as percentage adhering versus time curves, are shown in Figure 3.

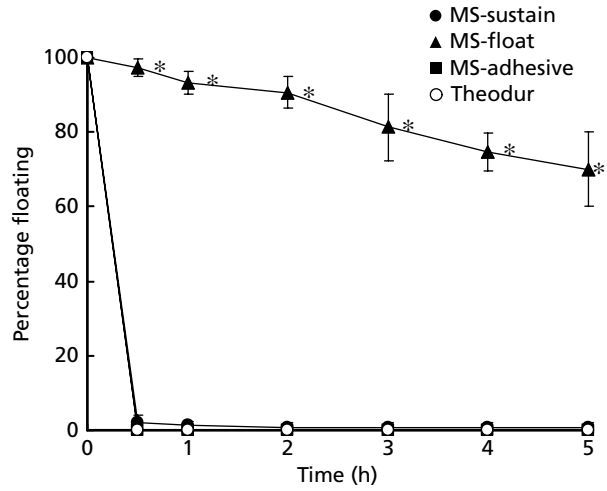

Figure 2 Floating behaviours of various microsphere (MS) preparations in JPXIV first fluid containing 0.02% (w/v) Tween 20 (pH 1.2). Data are mean ± s.d. (n = 3). *$P < 0.01$.

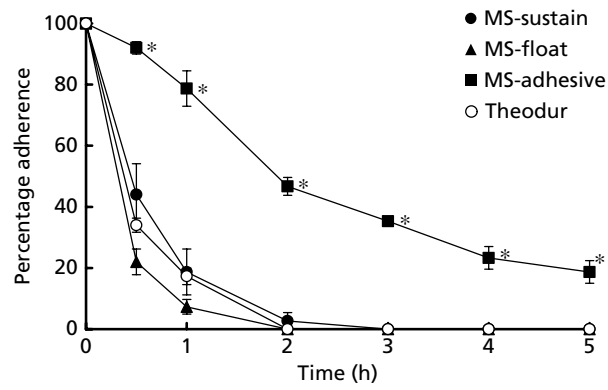

Figure 3 In-vitro mucoadhesion of various microsphere (MS) preparations to the rat small intestinal mucosa. Data are mean ± s.e. (n = 3). *$P < 0.01$.

A high percentage of adhesion indicates that a dosage form has excellent mucoadhesion to mucosal tissue. MS-adhesive separated from the sacs gradually, maintaining 47% adherence until 2 h, whereas detachment of the other preparations was rapid, reaching almost 0% adherence at 2 h. Significant differences were seen between MS-adhesive and the others ($P < 0.01$) at all observation times. These results indicated that only MS-adhesive showed mucoadhesive ability.

In-vivo pharmacokinetics

Plasma theophylline levels were monitored for 12 h after oral administration of 100 mg theophylline to Beagle dogs (Figure 4). The pharmacokinetic parameters are presented in Table 4. The bulk powder had a high C_{max} and a short T_{max} (1.8 h). Compared with the bulk powder, Theodur showed a smaller C_{max} ($P < 0.01$) and larger MRT ($P < 0.01$), indicating excellent sustained-release performance. MS-sustain also showed a smaller C_{max} ($P < 0.01$) and a larger MRT ($P < 0.05$). The AUC value of MS-sustain, however, was lower than that of the bulk powder ($P < 0.01$). This was because almost all of MS-sustain passed through the GI tract, despite incomplete drug release (owing to the slower release rate), as shown in Table 3. On the other hand, MS-float showed a higher MRT than the bulk powder ($P < 0.01$), indicating sustained-release ability. Moreover, MS-float showed a larger AUC value than Theodur ($P < 0.05$) but a similar in-vitro drug release rate. This result indicated that MS-float remained in the GI tract longer than Theodur owing to its floatability.

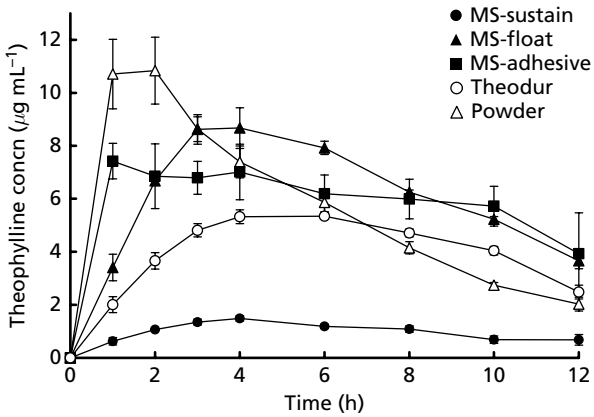

Figure 4 Plasma drug concentration–time profiles after oral administration of theophylline (100 mg) to Beagle dogs. Data are mean ± s.e. (n = 3).

MS-adhesive showed good control of the plasma theophylline level, with a constant level for a longer period of time. The pharmacokinetic parameters showed an increase in AUC and no decrease in C_{max} or T_{max}. The initial phase of the plasma level profile of MS-adhesive implied that some burst release occurred, resulting from an in-vivo situation such as contact with mucin and gastric motility (Vasir et al 2003). It is suggested that mucoadhesive polymers are largely borne off on first contact with the gastric mucosa. Thereafter, the constant drug level indicated a constant drug release rate. With both MS-float and MS-adhesive, the retention in the stomach prolonged overall gastrointestinal transit time, thereby improving the oral bioavailability of theophylline.

Discussion

In this study, we demonstrated that gastroretentive delivery systems such as mucoadhesive and floating systems have an advantage over sustained-release systems, providing improved bioavailability and prolonged plasma drug levels, using theophylline as a representative drug.

Previously, gastroretentive drug delivery systems have been used for drugs with a narrow absorption window, such as sulpiride (Kohri et al 1996), riboflavin (Sato et al 2004) and thiamine disulfide (Miyazaki et al 2003a), achieving improved bioavailability. This was because the absorption window of such drugs was in the upper GI tract, making drug release in the upper GI tract a critical factor that determined drug absorption. By contrast, theophylline is well absorbed, with a broad absorption window. Miyazaki et al (2003a) reported that mucoadhesive theophylline microspheres showed no improvement in bioavailability compared with non-adhesive microspheres because there was no change in the overall GI transit time. In general, prolonging the gastric retention time of a dosage form offers no advantage in terms of the extent of bioavailability for drugs with a broad absorption window in the GI tract (Rouge et al 1996).

Table 4 Pharmacokinetic parameters for various microspheres, Theodur and bulk powder after oral administration at a dose of 100 mg to Beagle dogs

Preparation	C_{max} (μg mL^{-1})	T_{max} (h)	$AUC_{0-12\,h}$ (μg h mL^{-1})	$MRT_{0-12\,h}$ (h)
Powder	11.5 ± 2.8	1.8 ± 0.4	69.3 ± 9.7	4.8 ± 0.3
Theodur	5.48 ± 0.35**	4.7 ± 1.2	48.4 ± 2.0	6.3 ± 0.2**
MS-sustain	1.51 ± 0.17**	5.3 ± 2.3	11.8 ± 1.4**††	5.8 ± 0.5*
MS-float	8.98 ± 1.4	4.3 ± 1.5	74.5 ± 4.1†	6.0 ± 0.1**
MS-adhesive	9.47 ± 1.2	2.7 ± 2.9	88.9 ± 17††	5.5 ± 0.3

C_{max}, maximum plasma theophylline concentration; T_{max}, time to C_{max}; $AUC_{0-12\,h}$, area under the 0–12 h plasma concentration–time curve; $MRT_{0-12\,h}$, 0–12 h mean residence time.
Data are mean ± s.d. (n = 3). *$P < 0.05$, ** $P < 0.01$ vs powder; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs Theodur.

However, the present results provide evidence that gastric retention improves the AUC of theophylline (Table 4). In MS-float, both the rate and extent of absorption were increased compared with Theodur, whereas MS-float and Theodur showed similar release rates in-vitro (Table 3). Sawamoto et al (1997) reported that absorption of theophylline is much faster in the upper segment than in the lower segments of the GI tract. MS-float remained in the stomach, releasing the drugs in the upper GI tract. Thus, the AUC of MS-float was larger than that of Theodur. In addition, the plasma concentration–time curve of MS-float indicated that drug absorption almost ended in the first half (by 6 h). By contrast, the absorption from Theodur was suppressed in the later phase despite excellent release performance in-vitro. This decreased absorption rate suggests that drug release from dosage forms was depressed in the colon (as theophylline is absorbable even in the colon). It has been reported that the lower bioavailability after dosing of oral sustained-release formulations derives from reduced release in the lower GI tract (Shameen et al 1995; Sako et al 1996).

By contrast, drug absorption from MS-adhesive was not changed in the late phase, indicating that drug release from the particles was not altered in the lower GI tract. Using metoprolol, which is absorbable drug from the whole GI tract, Nakamura et al (2006) reported that granules coated with a mixture of ethylcellulose, carbopol and hydroxypropylmethylcellulose achieved a higher plasma metoprolol level over protracted periods compared with granules coated with a mixture of ethylcellulose and hydroxypropylmethylcellulose. They suggested that the swelling of carbopol in the coating layer contributed to continuous drug release in the lower GI tract where only a small volume of water was available. In MS-adhesive, the mixture of DS/EA incorporated into the microspheres would play a role in providing water channels for the drug to diffuse out through. Thus, the amount of drug released from MS-adhesive was thought to be larger than that from Theodur during transit of the colon.

Conclusion

Sustained-release preparations often result in reduced bioavailability, even in the case of theophylline, which is well absorbed from the entire GI tract, because of the shortage of transit time in the GI tract to allow complete drug release. We employed floating and mucoadhesive systems for theophylline in order to prolong the residence time of the preparations in the stomach, resulting in improved bioavailability. The systems would be useful for ensuring the performance of sustained-release preparations in-vivo.

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