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Evaluation of Oral Mucoadhesive Microspheres in Man on the Basis of the Pharmacokinetics of Furosemide and Riboflavin, Compounds with Limited Gastrointestinal Absorption Sites

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Abstract

When sustained-release adhesive and non-adhesive microspheres which release the same drugs at similar rates are administered orally, drug absorption after administration of adhesive microspheres should, if the gastrointestinal residence of adhesive microspheres is prolonged as a result of mucoadhesion, be higher than that after administration of non-adhesive microspheres. The gastrointestinal transit of oral adhesive microspheres in man has been evaluated pharmacokinetically using furosemide and riboflavin, compounds with limited absorption sites in the upper small intestine.

In a preliminary experiment with fasted rats it was confirmed that a higher percentage of the drug remained in the stomach and that plasma drug levels were higher when furosemide was administered in the form of adhesive rather than non-adhesive microspheres. Two kinds of sustained-release microsphere, adhesive and non-adhesive, containing furosemide and riboflavin in hard gelatin capsules were prepared and orally administered to 10 healthy fasted volunteers in a cross-over design. Areas under the plasma concentration–time curves (AUC) were 1·8 times larger for furosemide and urinary recovery was 2·4 times higher for riboflavin when adhesive microspheres rather than when non-adhesive microspheres were used. When adhesive microspheres containing riboflavin were administered to fed volunteers, urinary recovery was 2·1 times higher and mean residence time (MRT) was more prolonged than when the microspheres were administered to fasted volunteers.

Adhesive microspheres were found to adhere to the gastric or intestinal mucosa with high affinity in man and rats, resulting in prolonged gastrointestinal residence.

Recent pharmaceutical investigations have produced several oral mucoadhesive drug-delivery systems which retain drugs in the gastrointestinal tract (Lehr et al 1990; Lenaerts et al 1990; Mathiowitz et al 1997). Mucoadhesive dosage-forms are useful both for drugs with bioavailability problems, because of a specific absorption window, and for drugs which have pharmacological effects as a result of direct contact with mucin or epithelial cell membranes, e.g. drugs for eradication of *H. pylori* (Kimura et al 1995). Although prolongation of gastrointestinal transit time and high bioavailability of chlorothiazide contained in a mucoadhesive dosage-form were confirmed in experiments using rats (Longer et al 1985), the gastrointestinal residence of mucoadhesive dosage-forms in man was not prolonged (Khosla & Davis 1987; Harris et al 1990).

We have already confirmed by direct observation of the inner gastrointestinal tract and evaluation of gastrointestinal transit patterns of microspheres after oral administration (Akiyama et al 1995) that mucoadhesive microspheres, referred to as adhesive micro-matrix system, 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, adhere to the rat gastrointestinal mucosa and prolong gastrointestinal residence.

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However, it is impossible to confirm directly the adhesion of mucoadhesive dosage-forms to the gastrointestinal mucosa in man without endoscopy or γ-scintigraphy (Wilding et al 1991). γ-Scintigraphy can be performed with formulations containing radioisotopes, e.g. ^{99m}Tc and ^{111}In, or the use of stable isotopes (e.g. ^{152}Sm and ^{170}Er in the oxide form) and neutron activation methods. Although the use of stable isotopes eliminates the need to handle radioactive materials and enables dosage-form manufacture to be conducted under normal production conditions, the effects of γ-emitting isotopes such as ^{153}Sm and ^{171}Er on the characteristics of formulations cannot be avoided—i.e. it is difficult to retain radiolabels within the mucoadhesive microspheres in the form of a spherical micro-matrix whose appearance is a powder, yet monitoring a dosage form in the body cannot be performed unless the radiolabels can be prevented from leaching out (Khosla et al 1989). Endoscopic procedures can cause significant discomfort to volunteers in addition to their being inappropriate for obtaining periodic information. Therefore, we have designed an experiment which could indirectly confirm the ability of a mucoadhesive dosage-form to adhere to the gastrointestinal mucosa and prolong the residence of a drug in the gastrointestinal tract, i.e. we planned to compare the pharmacokinetics of a drug, used as a marker, after its administration in both adhesive and non-adhesive microspheres. In this study, we selected as model drugs furosemide and riboflavin, whose absorption is limited to the upper part of the gastrointestinal tract (Stripp 1965; Graul et al 1985). When a sustained-release dosage-form containing furosemide or riboflavin was administered it could pass through the absorption window and reach the colon before releasing all its furosemide or riboflavin content. However, when the adhesive dosage form could, because of its adhesion to the mucosa, reside for longer periods in the stomach or the upper small intestine, close to the absorption window, the bioavailability would be expected to improve compared with that of a non-adhesive sustained-release dosage-form. In other words, if the bioavailability of furosemide or riboflavin from the adhesive sustained-release dosage-form is higher than that from a non-adhesive sustained-release dosage-form which releases furosemide or riboflavin at the same rate, we could conclude that the adhesive form really does adhere to the gastrointestinal mucosa, has extended residence in the gastrointestinal tract and enables the drug to be absorbed more effectively.

The purpose of this study was to evaluate the ability of the mucoadhesive microspheres to prolong gastrointestinal residence, i.e. to adhere to the gastrointestinal mucosa in man, by using two drugs with narrow absorption windows.

Materials and Methods

Materials

Tetraglycerol hexabehenate, tetraglycerol pentastearate 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, Japan) and furosemide from Hoechst, Japan. Riboflavin, Japanese Pharmacopoeia (JP) XII grade, was purchased from Tokyo Tanabe (Tokyo, Japan). Lactose was of JP grade. Other chemicals were of analytical grade.

Preparation of microspheres

Non-adhesive microspheres. Furosemide (10% w/w) or riboflavin (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-melted non-adhesive microspheres were prepared by dropping the molten mixture on to a 15-cm diameter aluminium disc rotating at 2000–3000 rev min^{-1}. 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 1993).

Adhesive microspheres. Furosemide (10% w/w) or riboflavin (10% w/w) and carboxyvinyl polymer (15% w/w and 20% w/w for furosemide and riboflavin, respectively) were dispersed in a molten mixture (65 : 10 for furosemide and 60 : 10 for riboflavin) of tetraglycerol hexabehenate–tetraglycerol monostearate (75% w/w for furosemide and 70% w/w for riboflavin) at 90 °C. Adhesive microspheres were prepared by the method used for non-adhesive microspheres. Non-adhesive and adhesive microspheres which release drugs at a similar rate were prepared by mixing tetraglycerol hexabehenate, tetraglycerol pentastearate and tetraglycerol monostearate (hydrophile–lipophile-balance values 1·8, 2·6 and 8·4, respectively) in the appropriate ratio.

Both non-adhesive and adhesive microspheres in the size range 177–500 μm, obtained by sieving, were used for animal experiments, and #3 gelatin capsules filled with 100 mg of the microspheres containing 10 mg furosemide or riboflavin were used for release studies and studies with man. The formulations of capsules containing non-adhesive and adhesive microspheres are shown in Table 1.

Table 1. Formulations of capsules filled with non-adhesive or adhesive microspheres containing furosemide or riboflavin.

	Contents of one capsule (total 100 mg)				
	Furosemide (mg)	Riboflavin (mg)	Polyglycerol ester of fatty acid (mg)	Carboxyvinyl polymer (mg)	Lactose (mg)
Non-adhesive; furosemide	10	–	60	–	30
Non-adhesive; riboflavin	–	10	60	–	30
Adhesive; furosemide	10	–	75	15	–
Adhesive; riboflavin	–	10	70	20	–

In-vitro release test

The in-vitro release of each drug from the capsule was measured using the USP XXII paddle apparatus (100 rev min^{-1}) at 37 °C in 900 mL of the 1st fluid (pH 1·2) specified in JP XII containing sodium dodecylsulphate (3·0 or 5·0% for furosemide and riboflavin, respectively) to improve wettability and solubility. The amount of furosemide released was determined by high-performance liquid chromatography (HPLC) with fluorimetric detection (λ_{ex} 250 nm and λ_{em} 389 nm) using a YMC-Pack ODS-A column (YMC, Japan) with 65 : 35 phosphate buffer (0·08 M, pH 7·2)–CH_3CN as mobile phase (flow rate 1·0 mL min^{-1}). The amount of riboflavin released was determined by HPLC with fluorimetric detection (λ_{ex} 450 nm and λ_{em} 530 nm) using a YMC-Pack ODS-A column with 265 : 35 KH_2PO_4 (0·05 M)–CH_3CN as mobile phase (flow rate 1·2 mL min^{-1}).

Administration of suspensions and microspheres to rats

Male Sprague-Dawley rats, 8 weeks old, were fasted overnight. Non-adhesive and adhesive microspheres containing furosemide or riboflavin (10 mg kg^{-1}) were placed in a polyethylene tube (PF260; Nippon Becton Dickinson) with one end covered with hydroxypropylcellulose film and administered orally by attaching the tube to a gastric sonde which was also attached to a microsyringe containing 0·2 mL of water. The microspheres were pushed through the polyethylene tube and orally administered to conscious rats. A methylcellulose suspension of furosemide (0·5% w/v, 0·5 mL) was administered using a gastric sonde.

Determination of amount of drug remaining in the rat's stomach after oral administration of microspheres

Two hours after oral administration of non-adhesive or adhesive microspheres to fasted rats, each rat was killed with ether, and the stomach was excized. Each stomach was put into a mixture of phosphate buffer (0·08 M, pH 7·2) and CH_3CN (65 : 35; 100 mL) for determination of furosemide or into distilled water–CH_3CN (50 : 50; 100 mL) for determination of riboflavin, incubated at 80 °C for 15 min, sonicated for 15 min and, after cooling to room temperature, filtered through a 0·45-μm membrane filter (Acrodisc LC PVDF; Gelman Sciences). The amounts of furosemide and riboflavin were determined by the HPLC methods used for the in-vitro dissolution test; before analysis the riboflavin extract was diluted 11-fold with 0·05 M acetate buffer (pH 4·7).

Determination of plasma concentrations of furosemide after oral administration to rats

Blood samples (200 μL) were withdrawn periodically from the rat's tail vein and added to CH_3CN (400 μL). The mixture was agitated vigorously for 1 min and centrifuged at 3000 rev min^{-1} for 15 min. The concentration of furosemide in the supernatant was determined by the HPLC method described for the in-vitro dissolution test.

The maximum plasma concentration, C_{max}, and the time, T_{max}, required to reach C_{max} were obtained from the individual plasma furosemide concentration curves. The area under the plasma concentration–time curve up to 24 h after administration, AUC_{0-24h}, was calculated by the trapezoidal method, and the mean residence time, MRT, was calculated by model-independent statistical moment analysis (Yamaoka et al 1978).

Administration to volunteers of capsules containing microspheres

Two kinds of study were performed on man. Step I was performed after fasting to evaluate the adhesiveness of adhesive microspheres. Step II was performed to evaluate the effect of food on the adhesiveness of riboflavin adhesive microspheres. The protocols of the Step I and Step II studies were approved by the IRB of Osaka Pharmacology Research Clinic. Freely given informed consent was obtained from every subject before participation in the trial.

Ten healthy male volunteers, 20–26 years old, participated in both the Step I and Step II absorption studies. Volunteers were judged to be healthy on the basis of their medical history, physical examination and absence of clinically significant abnormal laboratory values, i.e., full blood exam-

ination and urine analysis were performed. The volunteers were instructed not to drink other than that indicated and not to smoke from 10 h before (2300 h) to 12 h after administration. Volunteers were also instructed not to take any medication 1 week before and during the study period. Participants in the Step I study fasted for 10 h before the dose and for a further 6 h afterwards. Participants in the Step II study received a standard breakfast (641 kcal total; 33·4 g protein, 13·9 g fat and 89 g carbohydrate) 30 min before the dose and fasted for 6 h afterwards. Each volunteer received 200 mL water every 2 h up to 10 h after administration of the capsule. In each step, absorption studies were performed in a 2×2 cross-over design.

Step I study. Ten healthy volunteers were separated into two groups. Two capsules, a capsule filled with adhesive microspheres containing furosemide (adhesive/furosemide-capsule) and a capsule filled with adhesive microspheres containing riboflavin (adhesive/riboflavin-capsule), were administered with 200 mL water to each volunteer in the first group, and two capsules, a capsule filled with non-adhesive microspheres containing furosemide (non-adhesive/furosemide-capsule) and a capsule filled with non-adhesive microspheres containing riboflavin (non-adhesive/riboflavin-capsule), were administered in the same manner to each of the five volunteers in the second group. Two weeks later an adhesive/furosemide-capsule and an adhesive/riboflavin-capsule were administered to each of the five volunteers in the second group and a non-adhesive/furosemide-capsule and a non-adhesive/riboflavin-capsule were administered to each of the five volunteers in the first group.

Step II study. The Step II study was performed in the same way as the Step I study. Ten healthy volunteers were separated into two groups. An adhesive/riboflavin-capsule was administered to each volunteer in the first, fasted, group and an adhesive/riboflavin-capsule was administered to each volunteer in the second group 30 min after breakfast. Two weeks later an adhesive/riboflavin-capsule was administered after breakfast to each of the five volunteers in the first group and each of the five volunteers in the second group after fasting.

In each study plasma and urine were collected 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after administration, and stored at $-20\,^{\circ}C$ until analysis for furosemide or riboflavin.

Determination of plasma concentrations of furosemide and urinary concentrations of riboflavin after oral administration to volunteers

All determinations of furosemide and riboflavin in plasma and urine were performed by Takeda Analytical Laboratories (Osaka, Japan). Plasma furosemide levels were determined by the analytical procedure described for determination of levels of furosemide in rat plasma. Urinary riboflavin concentrations were determined by the HPLC method described for the in-vitro dissolution test, after appropriate dilution. Urinary recovery of riboflavin during the first 24 h after administration, $Recovery_{0-24h}$, the maximum urinary excretion rate, R_{max}, and the time, T_{max}, required to reach R_{max} were determined from the individual urinary excretion rate–time curves, a plot of urinary excretion rate against the mid-point of a urine collection interval. MRT was also calculated on the basis of the urinary excretion rate–time curve.

Statistical analysis

Analysis of variance was performed by use of the SAS GLM procedure (SAS/STAT User's Guide, Version 6, 4th Edition, SAS Institute, USA, 1990) on C_{max}, T_{max}, AUC_{0-24h} and MRT for plasma data and on $Recovery_{0-24h}$, R_{max}, T_{max} and MRT for urinary recovery data.

Results

The amount of furosemide and riboflavin remaining in the stomach of rats after oral administration of non-adhesive and adhesive microspheres

Adhesion of adhesive microspheres was observed for both furosemide and riboflavin; after fasting the amount of the drugs remaining in the rat stomach 2 h after administration of adhesive microspheres, $43{\cdot}9 \pm 18{\cdot}8\%$ and $55{\cdot}1 \pm 12{\cdot}1\%$, respectively, was higher than after administration of non-adhesive microsphere, $4{\cdot}0 \pm 3{\cdot}0\%$ and $0{\cdot}6 \pm 0{\cdot}6\%$, respectively (values are means $\pm$ s.e.). Because the in-vitro release profiles of furosemide and riboflavin in pH 1·2 simulated gastric juice (JP XII) were similar for adhesive and non-adhesive microspheres (Figure 1), it is inferred that the difference between the amounts remaining in the stomach results from differences between the adhesiveness of the two kinds of microsphere.

Absorption of furosemide after administration of non-adhesive and adhesive microspheres to rats

The plasma profiles after administration of a methylcellulose suspension, non-adhesive or adhesive microspheres containing $10\,mg\,kg^{-1}$ furosemide to fasted rats are shown in Figure 2. After administration of the methylcellulose suspension the plasma concentration of furosemide reached C_{max} ($2{\cdot}4 \pm 0{\cdot}6\,\mu g\,mL^{-1}$) at $0{\cdot}8 \pm 0{\cdot}4$ h and then decreased rapidly whereas both non-adhesive and adhesive microspheres resulted in furosemide

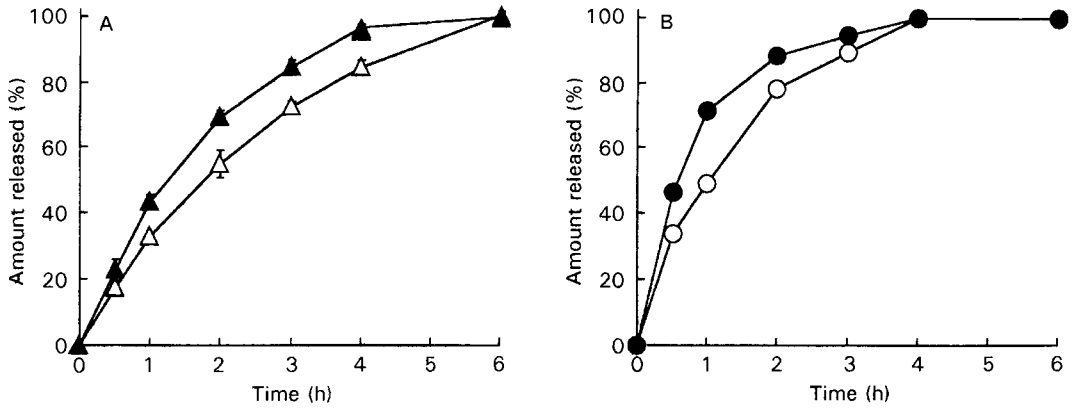

Figure 1. Release profiles of (A) furosemide from non-adhesive (△) and adhesive (▲) microspheres, and (B) riboflavin from non-adhesive (○) and adhesive (●) microspheres (mean ± s.d., n = 3).

plasma profiles characteristic of a sustained-release formulation. The mean pharmacokinetic parameters in Table 2 show that the AUC value after administration of adhesive microspheres was 1·8 times that after administration of non-adhesive microspheres, and the MRT value was longer after administration of adhesive microspheres than after administration of non-adhesive microspheres.

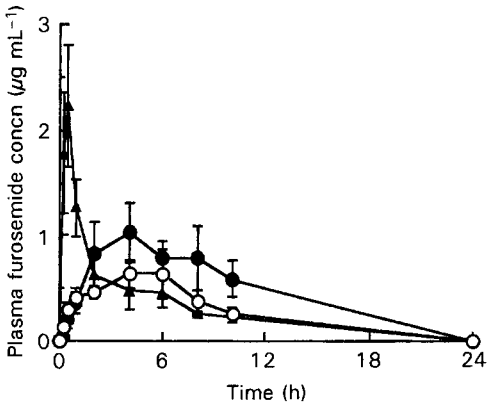

Figure 2. Plasma levels of furosemide after oral administration of 10 mg kg^{-1} as a 0·5% methylcellulose suspension (▲), as non-adhesive microspheres (○) and as adhesive microspheres (●) to fasted rats (mean ± s.e., n = 5).

Study with man

Administration of non-adhesive and adhesive microspheres containing furosemide or riboflavin to fasted volunteers. Pharmacokinetic parameters measured after administration of furosemide are shown in Table 3 and Figure 3. Statistical comparison of AUC_{0-24h} values indicated a significant difference ($P < 0·01$) between results obtained from non-adhesive and adhesive microspheres, i.e. the AUC_{0-24h} measured after administration of adhesive microspheres was 1·8 times that for non-adhesive microspheres. Statistical comparison of C_{max} parameters also indicated a significant difference ($P < 0·01$) between the results obtained from non-adhesive and adhesive microspheres. The absorption of riboflavin was evaluated from urinary excretion data (Table 4 and Figure 4). Statistical comparison of $Recovery_{0-24h}$ parameters indicated a significant difference ($P < 0·01$) between results from non-adhesive and adhesive microspheres, i.e. $Recovery_{0-24h}$ for adhesive microspheres was 2·27 ± 0·38 mg, whereas that for non-adhesive microspheres was 0·96 ± 0·15 mg. Statistical comparison of R_{max} parameters also indicated a significant difference ($P < 0·05$) between results from non-adhesive (0·18 ± 0·02 mg h^{-1}) and adhesive

Table 2. Pharmacokinetic parameters of furosemide after oral administration of 10 mg kg^{-1} as a 0·5% methylcellulose suspension or as non-adhesive or adhesive microspheres, to fasted rats.

	Formulation		
	Suspension	Non-adhesive microspheres	Adhesive microspheres
Area under the plasma concentration–time curve from 0–24 h (μg h mL^{-1})	7·41 ± 0·98	6·56 ± 0·93	11·57 ± 1·84
Maximum plasma concentration (μg mL^{-1})	2·4 ± 0·6	0·9 ± 0·2	1·5 ± 0·1
Time of maximum plasma concentration (h)	0·8 ± 0·4	5·0 ± 1·3	4·0 ± 1·0
Mean residence time (h)	4·3 ± 0·4	6·1 ± 0·6	6·7 ± 0·7

Each value is the mean ± s.e.

Table. 3 Pharmacokinetic parameters of furosemide after oral administration of 10 mg in non-adhesive or adhesive microspheres to fasted volunteers.

	Non-adhesive	Adhesive
Area under the plasma concentration–time curve from 0–24 h ($\mu g\,h\,mL^{-1}$)	0·328 ± 0·035	0·591 ± 0·034*
Maximum plasma concentration ($\mu g\,mL^{-1}$)	0·077 ± 0·009	0·202 ± 0·036*
Time of maximum plasma concentration (h)	3·3 ± 0·4	2·5 ± 0·5
Mean residence time (h)	4·4 ± 0·2	3·5 ± 0·4

Each value is the mean ± s.e. (n = 10). *$P < 0·01$, significantly different from result for non-adhesive microspheres.

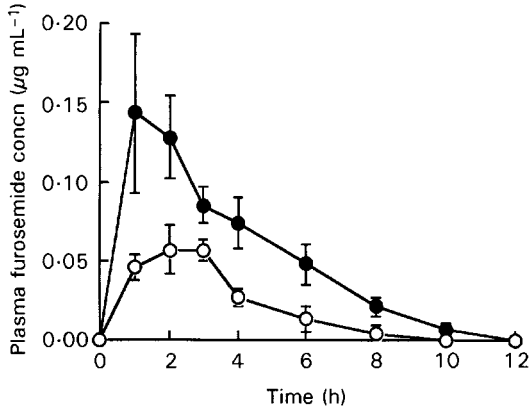

Figure 3. Plasma levels of furosemide after oral administration of 10 mg as non-adhesive (○) or adhesive (●) microspheres to fasted volunteers (mean ± s.e., n = 10).

($0·60 \pm 0·15\,mg\,h^{-1}$) microspheres. There was no significant difference between T_{max} or MRT for riboflavin or furosemide.

Effect of food on absorption of riboflavin after administration of adhesive microspheres. The Step II study was performed to determine whether adhesive microspheres would work appropriately when volunteers were fed. As shown in Table 5, $Recovery_{0-24h}$ of riboflavin was more than twice as high for fed volunteers than for fasted. After administration to fed volunteers both T_{max} and MRT were prolonged (Table 5 and Figure 5). Statistical comparison of $Recovery_{0-24h}$, T_{max} and MRT values indicated that there were significant differences between the absorption profiles obtained from fasted and fed volunteers, and absorption from the adhesive microspheres was greater.

Discussion

The improved absorption of furosemide obtained in our experiment with rats suggests that furosemide was released slowly from adhesive microspheres adhering to a more proximal area of the gastrointestinal tract, rather than the absorption window, and was effectively absorbed from the absorption window. Lehr et al (1992) reported that the in-vivo absorption of 9-desglycineamide, 8-arginine vasopressin from the rat small intestine was not improved after administration in the form of controlled-release microspheres coated with an adhesive polyacrylic acid derivative, whereas it was improved when administered in a liquid dispersion of the polyacrylic acid derivative. Therefore, there is little possibility that the polyacrylic acid derivative in the solid form dispersed in adhesive microspheres could work as an absorption enhancer. The reason the AUC_{0-24h} value of furosemide was smaller after administration as non-adhesive microspheres might be because the microspheres passed through the absorption window before all the furosemide was released, i.e. the assumption mentioned above seems to be correct. These results indicate that mucoadhesiveness can be evaluated by comparing availability, as determined by measurement of AUC or urinary recovery, after the administration of adhesive and non-adhesive

Table 4. Pharmacokinetic parameters of riboflavin after oral administration of 10 mg in non-adhesive or adhesive microspheres to fasted volunteers.

	Non-adhesive	Adhesive
Recovery from 0–24 h (mg)	0·96 ± 0·15	2·27 ± 0·38†
Maximum urinary excretion rate ($mg\,h^{-1}$)	0·18 ± 0·02	0·60 ± 0·15*
Time of maximum urinary excretion rate (h)	2·6 ± 0·4	2·1 ± 0·3
Mean residence time (h)	5·6 ± 0·4	4·8 ± 0·3

Each value is the mean ± s.e. (n = 10). *$P < 0·05$, †$P < 0·01$, significantly different from result for non-adhesive microspheres.

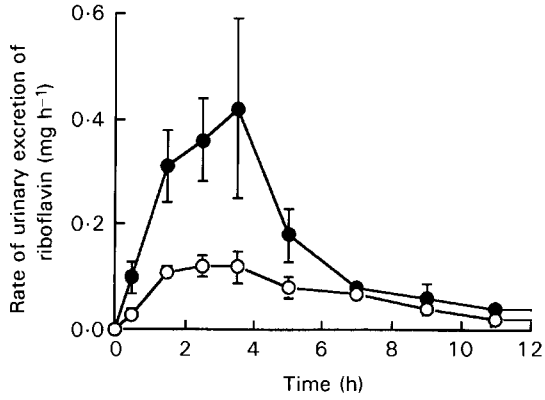

Figure 4. Urinary excretion of riboflavin after oral administration of 10 mg as non-adhesive (○) and adhesive (●) microspheres to fasted volunteers (mean ± s.e., n = 10).

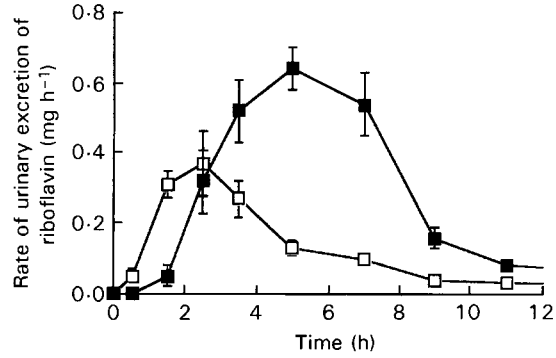

Figure 5. Urinary excretion of riboflavin after oral administration of 10 mg as adhesive microspheres to fasted (□) and fed (■) volunteers (mean ± s.e., n = 10).

microspheres. In the Step I test the greater bioavailability of both furosemide and riboflavin after administration in adhesive microspheres rather than the non-adhesive variety suggests that the adhesive microspheres adhered to the stomach and upper small intestine mucosa in man and released the drug slowly, and consequently the released drug passed gradually through the absorption site and was absorbed more efficiently.

Levy & Jusko (1966) reported that T_{max} was in the range 0·5–1·5 h when riboflavin was administered as a solution to fasted volunteers. In comparison with the shorter T_{max} after administration in solution, riboflavin was absorbed more slowly after administration as adhesive microspheres ($T_{max} = 2{\cdot}1$ h). Taking into consideration the results reported by Middleton (1990) that uptake of riboflavin occurred throughout the rat small intestine in-vitro, a possible reason the MRT of riboflavin was no greater after administration in adhesive microspheres than after administration in the non-adhesive variety is that riboflavin was slightly absorbed from the lower part of the small intestine in addition to the upper part.

There was a steep increase in the average plasma concentration of furosemide 1 h after administration of adhesive microspheres to man, in contrast with the result obtained for rats. This is possibly because of: lack of adhesion of adhesive microspheres to the stomach; absorption of furosemide from the stomach (Chungi et al (1979) and Lee & Chiou (1983) reported the absorption of furosemide from the rat stomach); shortened gastric emptying, i.e. adhesive microspheres arrived at the small intestine after a short residence time in the stomach, because turnover of mucin and phase III myoelectric migrating contraction activity occurred soon after adhesive microsphere administration; and the high gastric pH of the volunteers. The higher pH environment occurred soon after administration because weak acidity, e.g. hypoacidity, in the stomach or the shortened gastric emptying in some volunteers might have resulted in both over-swelling of the carboxyvinyl polymer and high solubility of furosemide, which has a pK_a value of 3·9. Therefore, it is expected that furosemide was released rapidly from the adhesive microspheres, the steep increase in furosemide plasma concentration occurred despite adhesion of the adhesive microspheres to the gastrointestinal tract. When riboflavin was co-administered with furosemide, a steep increase in the rate of urinary excretion was not observed. This might be because of the low and pH-independent solubility of riboflavin.

After experiments with indomethacin- and polycarbophil (calcium salt of carboxyvinyl polymer)-containing bioadhesive tablets Hosny et al (1994) suggested that the drastic reduction of indomethacin absorption after food intake might have occur-

Table 5. Pharmacokinetic parameters of riboflavin after oral administration of 10 mg in adhesive microspheres to fasted and fed volunteers.

	Non-adhesive	Adhesive
Recovery from 0–24 h (mg)	2·05 ± 0·26	4·22 ± 0·21‡
Maximum urinary excretion rate (mg h^{-1})	0·49 ± 0·06	0·82 ± 0·03*
Time of maximum urinary excretion rate (h)	2·3 ± 0·2	4·9 ± 0·5†
Mean residence time (h)	4·9 ± 0·2	6·3 ± 0·3‡

Each value is the mean ± s.e. (n = 10). *$P < 0{\cdot}05$, †$P < 0{\cdot}01$, ‡ $P < 0{\cdot}001$, significantly different from result for fasted volunteer.

red as a result of complexation of the bioadhesive material with food components and a consequent lack of bioadhesion. Food intake might affect the pH of the gastric contents and the extent of hydration of bioadhesive polymer, resulting in reduced polycarbophil adhesiveness. After administration of the capsule filled with adhesive microspheres containing riboflavin, higher urinary excretion rates were maintained from 3·5 h to 11·0 h and the urinary recovery of riboflavin after feeding was more than twice that measured after fasting. Levy & Jusko (1966) reported that food intake increased urinary recovery of riboflavin after administration to man in solution; urinary recoveries after fasting and feeding were 30 and 63%, respectively. After administration as a solution MRT after feeding, 2·2 h, was not so significantly prolonged in comparison with that after fasting, 1·9 h, although intake of food usually prolongs the gastric emptying of a drug. On the other hand, the prolonged MRT after administration of adhesive microspheres, $6{\cdot}3 \pm 0{\cdot}3$ h, might have resulted from longer residence in the gastrointestinal tract. Consequently, these results indicate that administration of adhesive microspheres after feeding resulted neither in reduction in riboflavin absorption as a result of interaction with the food nor in rapid riboflavin absorption as a result of destruction of the microspheres.

When furosemide and riboflavin, drugs with a narrow absorption window, were administered as adhesive microspheres to volunteers, absorption was enhanced owing to prolongation of gastrointestinal residence. In addition, it is unlikely that adhesive microspheres were destroyed even after feeding. This suggests that adhesive microspheres would work as a non-specific targeting drug-delivery system when used for drugs which rely for their pharmacological effects on direct contact with the mucosa of the gastrointestinal tract and when used as an oral sustained-release delivery system for a drug with a narrow absorption window.

It is concluded that adhesive microspheres could adhere to the gastrointestinal mucosa in man, resulting in prolonged gastrointestinal residence.

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