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Improvement in site-specific intestinal absorption of furosemide by Eudragit L100-55

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Abstract

Furosemide (frusemide) is a weakly acidic diuretic drug. Its absorption is poor and variable, in part due to its restricted sites of absorption, mainly the stomach. The narrow absorption window of this drug can be explained by pH partition theory. The purpose of this study was to investigate the feasibility of widening the absorption window of furosemide by controlling the pH in distal portions of the gastrointestinal tract with officially used additives. Methacrylate copolymer (Eudragit L100-55), hydroxypropylmethylcellulose phthalate (HP-55) and hydroxypropylmethylcellulose acetate succinate (AS-MF) were selected as additives. The pH of suspensions of these additives was about 4, and the pH was adjusted to about 6–7 by the addition of NaOH. The Eudragit L100-55 suspension was found to be the most resistant to NaOH titration. When Eudragit L100-55 was used in an in-situ ileal loop experiment in rats, the pH of the intestinal contents was significantly reduced, from 7.9 ± 0.1 to 5.7 ± 0.1, and the plasma concentration of furosemide 15 min after administration was about 3 times higher than that in controls, 1.81 ± 0.42 μg mL^{-1} vs 0.63 ± 0.08 μg mL^{-1}. However, the plasma concentration of [^{14}C] mannitol was not changed by the co-administration of Eudragit L100-55. Furthermore, the AUC of furosemide was significantly increased by a factor of about 1.6 relative to that in controls by the co-administration of Eudragit L100-55, to 21.4 ± 4.0 μg h mL^{-1} from 13.3 ± 3.9 μg h mL^{-1}, and the gastrointestinal pH in the midgut and ileum was significantly reduced, with most of the furosemide remaining in these segments at 2 h following the oral administration of furosemide with Eudragit L100-55 to rats. These findings clearly demonstrate that the addition of Eudragit L100-55 can increase the absorption of furosemide in distal portions of the gastrointestinal tract. In conclusion, it is feasible to widen the absorption window of furosemide by controlling the pH in distal portions of the gastrointestinal tract by the co-administration of Eudragit L100-55.

Introduction

Furosemide (frusemide), a loop diuretic agent, is widely used as an antihypertensive drug. The fractional dose of furosemide absorbed after oral administration is low, at about 60 %, and is quite variable (Benet 1979). One reason for the poor bioavailability of furosemide is thought to be its site-specific absorption (Ponto & Schoenwald 1990). The stomach is the major site for the absorption of furosemide, followed by the duodenum, and this limited absorption window can be explained by pH partition theory (Ritschel et al 1991). Several modified-release formulations (e.g. a floating system (Menon et al 1994), a non-disintegrating single-unit formulation (Smal et al 1996) and mucoadhesive microspheres (Akiyama et al 1998)) to improve the bioavailability of furosemide have been described in the

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literature. However, due to the narrow absorption window, these formulations are designed to remain in the upper gastrointestinal tract until all of the drug has been released.

We have investigated a new approach in which the absorption ratio of furosemide in distal portions of the gastrointestinal tract is increased by reducing the gastrointestinal pH by the co-administration of officially used non-absorbed acidic additives. Enteric polymers such as hydroxypropylmethylcellulose phthalate, hydroxypropylmethylcellulose acetate succinate and methacrylate copolymer were selected because they are large molecules containing many carboxylate groups and their suspensions are therefore expected to promote an acidic pH. In addition, one of these polymers, methacrylate copolymer (Eudragit L), is not absorbed after oral administration to the rat. The purpose of this study was to determine whether it is possible to widen the absorption window of furosemide by reducing the gastrointestinal pH in distal portions of the gastrointestinal tract by the co-administration of such acidic polymers.

Materials and Methods

Materials

[^{14}C] Mannitol (56 mCi mmol^{-1}) was purchased from Maravek Biochemicals, Inc. (Brea, CA). Furosemide was obtained from Sigma Chemical Co. (St Louis, MO). Hydroxypropylmethylcellulose, hydroxypropylmethylcellulose phthalate (HP-55) and hydroxypropylmethylcellulose acetate succinate (AS-MF) were obtained from Shin-Etsu Chemical Co., Ltd (Toyama, Japan). Methacrylate copolymers (Eudragit L100 and Eudragit L100-55) were obtained from Röhm GmbH (Darmstadt, Germany). All other chemicals used were of reagent grade and were obtained from commercial sources.

Measurement of intestinal absorption by the intestinal loop method

Male Sprague-Dawley rats (Charles River Japan, Kanagawa, Japan), fasted overnight, were anaesthetized by injection of sodium pentobarbital (50 mg kg^{-1}). A midline incision was made to expose the small intestine and a 10-cm loop of ileum, approximately 5 cm proximal to the caecum, was prepared by ligating both ends. Furosemide (15 mM) or [^{14}C] mannitol (2 mM, 6 μCi mL^{-1}), dissolved in phosphate buffer containing (mM): 22 Na_2HPO_4 12H_2O, 22 KH_2PO_4 and 110 NaCl (pH 7.0, 0.5 mL), was injected into the ileal loop (final concentrations: furosemide, 7.5 mM; [^{14}C] mannitol, 1 mM, 3 μCi mL^{-1}), which was kept in the body for 45 min (furosemide) or 120 min ([^{14}C] mannitol) during the absorption experiments. Heparinized blood samples (0.5 mL) were obtained from the jugular vein at 15-min intervals (furosemide) or 30-min intervals ([^{14}C] mannitol) during the experiment. All blood samples were immediately centrifuged to obtain the plasma, and the plasma concentrations of the test compounds were determined. At the end of the experiment on furosemide, the intestine was thoroughly washed with 1 mL of saline solution and the pH was measured. The polymer under evaluation (200 mg mL^{-1}), suspended in 1% hydroxypropylmethylcellulose solution (0.5 mL), was injected together with the test compound solution into the ileal loop (final concentration, 100 mg mL^{-1}). In controls, 1% hydroxypropylmethylcellulose solution alone (0.5 mL) was injected instead of the polymer suspension.

Measurement of intestinal pH and gastrointestinal distribution

The rats were given an oral dose of furosemide (15 mg kg^{-1}) and Eudragit L100-55 (400 mg kg^{-1}) suspended in phosphate buffer, containing (mM): 22 Na_2HPO_4 12H_2O, 22 KH_2PO_4 and 110 NaCl (pH 7.5), via a stomach tube. The rats were then left free in metabolic cages at an ambient temperature of 23°C. Animals were killed at 2 or 4 h after administration for measurement of pH in the gastrointestinal tract, with the small intestine divided into the duodenum (about 1/10th of its total length) and three segments of equal length (corresponding to the jejunum, midgut and ileum). Each segment was cut open axially and placed on a flat glass plate. The pH value of the mucosal side of the segment was then measured with a pH meter (F-14; HORIBA Ltd, Kyoto, Japan) equipped with a flat pH electrode (6261-10C, HORIBA Ltd, Kyoto, Japan; tip diameter, 0.6 mm). The gastrointestinal contents were obtained by washing out stomach or intestinal segments on the glass plate with 5 mL of 0.1 N NaOH. The washing solution was homogenized at 30 000 rev min^{-1} for 2 min at < 10 °C with a homogenizer (Model 395 type 5, DREMEL, Racine, WI) and centrifuged at 2000 *g* for 10 min at 4°C (8800 centrifuge; Kubota, Tokyo, Japan), and the supernatant obtained was neutralized by the addition of an equal volume of 0.1 N HCl. Subsequently, the concentration of furosemide in each sample of supernatant was determined. Furosemide (15 mg kg^{-1}) dissolved in phosphate buffer (pH 7.5) was administered as the control.

Bioavailability study

The rats were given an oral dose of furosemide (15 mg kg^{-1}) and Eudragit L100-55 (400 mg kg^{-1}) suspended in phosphate buffer (pH 7.5) via a stomach tube. The rats were then left free in metabolic cages at an ambient temperature of 23°C. Heparinized blood samples (0.2 mL) were obtained via a tail vein at 0.5, 1, 2, 4, 6, 8 and 24 h after administration. All blood samples were immediately centrifuged to obtain the plasma, and the plasma concentration of the test compound was determined. Furosemide (15 mg kg^{-1}) dissolved in phosphate buffer (pH 7.5) was administered as the control.

Analytical methods

To assay [^{14}C] mannitol, all samples were transferred to counting vials, mixed with scintillation fluid (ACSII, Amersham International plc, Buckinghamshire, UK), and measured with a liquid scintillation counter (TRI-CARB Model 4530, Packard Instruments Co., Meriden, CT). Furosemide was measured by HPLC. The HPLC system was equipped with a constant flow pump (CCPM; Tosoh, Tokyo, Japan), a fluorescence detector (FS-8010; Tosoh, Tokyo, Japan), an integrator (Chromatopac C-R5A; Shimazu, Kyoto, Japan) and an automatic sample injector (AS-8010; Tosoh, Tokyo, Japan). The analytical column was a reversed-phase SP-120-5-ODS-BP (4.6 mm × 25 cm; Disoh, Tokyo, Japan). The mobile phase used was a gradient from mobile phase A (10 mM KH_2PO_4 (pH 3)–acetonitrile, 2:8) to mobile phase B (100% acetonitrile). The linear time program for A was from 99% at 0 min to 25% at 30 min. The eluent was analysed using a fluorescence detector with $\lambda_{em} = 345$ nm and $\lambda_{ex} = 418$ nm.

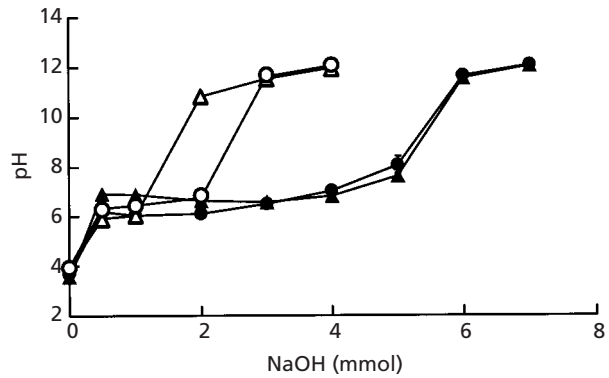

Figure 1 Titration curves for 1% HP-55 (○), AS-MF (△), Eudragit L100-55 (●) and Eudragit L100 (▲) suspensions using 5 N NaOH. Each point represents the mean ± s.d. of 3 experiments.

Data analysis

The maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (t_{max}) after oral administration were read directly from the mean plasma concentration data. The area under the plasma concentration–time curve from 0 to 24 h ($AUC_{0-24\,h}$) after oral administration was calculated according to the trapezoidal rule (Yamaoka et al 1978). Data are expressed as mean ± s.d., and statistical analysis was performed using the one-tailed Student's *t*-test. The level of significance was set at $P < 0.05$ or < 0.01.

Results

Titration of polymer suspensions

To identify the most suitable polymer for the control of gastrointestinal pH, 1 g of polymer (HP-55, AS-MF, Eudragit L100 or Eudragit L100-55) suspended in 100 mL of water was titrated with 5 N NaOH. The titration curves are shown in Figure 1. The pH of the suspension of each polymer was less than 4 at the start of titration. The titration curves of all of the polymer suspensions showed a two-step pH shift: first, from pH 3–4 to pH 6–7, and second, from pH 6–8 to pH 11–12. The amounts of NaOH added before the second pH shift in the titration curves for the polymers HP-55, AS-MF, Eudragit L100 and Eudragit L100-55 were 2, 1, 5 and 5 mmol, respectively.

Effect of the polymers on intestinal absorption

To determine whether the order of resistance to NaOH titration in in-vitro studies is the same as that of effects on gastrointestinal pH and furosemide absorption, two typical polymers, Eudragit L100-55 (most resistant to NaOH titration) and AS-MF (least resistant to NaOH titration), were selected, and the intestinal permeability of furosemide with or without Eudragit L100-55 or AS-MF was examined by the in-situ rat intestinal loop method. Plasma concentrations in all groups reached a plateau at 15 min after administration, and the concentration with co-administration of Eudragit L100-55 ($1.81 \pm 0.42\ \mu g\,mL^{-1}$) was significantly higher ($P < 0.01$) than that in controls ($0.63 \pm 0.08\ \mu g\ mL^{-1}$) or that with co-administration of AS-MF ($0.65 \pm 0.03\ \mu g\ mL^{-1}$) (Figure 2). However, no significant differences were found between the plasma concentration of [^{14}C] mannitol with the co-administration of Eudragit L100-55 and that in controls at any time points (Figure 3).

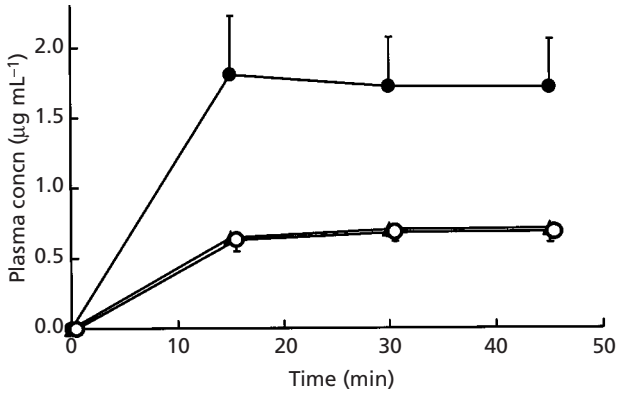

Figure 2 Plasma concentrations of furosemide after administration of the drug (final concentration, 7.5 mM) to rat ileal loops in the absence (○) and presence of 100 mg mL^{-1}(final concentration) of AS-MF (△) or Eudragit L100-55 (●). Each point represents the mean ± s.d. of 3 experiments.

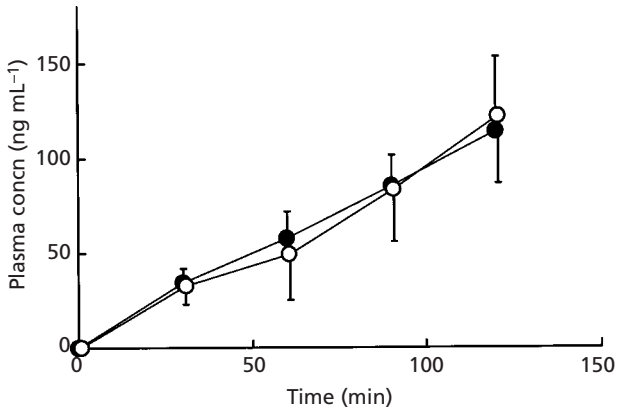

Figure 3 Plasma concentrations of [^{14}C] mannitol after administration of the drug (final concentration, 1 mM) to rat ileal loops in the absence (○) and presence of 100 mg mL^{-1} of Eudragit L100-55 (●). Each point represents the mean ± s.d. of 3 experiments.

At the end of the experiments, the intestinal pH with co-administration of Eudragit L100-55 (5.7 ± 0.1) or AS-MF (6.7 ± 0.5) was significantly ($P < 0.05$) lower than that in controls (7.9 ± 0.1).

Effect of Eudragit L100-55 on the bioavailability of furosemide

Furosemide was administered orally with or without Eudragit L100-55 to rats to evaluate the effects of co-administration of Eudragit L100-55 on the bioavailability of furosemide. The plasma concentrations of

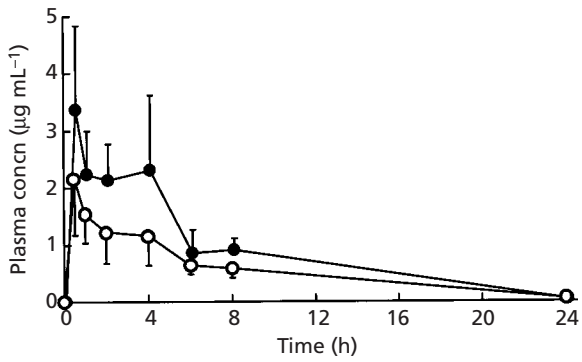

Figure 4 Plasma concentrations of furosemide after oral administration of the drug (15 mg kg^{-1}) to rats in the absence (○) and presence of 400 mg kg^{-1} of Eudragit L100-55 (●). Each point represents the mean ± s.d. of 4 experiments.

Table 1 Pharmacokinetic parameters of furosemide after oral administration with or without Eudragit L100-55 to rats.

Parameter	Control	With Eudragit L100-55 (400 mg kg^{-1})
$AUC_{0-24\,h}$ (μg h mL^{-1})	13.3 ± 3.9	21.4 ± 4.0*
C_{max} (μg mL^{-1})	2.2 ± 1.2	3.5 ± 0.9
t_{max} (h)	0.9 ± 0.8	0.9 ± 0.8

Furosemide (15 mg kg^{-1}) was administered orally with or without Eudragit L100-55 suspension (400 mg kg^{-1}) to rats. Each value represents the mean ± s.d. of 4 experiments. *$P < 0.05$, compared with control value.

furosemide are shown in Figure 4 and the pharmacokinetic parameters are shown in Table 1. The $AUC_{0-24\,h}$ of furosemide with co-administration of Eudragit L100-55 (21.4 ± 4.0 μg h mL^{-1}) was significantly ($P < 0.05$) higher than that in controls (13.3 ± 3.9 μg h mL^{-1}).

Effect of Eudragit L100-55 on intestinal pH and gastrointestinal distribution

The gastrointestinal pH and gastrointestinal distribution of furosemide at 2 and 4 h after the administration of furosemide with Eudragit L100-55 were examined under the same conditions as in the bioavailability study. The pH in the midgut and the ileum at 2 h after administration was significantly ($P < 0.05$) reduced by the co-administration of Eudragit L100-55 (Table 2).

The gastrointestinal distribution of the drug at 2 and 4 h after administration was also examined. In controls,

Table 2 Gastrointestinal pH at 2 and 4 h after oral administration of furosemide with or without Eudragit L100-55 to rats.

	2 h		4 h	
	Control	With Eudragit L100-55	Control	With Eudragit L100-55
Stomach	4.14 ± 0.83	4.33 ± 0.32	3.76 ± 0.66	3.87 ± 0.79
Duodenum	6.16 ± 0.11	5.95 ± 0.27	6.43 ± 0.48	6.05 ± 0.11
Jejunum	6.22 ± 0.09	6.15 ± 0.09	6.34 ± 0.16	6.24 ± 0.12
Midgut	6.62 ± 0.21	6.27 ± 0.21*	6.55 ± 0.19	6.59 ± 0.30
Ileum	7.42 ± 0.14	6.98 ± 0.29*	7.35 ± 0.22	7.15 ± 0.52
Caecum	6.05 ± 0.34	6.00 ± 0.27	6.29 ± 0.22	6.18 ± 0.26
Colon	6.59 ± 0.45	6.20 ± 0.37	6.67 ± 0.28	6.26 ± 0.23

Data show oral administration of furosemide (15 mg kg^{-1}) to rats with or without Eudragit L100-55 suspension (400 mg kg^{-1}). Each value represents the mean ± s.d. of 4 experiments. *$P < 0.05$ compared with control value.

Table 3 Percent of furosemide distributed in the gastrointestinal tract and plasma concentration at 2 and 4 h after oral administration of furosemide with or without Eudragit L100-55 to rats.

	2 h		4 h	
	Control	With Eudragit L100-55	Control	With Eudragit L100-55
% recovered from gastrointestinal tract				
Stomach	21.1 ± 9.6	31.0 ± 0.7	10.9 ± 9.7	11.9 ± 4.3
Duodenum	0.4 ± 0.4	0.2 ± 0.4	0.1 ± 0.2	0.5 ± 0.5
Jejunum	1.8 ± 0.7	4.5 ± 0.9**	1.3 ± 0.5	1.2 ± 0.8
Midgut	25.5 ± 12.7	32.3 ± 10.1	17.1 ± 12.4	7.7 ± 7.5
Ileum	50.3 ± 10.6	28.3 ± 7.5**	28.5 ± 14.5	21.5 ± 4.3
Caecum	0.5 ± 0.3	3.3 ± 5.4	29.5 ± 16.5	49.0 ± 11.3*
Colon	0.4 ± 0.5	0.5 ± 0.2	12.6 ± 16.0	8.2 ± 7.7
Plasma concn ($\mu g \cdot mL^{-1}$)	1.03 ± 0.23	2.54 ± 0.37**	1.17 ± 0.31	1.27 ± 0.43

Furosemide (15 mg kg^{-1}) was administered orally with or without Eudragit L100-55 suspension (400 mg kg^{-1}) to rats. The percent distributed in the gastrointestinal tract is normalized to the value remaining in the entire gastrointestinal tract. Each value represents the mean ± s.d. of 4 experiments. *$P < 0.05$, **$P < 0.01$, compared with control value.

the maximum distribution of furosemide was seen in the ileum at 2 h and in the caecum at 4 h. With the co-administration of Eudragit L100-55, the maximum distribution of furosemide was seen in the midgut at 2 h and in the caecum at 4 h (Table 3). The total recovery rates of furosemide in these segments in controls and with the co-administration of the polymer were, respectively, 51 ± 12% and 43 ± 5% at 2 h and 39 ± 7% and 40 ± 5% at 4 h. There was no significant difference in the recovery of furosemide in controls vs with the co-administration of Eudragit L100-55 at 2 h or 4 h. In this experiment, the plasma concentration of furosemide at 2 h was significantly ($P < 0.01$) increased by the co-administration of Eudragit L100-55, but no significant difference was seen between the concentration with co-administration of Eudragit L100-55 and that in controls at 4 h (Table 3).

Discussion

The findings of this study, which involved three experiments (i.e., in-vitro titration of polymer suspensions, in-

Table 4 Fraction of non-ionized furosemide distributed in the gastrointestinal tract of rats.

	2 h		4 h	
	Control	With Eudragit L100-55	Control	With Eudragit L100-55
Stomach	20.67 ± 15.49	21.82 ± 9.00	9.05 ± 7.90	9.89 ± 5.51
Duodenum	0.01 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.03
Jejunum	0.21 ± 0.07	0.64 ± 0.27*	0.13 ± 0.08	0.13 ± 0.08
Midgut	1.21 ± 0.55	3.61 ± 2.09	0.81 ± 0.30	0.58 ± 0.78
Ileum	0.52 ± 0.26	0.72 ± 0.48	0.27 ± 0.14	0.61 ± 0.80
Small intestine	1.95 ± 0.72	4.97 ± 1.64*	1.22 ± 0.20	1.34 ± 1.61
Total	22.62 ± 15.10	26.79 ± 9.74	10.27 ± 7.76	11.23 ± 6.86

Small intestine: total fraction in the duodenum, jejunum, midgut, and ileum. The fraction of un-ionized furosemide distributed in the gastrointestinal tract was calculated using equation 2. Each value represents the mean ± s.d. of 4 experiments. $*P < 0.05$ compared with control value.

situ intestinal absorption and in-vivo bioavailability), demonstrated that Eudragit L100-55 is suitable for widening the absorption window of furosemide in modified-release form by reducing the pH in distal portions of the gastrointestinal tract.

Initially, the titration of polymer by NaOH was investigated to assess the feasibility of reducing the pH in distal portions of the gastrointestinal tract by the administration of enteric polymer. All of the polymer suspensions were acidic, and more NaOH was needed to increase the pH of the suspensions of methacrylate copolymer (Eudragit L100-55 and Eudragit L100) than the suspensions of cellulose polymer (AS-MF and HP-55). This finding can be explained by the differences in molecular structure between methacrylate and cellulose polymers. One gram of Eudragit L100-55, Eudragit L100, AS-MF or HP-55 contains 4.1, 3.9, 0.2 or 0.6 mol of carboxylate groups, respectively. This suggests that methacrylate copolymer is preferable for maintaining an acidic pH in the distal portions of the gastrointestinal tract.

In the second part of these investigations, the in-situ loop study, the pH values in the ileum in controls and with the co-administration of Eudragit L100-55 and AS-MF were 7.9, 5.7 and 6.7, respectively. Equation 1 (Ritschel et al 1991) was used to calculate the un-ionized fraction.

$$\text{percent un-ionized} = 100 \times [1 - (1 + \text{antilog}(\text{pKa} - \text{pH}))^{-1}] \quad (1)$$

Solving this equation using the pH and pKa of furosemide (4.7), the values for the un-ionized fraction of furosemide in controls and with the co-administration of Eudragit L100-55 and AS-MF are calculated as 0.1 %, 8.6 % and 1.0 %, respectively. However, the mean plasma concentration of furosemide at 15 min after co-administration of the drug with Eudragit L100-55 was only about 3 times higher than that in controls or with the co-administration of AS-MF. This difference between the increase ratio in the un-ionized fraction and that in plasma concentration is adequately explained by the surface pH. The surface pH of the ileum has been reported to be pH 7.0 (McEwan et al 1988), and if the mucosal bulk pH is reduced to pH 5.7 by the co-administration of Eudragit L100-55, the surface pH is thought to be closer to that in controls.

In this experiment, the initial concentration of Eudragit L100-55 or AS-MF was 200 mg mL^{-1}. The maximum feasible concentration of the polymer suspension, 200 mg mL^{-1}, was selected because these polymers are extremely safe, with LD50 (lethal dose 50 %) values of not less than 10000 mg kg^{-1} for Eudragit L100-55 and not less than 25000 mg kg^{-1} for AS-MF. Furthermore, the permeability of [^{14}C] mannitol was not affected by the co-administration of Eudragit L100-55, and the permeability of furosemide was not affected by the co-administration of AS-MF. These results indicate that Eudragit L100-55 and AS-MF do not damage the intestinal membrane at the concentration used in this experiment.

Finally, a bioavailability study was conducted by administering furosemide with or without Eudragit L100-55 to rats. With the co-administration of Eudragit L100-55, the AUC was significantly increased by a factor of 1.6 relative to controls. There was no effect similar to that of absorption enhancers, which damage the intestinal membrane, because the dose of Eudragit L100-

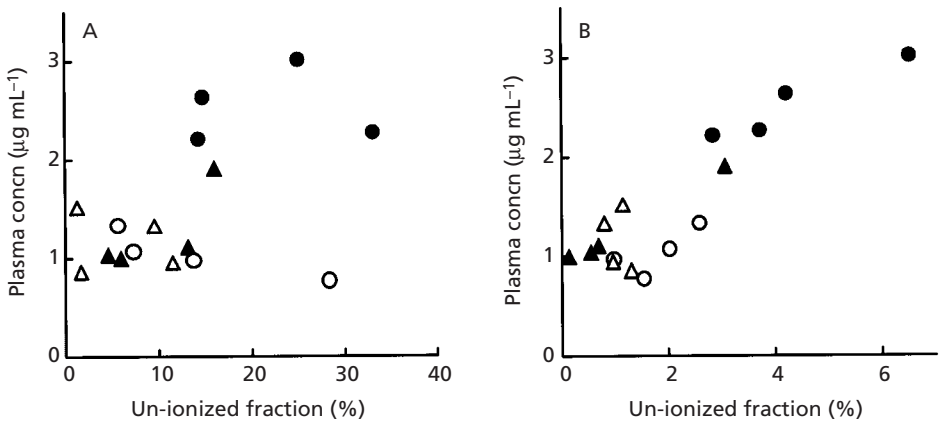

Figure 5 Scatter diagram showing the relationship between the plasma concentration and the un-ionized fraction of furosemide distributed in the stomach (A) and small intestine (B) after oral administration of the drug (15 mg kg^{-1}) to rats in the absence (○, 2 h; △, 4 h) and presence of 400 mg kg^{-1} of Eudragit L100-55 (●, 2 h; ▲, 4 h).

55 in this experiment was at least about 25 times lower than the LD50, and Eudragit L100-55 is thought not to damage the intestinal membrane at this dose in in-situ experiments. The gastrointestinal distribution and gastrointestinal pH were also evaluated under the same conditions. The total recovery rates of furosemide in these segments in controls and with the co-administration of the polymer were, respectively, $51 \pm 12\%$ and $43 \pm 5\%$ at 2 h and $39 \pm 7\%$ and $40 \pm 5\%$ at 4 h. Similar values were reported by Lee & Chiou (1983). They reported that the percentages remaining in the entire gastrointestinal tract at 2 h and 9 h after administration were 47.1 % and about 40 %, respectively. The pH in the midgut and ileum at 2 h was significantly ($P < 0.05$) reduced by the co-administration of Eudragit L100-55, and the percentages of furosemide distributed in the midgut and ileum were 32.3 % and 28.3 %, respectively, at that time point. These results indicate that Eudragit L100-55 and furosemide were distributed in the same segment. Equation 2 was used to calculate the un-ionized fraction of furosemide distributed in the gastrointestinal tract, to clarify its effects.

$$\begin{array}{l}\text{Un-ionized fraction of furosemide distributed in the gastrointestinal tract}\end{array} = \begin{array}{l}[1-(1+\text{antilog}(\text{pKa}-\text{pH}))^{-1}] \\ \times \text{percentage of furosemide distributed in the gastrointestinal tract}\end{array} \quad (2)$$

Solving equation 2 using the gastrointestinal pH and pKa of furosemide (4.7) and the distribution data, the value for the un-ionized fraction of furosemide distributed in each segment was calculated (Table 4), and these values were plotted against plasma concentrations (Figure 5).

The fraction in the small intestine was significantly increased by the co-administration of Eudragit L100-55, and a good correlation ($r = 0.898$) was seen between the un-ionized fraction of furosemide distributed in the small intestine and the plasma concentration. Although the percent distributed in distal portions of the gastrointestinal tract (midgut and ileum) 2 h after the co-administration of Eudragit L100-55 ($60.5 \pm 12.1\%$) was significantly ($P < 0.05$) lower than that in controls ($75.9 \pm 9.4\%$), the fraction in distal portions of the gastrointestinal tract ($4.32 \pm 1.68\%$), due to the reduction in pH in these segments, was significantly higher than that in controls ($1.73 \pm 0.72\%$), and these fractions were the main fractions in the small intestine. However, the fractions in the stomach and in all segments were not affected by the co-administration of Eudragit L100-55, and there was a poor correlation ($r = 0.220$) between the fraction in the stomach and the plasma concentration. The fraction in the small intestine was lower than that in the stomach, which is understandable given the greater surface area and slower gastrointestinal transit rate of the small intestine compared with the stomach. These results clearly indicate that the increase in the bioavailability of furosemide was due to improved absorption in distal portions of the gastrointestinal tract due to the reduction in pH induced by the co-administration of Eudragit L100-55.

In conclusion, the findings of this study clearly demonstrate the feasibility of widening the absorption window of furosemide to increase its bioavailability.

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