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Segmental difference in transmucosal fluid movement and its effect on gastrointestinal drug absorption in rabbits

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Summary

Segmental differences in transmucosal fluid movement and their effects on gastrointestinal drug absorption in rabbits were studied with aspirin as an acidic drug and metoclopramide as a basic compound. The segments used were stomach, duodenum, jejunum and ileum. The ligation method was employed in the stomach study and other segments were studied by using an in situ loop method. All segments were investigated simultaneously.

Segmental differences in transmucosal fluid movement was statistically observed with a one-way analysis of variance, although the initial drug solutions were adjusted to isotonicity. The net fluid absorption was increased as the distance from the mouth increased. A good positive correlation between transmucosal fluid movement and drug absorption was found in both drug studies. We conclude that segmental differences in drug absorption are partly dependent upon the transmucosal fluid movement in each gastrointestinal segment.

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Introduction

It has been reported from our laboratories that the absorption of drugs was increased with increasing transmucosal fluid movement using the entire small intestine of rats (Kitazawa et al., 1975). A generality in the effect of fluid movement on drug absorption has been verified with 23 different drugs (Kitazawa et al., 1978). This phenomenon was also observed in rat intestinal segments including duodenum, jejunum, ileum, colon and rectum, but not in the stomach (Kitazawa et al., 1977a and b). Similar observations with the jejunum of rats have been reported by Ochsenfahrt and Winne (1974a and b). These observations may have been caused by a mechanism such as solvent drag which had first been used by Anderson and Ussing (1957). In addition, evidence has been presented for a segmental difference in drug absorption and suggested that different ion species of drugs showed different absorptions between the jejunum and ileum of the rat (Kitazawa et al., 1977a). In these studies, various osmolalities of drug solution were employed to manipulate fluid movement and the findings were only from rats. Another question has been raised whether these phenomena might also be observed among different species of experimental animals.

The present study was undertaken to examine these phenomena in rabbits and to make correlations between transmucosal fluid movement and drug absorption using aspirin (ASP) as an acidic drug and metoclopramide (MCP) as a basic compound.

Experimental

Animals

Male Japanese white rabbits weighing 2.5–3.5 kg were purchased from Shimizu Shoten (Kyoto, Japan). The animals were fed a specially prepared laboratory diet without alphalpha (Nihon Clea, Tokyo, Japan) (Maeda et al., 1977) for at least 1 week. Animals fed the diet showed a faster gastric emptying rate and the GI tract could be easily washed. The rabbits were fasted with free access to water overnight prior to the absorption studies.

Drug solutions

ASP of J.P.X. grade produced by Yoshitomi Pharmaceuticals (Osaka, Japan) was purchased from commercial sources. MCP was kindly supplied by Fujisawa Pharmaceuticals (Osaka, Japan). All other chemicals used were of reagent grade and were used without further purifications.

ASP was dissolved in isotonic sodium chloride solution (0.9%). Because of the instability of the drug in the solution, the drug solution was freshly prepared. The pH was adjusted to 2.3 with 1 N HCl to minimize ASP hydrolysis (Edwards, 1950). MCP was dissolved in 0.9% NaCl solution by adding concentrated hydrochloric acid and the pH value of resultant solution was around 3.4. Each drug concentration was 1 mM and the drug solution contained 20 μg/ml (0.056 mmol) phenol red which is a non-absorbable indicator. Initial and final pH values of drug solutions were mea-

sured with a pH meter model HM-5A (Toa Electric Measuring Instruments, Tokyo, Japan). The osmolality of drug solution was obtained with Advanced digimatic osmometer model 3D (Advanced Instruments, MA, U.S.A.).

Drug and fluid absorption studies

The rabbits were anesthetized with intraperitoneal sodium pentobarbital (Nembutal, 40 mg/kg of body weight), the abdomen opened, and the common bile duct ligated. For the gastric study, the stomach was exposed and the cardiac opening was ligated with strings on the esophagus to prevent leakage of the test solution. The pyrolus end was cannulated with a silicon tubing (o.d. 5 mm; i.d. 3 mm) and the cannula was fastened with strings. The stomach was washed twice using a syringe with 50 ml of physiological saline solution which had been kept at 37°C. For the small intestine, three segments were used. A segment of 30 cm long just distal to the pylorus was assumed to be the duodenum. The ileum (30 cm long) was just proximal to the ampulla ilei. The jejunal segment (30 cm in length) was between the duodenum and the ileum and the distal end was 40 cm from the proximal end of ileal segment. Inlet and exit cannulas of silicon tubing were introduced into each segment through small incisions and tied in place. The segments were washed twice with 30 ml each of physiological saline solution which had been kept at 37°C. A ligation method (Kitazawa et al., 1977a) and an in situ loop method (Barr and Riegelman, 1970) were employed in the gastric and the small intestinal drug absorption studies, respectively. The period of the absorption studies was 1 h and all the segments were studied simultaneously. The volumes of the test solutions used were 50 ml for the stomach study and 30 ml each for the small intestinal segments. After instillation of the test solution into each segment, the abdominal incision was closed. During the procedure the animals were warmed with heating lamps and the abdomen was maintained moist using saline-soaked gauze.

After the absorption studies had been completed, intraluminal drug solution was recovered carefully and was used for the assay. The GI tract segments used were isolated and the wet weight was measured. Then the segments were dried for 24 h at 100°C (Kitazawa and Johno, 1976) to obtain the dry weight.

Analysis

ASP was analyzed as salicylate following our previous paper (Kitazawa et al., 1974). MCP and phenol red were determined colorimetrically (Kitazawa et al., 1975). The developed color was determined spectrophotometrically using a Hitachi spectrophotometer model 200-20.

Calculations

The absorption rate of drug or fluid was calculated as follows:

$$\text{Drug absorption rate} = \frac{C_{\text{drug init.}}\left[1 - \left(\frac{C_{\text{drug final}}}{C_{\text{drug init.}}} \times \frac{C_{\text{indicator init.}}}{C_{\text{indicator final}}}\right)\right]}{T_i \times \text{dry weight}} \quad (1)$$

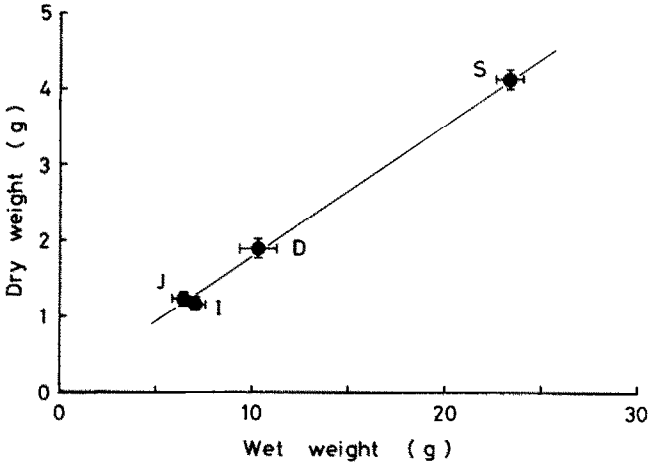

Fig. 1. Relationship between wet and dry weight in gastrointestinal segment of rabbit. S = stomach; D = duodenum; J = jejunum; I = ileum. Each plot indicates the mean ± S.E.M. of at least 3 runs.

$$\text{Fluid absorption rate} = \frac{V_0\left(1 - \frac{C_{\text{indicator init.}}}{C_{\text{indicator final}}}\right)}{T_i \times \text{dry weight}} \quad (2)$$

where C is the concentration of drug or non-absorbable indicator in the test solution in μM, T_i is the absorption time in hours, V_0 is the volume of the test solution in ml, and dry weight is the dry weight of GI segments in grams. For the fluid absorption rate, the fluid movement from lumen to blood was regarded as inflow, and the inflow shows positive values following Eqn. 2. On the other hand, the fluid secretion was also observed from blood to lumen and this was outflow and showed negative values by Eqn. 2.

Data were analyzed by standard statistical methods and P values of less than 0.05 were considered to be significant.

Results

Relationship between wet and dry weight of gastrointestinal tract

In this report, the rate of transmucosal fluid movement and drug absorption was calculated using the dry weight of each segment. Fig. 1 shows a correlation between wet and dry weight of the GI segments in rabbits. A good correlation was obtained and the regression equation was $y = 0.17x + 0.10$ ($n = 27$, $r = 0.986$, $P < 0.001$). In our previous report (Komuro et al., 1975), water content of rat entire small intestine was 84% and the result was in good agreement with rabbit GI water content obtained in this report. Gastric weights were the largest and 4 times larger than those of the jejunum or ileum.

TABLE 1
pH AND OSMOLALITY OF TEST DRUG SOLUTION

	Segment	pH		Osmolality (mOsm/kg)	
		Aspirin study	Metoclopramide study	Aspirin study	Metoclopramide study
Initial		2.28 ± 0.02	3.39 ± 0.04	285.0 ± 0.6	283.3 ± 4.3
Final	stomach	2.05 ± 0.29	2.51 ± 0.37	291.0 ± 5.5	293.5 ± 10.5
	duodenum	8.01 ± 0.05	8.15 ± 0.05	303.0 ± 4.5	304.0 ± 3.6
	jejunum	7.89 ± 0.12	7.99 ± 0.07	307.0 ± 5.5	303.0 ± 2.7
	ileum	7.61 ± 0.09	7.96 ± 0.12	301.0 ± 2.3	300.7 ± 3.0

The values are the mean ± S.E.M. of at least 3 determinations.

pH and osmolality changes during absorption studies

The initial and final pH values and osmolalities of test solutions are tabulated in Table 1. The pH values were lower in the stomach and increased largely in the intestinal segments. The duodenum showed the highest values (more than 8.0). There were no significant differences among the intestinal segments except between the duodenum vs the ileum in the ASP study. The osmolality of final luminal solution increased 2–8%. This shows that the osmolality of the initial test solution is close to that of plasma. Similar observations were found in rats (Miller et al., 1979).

Transmucosal fluid movement

The transmucosal fluid movement in each GI segment was illustrated in Fig. 2. As shown in Fig. 2, any statistical differences in the fluid movement with unpaired *t*-test were not observed in these drug studies, but segmental differences on the fluid

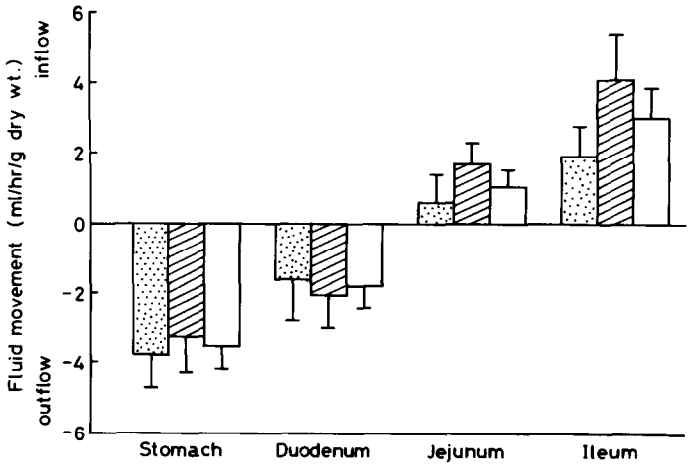

Fig. 2. Transmucosal fluid movement in each gastrointestinal segment of rabbit. ▩, metoclopramide study; ▨, aspirin study; □, metoclopramide and aspirin study. Each bar represents the mean ± S.E.M. There are no significant differences on the fluid movement between MCP and ASP study.

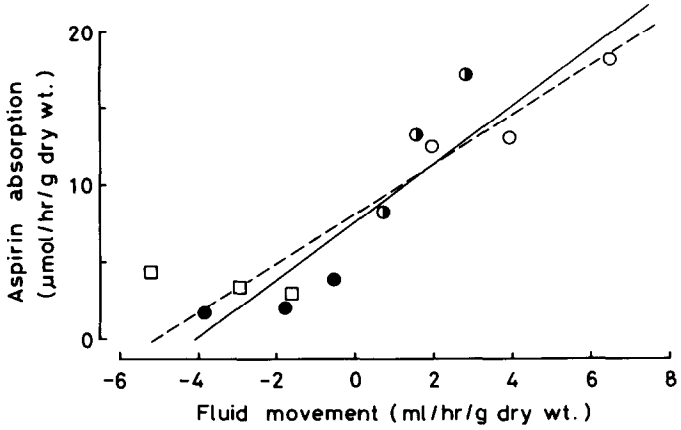

Fig. 3. Correlation between transmucosal fluid movement and aspirin absorption. □, stomach; ●, duodenum; ◑, jejunum; ○, ileum. The regression line was obtained using all data (dotted line) and without stomach data (solid line).

movement were statistically observed with one-way analysis of variance (ANOVA). In the gastric and duodenal segments, net fluid outflow was observed and the rate was greater in the gastric segment. In contrast, the net fluid inflow was observed in the jejunum and ileum, and was greater in the lower segment. These results showed that the net fluid absorption was increased with increasing distance from the mouth.

Correlation between transmucosal fluid movement and drug absorption

A significant positive linear correlation ($n = 12$, $r = 0.895$, $P < 0.001$) was ob-

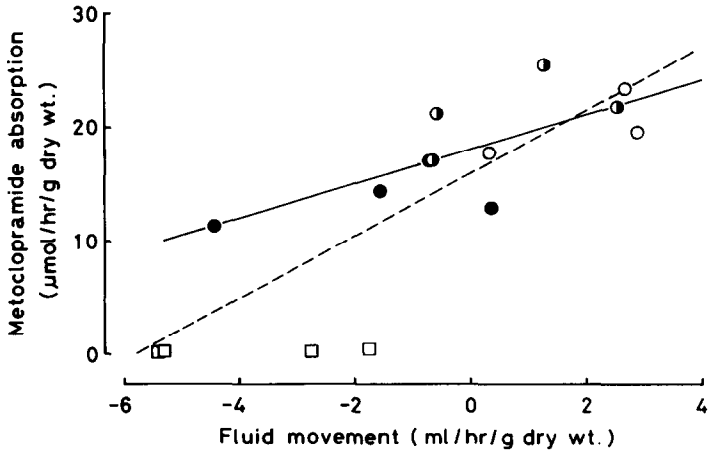

Fig. 4. Correlation between transmucosal fluid movement and metoclopramide absorption. □, stomach; ●, duodenum; ◑, jejunum; ○, ileum. The regression line was obtained using all data (dotted line) and without stomach data (solid line).

tained between transmucosal fluid movement and drug absorption (Fig. 3) over a wide range of the fluid movement, although the four different segments were used. The regression equation was $y = 1.60x + 8.22$ using all data depicted by the dotted line in Fig. 3. The regression equation without stomach data was $y = 1.87x + 7.69$ as shown by the solid line. These two lines are very close to each other and were considered statistically (Diem and Lentner, 1970) as being identical. This evidence shows that segmental differences in drug absorption are partly dependent upon the transmucosal fluid movement in each segment.

With the MCP absorption studies, a significant positive correlation ($n = 15$, $r = 0.816$, $P < 0.001$) was also obtained between transmucosal fluid movement and drug absorption as shown in Fig. 4. This phenomenon is similar to that of the ASP study. The regression equation was $y = 2.76x + 16.12$ for all data as shown by the dotted line, and $y = 1.52x + 18.26$ for the data minus the gastric data illustrated by the solid line in Fig. 4. These regression lines did not statistically coincide (Diem and Lentner, 1970).

Discussion

Segmental differences in transmucosal fluid movement were found and fluid absorption increased with increasing distance from the mouth. In the gastric segment, net water outflow was larger than in the other segments (Fig. 2). Even when a hypotonic solution was introduced into the segment, net transmucosal fluid outflow was observed in rats (Kitazawa et al., 1977a). It is well known that there are many secretory glands on the surface of the gastric epithelium and many kinds of digestive fluids are secreted into the lumen (Tidball, 1971). The results obtained show that fluid secretion always exceeds fluid absorption. In dogs, net fluid secretion in the duodenum was observed and ileal fluid absorption was superior to the duodenum (Grim, 1962). The upper intestine in humans showed higher water movement into the lumen than the lower small intestine (Fordtran et al., 1965). These results support our findings in rabbits described in the present report.

In our previous report (Kitazawa et al., 1975), the effect of the sodium chloride concentration in a luminal solution on transmucosal fluid movement in rats was examined and a good linear positive relationship between these two phenomena was obtained. Similar correlation between these two phenomena was observed using a test solution containing each drug used in the paper including MCP and there were no statistical differences in transmucosal fluid movement between an isotonic solution containing sodium chloride alone and that dissolving MCP (Kitazawa and Johno, unpublished data). This shows that MCP does not increase or decrease transmucosal fluid movement. In the present study, there were no statistical differences in the fluid movement between ASP and MCP study as shown in Fig. 2. These results may suggest that these two drugs do not affect the fluid movement in a concentration such as 1 mM or less.

It is widely accepted that ASP can be absorbed from the gastric segment. Segmental difference in ASP absorption rate was statistically observed using ANOVA

and the gastric ASP absorption, 3.60 ± 0.40 μmol/h/g dry weight (mean ± S.E.M.) occurred to the same extent statistically as that in the duodenum, 2.58 ± 0.68 μmol/h/g dry weight, but less than that in the other two segments (12.91 ± 2.57 and 14.52 ± 1.77 μmol/h/g dry weight for the jejunum and ileum). It has been observed that MCP was absorbed only in the small intestine of rats (Kitazawa et al., 1977a). In rabbits, segmental differences in MCP absorption were statistically found with ANOVA as well and the absorption rate in the intestine was more than 50 times greater than in the stomach (Fig. 4). In addition, duodenal absorption of MCP (14.04 ± 1.22 μmol/h/g dry weight; mean ± S.E.M.) was more than 5 times than that of ASP (see Figs. 3 and 4).

Archambault et al. (1967) have examined the in situ human duodenal pH and reported that the average value at the duodenal bulb was 4.5 in a fasting normal group and the pH value was lower after a meal. In the present study, duodenal pH was found to be greater than 8.0 in both drug studies (Table 1). Considering this evidence, ASP absorption may be increased after a meal in the duodenum according to the pH-partition hypothesis (Shore et al., 1957).

A good positive correlation between transmucosal fluid movement and drug absorption was found as shown in Figs. 3 and 4. However, the MCP absorption from the gastric segment was negligible as mentioned above (Fig. 4). The low MCP gastric absorption supports our previous report (Kitazawa et al., 1977a) and may explain why the two regression lines as shown in Fig. 4 do not statistically coincide. In our previous reports (Kitazawa et al., 1975, 1978; Kitazawa and Johno, 1976), it has been elucidated that the intestinal absorption of drugs was affected by transmucosal fluid movement in rats; that is, drug absorption was increased with increasing fluid absorption. We have used the entire small intestine and fluid movement was increased by decreasing the osmolality of drug solution. In the present study, the fluid absorption was different from each segment in spite of the fact that an isotonic drug solution was employed in all segments. This segmental difference in transmucosal fluid movement affected the drug absorption from the respective segments. Sandle and others (1982) have found a significant positive linear relationship between water absorption and hydrocortisone absorption. They have concluded that the increased bulk flow of water may have increased the diffusive movement of hydrocortisone. Kitazawa and his coworkers (1975) have demonstrated that the increased drug absorption by increasing fluid absorption might be due to facilitated transport such as solvent drag. We conclude that the results obtained by us support their findings and one of the segmental differences in drug absorption is dependent upon transmucosal fluid movement in each GI segment.

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