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Site of drug absorption after oral administration: Assessment of membrane permeability and luminal concentration of drugs in each segment of gastrointestinal tract

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ABSTRACT

This study was conducted to assess the site of drug absorption in the gastrointestinal (GI) tract after oral administration. Drug permeability to different regions of rat intestine, jejunum, ileum and colon, was measured by *in situ* single-pass perfusion method. It was revealed that the epithelial surface area should not be a determinant of the regional difference in the intestinal permeability of highly permeable drugs. Effects of the mucus layer at the surface of the epithelium and the fluidity of the epithelial cell membrane on the drug permeability were investigated. These factors are demonstrated to contribute to the regional differences in intestinal drug permeability. The luminal drug concentration in each segment of the GI tract after oral administration was measured directly in fasted rats. Water ingested orally was absorbed quickly in the jejunum and the luminal fluid volume was diminished in the middle to lower part of the small intestine. According to the absorption of water luminal concentration of atenolol, a drug with low permeability, was elevated and exceeded the initial dose concentration. In contrast, the concentration of highly permeable drugs, antipyrine and metoprolol, decreased quickly in the upper part of the intestine and a significant amount of drugs was not detected in the lower jejunum and the ileum. From the time-profiles of luminal drug concentration, fraction of dose absorbed from each segment of the GI tract was calculated. Both antipyrine and metoprolol were found to be absorbed quickly at the upper part of the small intestine. In addition, the possible contribution of gastric absorption was demonstrated for these drugs. The pattern of site-dependent absorption of atenolol showed the higher absorbability in the middle and lower portion of the jejunum. These informations on site-dependent absorption of drugs are considered to be important for effective oral delivery systems.

1. Introduction

Oral drug administration is the most convenient and common method of medication and now more than 60% of marketed drugs are used as oral products. Drugs administered orally are absorbed into systemic circulation mainly from the small intestinal tract. Although the upper part of the small intestine is considered to have the higher capacity for drug absorption,

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some drugs are known to be absorbed from other specific portions of the intestinal tract including the colon. In order to develop efficient dosage forms, therefore, detailed information on the site-dependent absorption of drugs should be necessary. Especially for sustained of controlled release products, high absorbability of drugs in the lower part of the intestine is desired.

Drug absorption rate and amount are expressed by the following equations as:

$$\text{absorption rate} = P_{\text{eff}} S C_{\text{GI}} \quad (1)$$

$$\text{absorption amount} = P_{\text{eff}} S \int_0^t C_{\text{GI}}\, dt = P_{\text{eff}} S\, \text{AUC}_{\text{GI}} \quad (2)$$

where P_{eff} is an effective permeability of drugs, S the surface area of the intestinal membrane and C_{GI} is a luminal concentration of drugs. AUC_{GI} expresses the area under the drug concentration–time curve in the GI tract during the intestinal transit time, t. In order to calculate the fraction absorbed of drugs in each segment of the GI tract, these parameters in the equations should be assessed.

Among above parameters, gastrointestinal transit time of drugs has been researched in detail using polyethylene glycol 4000 (Murata et al., 1987), and phenol red (Sawamoto et al., 1997) as non-absorbable markers. There are reviews indicating the importance of the gastrointestinal transit rate in considering the absorption kinetics and the bioavailability of orally administered drugs (Kimura and Higaki, 2002).

Generally, drug permeability to the intestinal membrane is considered to be higher in the upper region of the intestine than in the lower. Well-developed villous structure in the upper intestine spreads the surface area of the membrane. Relatively leaky structure of the membrane might also contribute to the high permeability of hydrophilic drugs to the upper intestinal membrane. However, some drugs have been reported to show fairly high permeability even to the lower part of the intestine, ileum and colon (Patel and Kramer, 1986; Gramatte, 1994). The pattern of regional difference in permeability should be dependent on the physicochemical properties of drugs.

Water volume for oral dosing is an important factor to determine the dose concentration of drugs. Usually, 250 mL of water is recommended in the clinical study to be ingested with drugs. However, drug concentration in the GI tract should be changing according to the fluid absorbed from the GI tract. Also, gastrointestinal secretion of fluids, such as bile and pancreatic juice, might affect the fluid volume in the intestine. However, since information on real fluid volume or the concentration of drugs in the each part of the GI tract *in vivo* are very limited, ingested water volume (250 mL) has been used to calculate the drug concentration in the intestine to estimate or simulate the oral drug absorption so far (Yu et al., 1996a).

In this report, first we have tried to clarify factors that contribute to the regional differences in the intestinal drug permeability by taking into account the physicochemical properties of drugs. Then, the luminal drug concentration in each segment of the GI tract after oral administration was measured directly in fasted rats. In addition, the change in water volume in each segment was estimated from the concentration of non-absorbable marker, FITC-dextran (FD-4).

2. Materials and methods

2.1. Materials

[^{14}C]Mannitol and [^{14}C]Urea was purchased from New England Nuclear (Boston, MA). Antipyrine, atenolol, griseofulvin, metoprolol and naproxen were purchased from WAKO Pure Chemical Industries, Ltd. (Japan). FITC-dextran (FD-4, MW 4400) was purchased from Sigma–Aldrich (St. Louis, MO). All other chemicals were commercial products of reagent grade.

2.2. Intestinal drug permeability

The permeability of rat intestinal membrane was evaluated by *in situ* single-pass perfusion method and closed loop method. Male Wistar rats (body weight, 200–250 g) fasted overnight were anesthetized with pentobarbital. The abdominal cavity was opened and an intestinal loop (length, 10 cm) was made at three regions (proximal jejunum, distal ileum, and colon) by cannulation with a silicone tube (i.d., 3 mm), then the intestinal contents were removed by a slow infusion of saline and air.

Following the above procedure, in the perfusion experiment, test solution (phosphate buffered solution, adjusted to pH 6.5) containing each compound (20 μM) and FD-4 (10 μM) was perfused with an infusion pump at a flow rate of 0.5 mL/min. The effluent was collected from 30 min after starting the perfusion to 90 min at 10-min intervals, because steady-state absorption usually was achieved by 30 min under these conditions. The drug permeability was calculated according to the following equation:

$$P_{\text{eff}} = \frac{Q(1 - C_{\text{out}}/C_{\text{in}})}{2\pi RL} \quad (3)$$

where Q is the flow rate and $C_{\text{out}}/C_{\text{in}}$ is the ratio of outlet/inlet drug concentration. The effect of water transport during perfusion on C_{out} was corrected using the concentration ratio of a non-absorbable marker (FD-4). L and R represent the length and the radius of the used segment of intestine, respectively, thus, the value of $2\pi RL$ corresponds to its surface area. As a radius of each intestinal segment, the value reported by Fagerholm et al. (1997) was used (0.18 cm for jejunum and ileum, 0.25 cm for colon).

In the closed loop experiment, test solution containing each compound (20 μM) was introduced into intestinal loops and both ends of the loop were ligated. After a certain period of time, the luminal solution in the loop was collected. The drug permeability was evaluated by the percentage of dose absorbed, by subtracting the remaining amount of the drug from the administered amount. The following equation was used to calculate the permeability:

$$P_{\text{eff}} = \frac{k_a V}{2\pi RL} \quad (4)$$

where k_a is the absorption rate constant of the drug estimated from the percentage of dose absorbed during the defined

period, assuming that the drug absorption follows the first-order rate kinetic. V is the volume of drug solution introduced to the loop.

To remove mucus layers, mucosal surface was exposed to 20 mM dithiothreitol solution (DTT) for 30 min before the perfusion study (Tockman et al., 1995). It has been confirmed that 20 mM of dithiothreitol solution did not induce damage to the intestinal epithelial (data not shown).

2.3. Pore radius of the paracellular pathway

Pore size was calculated by the Renkin molecular sieving function. The paracellular permeability coefficients of neutral compounds are given by the following equation:

$$P_p = \frac{\varepsilon D F(r/R)}{\delta} \quad (5)$$

where ε is the porosity of the monolayer, δ the tortuousity times the path length across the monolayer, D the aqueous diffusion coefficient of the solute and $F(r/R)$ is the Renkin function (Adson et al., 1994). The aqueous diffusion coefficient was calculated by the Stokes–Einstein equation for equivalent spherical molecules:

$$D = \frac{RT}{6\pi\eta r N} \quad (6)$$

where R is the gas constant, T the absolute temperature, η the viscosity of solvent and N is the Avogadro's number. The dimensionless Renkin molecular sieving function compares the molecular radius (r) and the cylindrical pore radius (R) and takes values of $0 < F(r/R) < 1$:

$$F\left(\frac{r}{R}\right) = \left(1 - \left(\frac{r}{R}\right)\right)^2 \times \left[1 - 2.104\left(\frac{r}{R}\right) + 2.09\left(\frac{r}{R}\right)^3 - 0.95\left(\frac{r}{R}\right)^5\right] \quad (7)$$

The aqueous pore radius of tight junctions was calculated from the ratio of the paracellular permeability of the pair of neutral compounds, mannitol and urea, by the following relationship:

$$\frac{P_{p,mannitol}}{P_{p,urea}} = \frac{r_{urea} F(r_{mannitol}/R)}{r_{mannitol} F(r_{urea}/R)} \quad (8)$$

The permeability coefficients are obtained experimentally. The molecular volume, and thereby molecular radii are obtained using QMPRplus™ (Simulation Plus, Inc., U.S.A.) which is a state-of-the-art computer program designed to estimate certain ADME properties of a drug from its structure (SMILES code). Unknown parameters without the pore radius are canceled.

2.4. Preparation of rat brush border membrane vesicles (BBMVs)

The BBMVs were prepared from intestinal segments by a $CaCl_2$ precipitation method, as previously described (Kessler et al., 1978; Vazquez et al., 1997). The mucosal surface of each segment was scraped off with a cover glass. The intestinal mucosa were collected and homogenized in a buffer containing 50 mM mannitol, 2 mM HEPES/Tris (pH 7.1) with a Waring blender. One molar of $CaCl_2$ was added to the suspension to a final concentration of 10 mM and mixture was stirred for 15 min on ice. The suspension was centrifuged 3000 rpm for 17 min at 4 °C. The supernatant was centrifuged at $27{,}000 \times g$ for 30 min. After successive centrifugations, final pellet containing purified BBMVs was resuspended in a buffer containing 270 mM mannitol and 25 mM HEPES/Tris (pH 7.4) to a final protein concentration of 10 mg/mL.

2.5. Membrane fluidity

The steady-state fluorescence polarization and fluorescence anisotropy were determined as previously described (Garriga et al., 2002), using the lipid-soluble fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH). BBMVs equivalent to 100 mg protein was incubated at 25 °C for 1 h in 2 mL of buffered saline containing 250 mM sucrose, 10 mM Tris/HCl (pH 7.4), and 2 mM DPH. Measurements were taken using Analyst GT (Nihon Molecular Devices Co.), a fluorescence spectrophotometer equipped with a polarizing filter. The excitation and emission wavelengths were 330 and 530 nm, respectively. The degree of fluorescence polarization (p) was defined as the following equation:

$$p = \frac{I_V - I_H}{I_V + I_H} \quad (9)$$

where I_V and I_H are observed intensities measured with polarizers, respectively, parallel to and perpendicular to the vertically oriented polarizer exciting beam. The fluorescence anisotropy (r) was calculated as follows:

$$r = \frac{2p}{3 - p} \quad (10)$$

2.6. Luminal drug concentration

Three drugs (atenolol, metoprolol, antipyrine) and FD-4 were dissolved in purified water and orally administered as a cassette dose to rat. The initial concentration of drugs was 200 μM and the volume of the solution administered was 1 mL. Then, rats were sacrificed at 5–10 min intervals during 140 min and their abdomen was opened immediately to take the sample of residual water from each segment of the GI tract. Since water volume remaining in each segment of the GI tract is very small, luminal water was sampled using a sponge by wiping off the surface of the GI membrane at each region. Then, the sponge was weighed and the amount of water was calculated by setting the relative density of water equal to 1. Drugs contained in the sponge were extracted using purified water. GI segments used in this study were the stomach, the duodenum (a 2-cm position distal to the stomach), the upper jejunum (a 20-cm position distal to the stomach), the lower jejunum (a 60-cm position distal to the stomach) and the ileum (a 10-cm position proximal to the caecum).

2.7. Analytical methods

The concentrations of [^{14}C]Mannitol and [^{14}C]Urea were determined by a liquid-scintillation counter (LSC 3500, Aloka, Tokyo, Japan).

The concentrations of drugs in perfusate samples were analyzed with a reversed-phase HPLC system (LC-10A Shimadzu Co., Kyoto, Japan) equipped with a variable wavelength ultraviolet detector (SPD-10A, Shimadzu Co., Kyoto, Japan). The column (J'sphere ODS-H80 75 mm × 4.6 mm i.d., YMC, Japan) was used with a mobile phase consisting of 50 mM phosphate buffer (pH 2.5) and acetonitrile. Antipyrine, atenolol, griseofulvin, metoprolol and naproxen were quantified with the variable ultraviolet detector at 250, 226, 325, 225 and 230 nm, respectively.

The concentration of FD-4 was determined fluorometrically (495 nm for excitation and 514 nm for emission) using a spectrofluoro-photometer (RF-5300PC, Shimadzu Co., Kyoto, Japan).

The drug concentrations of residual water sampled by sponge from lumens were analyzed with the reversed-phase HPLC system (LC-10AD, Shimadzu Co., Kyoto, Japan) equipped with a LCMS detector (LCMS-2010A, Shimadzu Co., Kyoto, Japan). MercuryMS (Luna5μ C18, 10 mm × 4.0 mm i.d., Phenomenex, CA) were used as an analytical column, and the mobile phase was composed of 0.1% formic acid in water and acetonitrile. Selected ion monitoring was used for detection of protonated molecules of antipyrine (*m*/*z* 189.00), atenolol (*m*/*z* 267.00), and metoprolol (*m*/*z* 268.00).

3. Results

3.1. Assessment of intestinal drug permeability

3.1.1. Regional difference in intestinal drug permeability

Drug permeability to different regions of rat intestine, jejunum, ileum and colon, was measured by the *in situ* single-pass perfusion method. Table 1 shows the regional differences in the permeability of drugs with their physicochemical properties. To avoid the effect of luminal pH, studies were performed under the fixed pH conditions in all segments using phosphate buffered solution (pH 6.5). In the case of atenolol, antipyrine, and metoprolol, the permeability to the jejunum was higher than that to the colon, following the order of epithelial surface area. Permeability in the ileum was also lower than jejunum for atenolol and antipyrine, but almost the same for metoprolol.

In contrast, the permeability of griseofulvin and naproxen was highest in the colon and lowest in the jejunum, suggesting that the epithelial surface area should not be a determinant of intestinal permeability of these drugs.

3.1.2. Pore radius of the paracellular pathway

In addition to the membrane surface area, difference in the cell junctional structure might be the factor of regional difference in drug permeability. In order to elucidate the difference in paracellular permeability, pore radius of the junctional pathway in each region of intestinal membrane was estimated from the permeability ratio of mannitol and urea by using Renkin equation. Since single-pass perfusion method sometimes fails to detect the permeability of poorly absorbable drugs such as mannitol, the loop method was applied to measure the permeability of mannitol and urea. As shown in Table 2, permeability of mannitol and urea in the colon was significantly lower than that in the jejunum or ileum. However, the calculated values of the pore radii in the jejunum, ileum and the colon were 6.19, 5.31 and 6.23 Å, respectively, indicating that the pore radius of the paracellular pathway is almost similar in all segments.

3.1.3. Effect of mucus layer

Fig. 1 shows the effect of mucus layer at the surface of the intestinal mucosa on the permeability of highly permeable drugs in three intestinal regions. After removing the mucus layer by dithiothreitol, jejunal permeability of griseofulvin was significantly enhanced and became almost the same with that in the ileum or colon. Removal of mucus layer did not affect the permeability of griseofulvin in the ileum and colon. This result indicates that, in the upper part of the intestine, mucus layer regulate the permeability of highly permeable drugs. However removal of mucus layer showed no effect on the permeability of naproxen in all segments. Also, in the case of antipyrine and metoprolol, permeability was not affected by the pretreatment with dithiothreitol in all segments (data not shown).

Table 1 – Physicochemical properties and permeability of drugs at various regions in rat intestine

Drug	MW	pK_a[a]	log *D*(6.5)[a]	Permeability (×10^{-4} cm/s)[b]		
				Jejunum	Ileum	Colon
Atenolol	266.38	10.8	−2.34	0.295 ± 0.062	0.244 ± 0.096	0.207 ± 0.038[c]
Metoprolol	267.41	10.08	−1.08	1.521 ± 0.213	1.603 ± 0.414	1.006 ± 0.389
Naproxen	230.28	4.06	0.83	0.964 ± 0.388	1.382 ± 0.445	1.409 ± 0.200
Antipyrine	188.25	1.6	1.79	1.496 ± 0.025	1.124 ± 0.180	1.078 ± 0.141[c]
Griseofulvin	352.79	Neutral	2.88	0.909 ± 0.243	1.243 ± 0.293	1.296 ± 0.094

[a] Predicted by Pallas.

[b] Permeability was estimated by *in situ* single-pass perfusion method. Solution of each drug (20 μM, flow rate: atenolol, 0.2 mL/min and others, 0.5 mL/min) containing 10 mM FITC-dextran was perfused through the each intestinal loop (10 cm). Phosphate buffer (2.54% $NaH_2PO_4 \cdot 2H_2O$ and 4.41% $Na_2HPO_4 \cdot 12H_2O$, pH 6.5) was used as the solvent. Each value represents the mean ± S.E. of four to eight experiments.

[c] $p < 0.05$ compared with jejunal permeability.

Table 2 – Physicochemical properties and permeability of drugs at various regions in rat intestine

Drug	MW	pK_a[a]	log D(6.5)[a]	Permeability ($\times 10^{-4}$ cm/s)[b]		
				Jejunum	Ileum	Colon
Mannitol	182.20	Neutral	−4.17	0.053 ± 0.008	0.042 ± 0.002	0.019 ± 0.001[c]
Urea	60.07	Neutral	−1.79	0.536 ± 0.054	0.616 ± 0.061	0.191 ± 0.033[c]

[a] Predicted by Pallas.
[b] Permeability was estimated by *in situ* closed loop method. Solution of each drug (20 μM, 1 mL) was injected into the each intestinal loop (10 cm). Phosphate buffer (2.54% $NaH_2PO_4 \cdot 2H_2O$ and 4.41% $Na_2HPO_4 \cdot 12H_2O$, pH 6.5) was used as the solvent. Each value represents the mean ± S.E. of four to eight experiments.
[c] $p < 0.05$ compared with jejunal permeability.

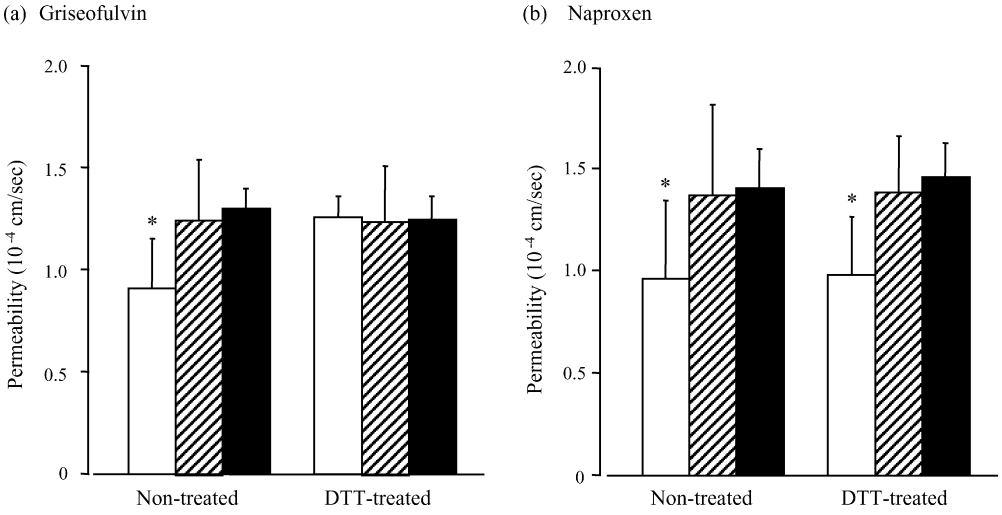

Fig. 1 – Effect of mucus layer on the permeability of (a) griseofulvin and (b) naproxen at various regions in rat intestine. The permeability was estimated by *in situ* single-pass perfusion method. To remove the mucus layer, intestinal epithelium was exposed to 20 mM dithiothreitol (DTT) for 30 min before perfusion experiments. Open bars represent permeability at jejunum, hatched bars represent at ileum, and filled bars represent at colon. The data are expressed as the mean ± S.E. of at least four experiments. * $p < 0.05$ compared with colonic permeability.

3.1.4. *Regional membrane fluidity*

As another factor for regional difference in drug permeability, membrane fluidity was measured by using the fluorescence depolarization method in rat intestinal BBMVs. The steady-state fluorescence polarization and fluorescence anisotropy data for DPH-labeled BBMVs preparations from each intestinal segment is given in Table 3. Both polarization and anisotropy were highest in duodenum, followed by the order of jejunum > ileum > colon. This result clearly indicated that the cellular membranes from proximal intestine were less fluid than distal one.

3.2. *Assessment of luminal drug concentration after oral administration*

3.2.1. *Luminal concentration of non-absorbable maker (FITC-dextran, FD-4)*

Fig. 2 shows the time course of the gastric concentration of non-absorbable marker, FD-4. In the stomach, FD-4 concentration at 5 min after oral ingestion was almost half of the initial dose concentration, and then decreased rapidly following the first-order rate kinetics. By extrapolating the time curve of FD-4 concentration, initial FD-4 concentration in the stomach at time 0 was estimated to be 153 μM. This indicated that, in rat stomach, approximately 0.3 mL of fluid existed before oral ingestion of drug solution. Since the half-life of gastric emptying of the solution in the fasted rat was reported to be around

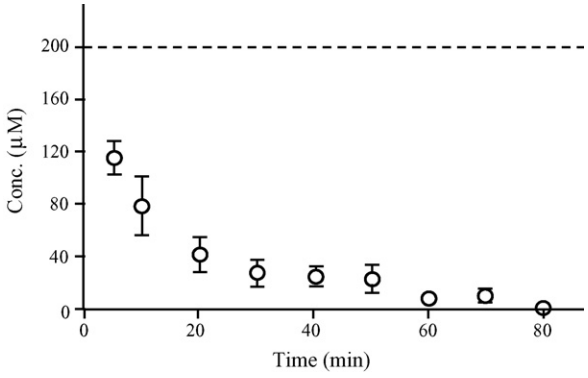

Fig. 2 – Time course of FD-4 concentration in the stomach. After oral administration of drug solution (dosage; 200 μM of each drugs, 1 mL), luminal residual water was sampled from the stomach directly. The data are expressed as the mean ± S.E. of at least four experiments.

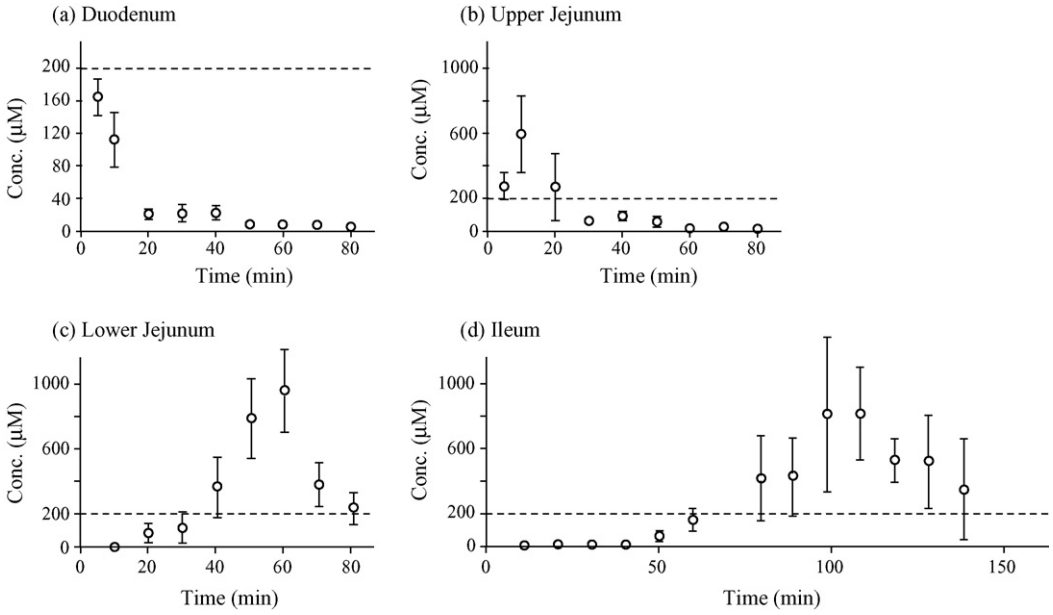

Fig. 3 – Time course of FD-4 concentration in each segment of the GI tract. After oral administration of drug solution (dosage; 200 μM of each drugs, 1 mL), luminal residual water was sampled from each segment. The data are expressed as the mean ± S.E. of at least four experiments.

10 min (Kimura and Higaki, 2002), rapid decrease in the gastric concentration of FD-4 was considered to represent the time-profile of gastric emptying of orally ingested solution.

The time course of FD-4 concentration in each segment of the small intestine is shown in Fig. 3. In the duodenum, FD-4 concentration decreased rapidly in a similar manner with that in the stomach, showing the movement of solution down to the jejunum. In the jejunum and ileum, FD-4 concentration reached the peak at 10, 60, and 110 min after oral ingestion in the upper jejunum, lower jejunum and ileum, respectively. In all segments of small intestine, the peak concentration of FD-4 was apparently higher than the initial dose concentration, approximately 2.5 times higher in the upper jejunum and 4–5 times higher in the lower jejunum and ileum. Since the change in FD-4 concentration reflects the change in the volume of water in which FD-4 was dissolved, this result clearly indicates that water ingested orally was absorbed quickly in the jejunum and luminal fluid volume was diminished especially in the middle to lower part of the small intestine.

3.2.2. *Luminal concentration of drugs*

In the case of drugs absorbable from the GI tract, luminal concentration should be determined not only by the fluid volume change but also by their absorption. Luminal concentrations of atenolol, a drug with relatively low permeability, in each segment is shown in Fig. 4. The time-profiles of luminal concentration of atenolol were similar to those of FD-4 in all segments. Atenolol concentration became higher than the initial dose concentration in upper and lower jejunum. However, the peak concentrations in both segments were only 1.2–1.4 times higher than the dose concentration and in the ileum, peak concentration was much lower than that of FD-4 (approximately 120 μM). This may be due to the absorption of atenolol itself from the GI tract.

In contrast, luminal concentration of highly permeable drugs such as metoprolol was lower than that of atenolol or FD-4 and a peak was not observed in all segments (Fig. 5). Furthermore, any significant concentration was not detected in the lower jejunum and ileum. In the case of antipyrine, luminal concentration was detected only in the stomach and the duodenum (Fig. 6). In the duodenum, luminal concentration at 5 min after oral ingestion was very low (about 20 μM), and thereafter, any significant concentration was not detected. It was apparent that both drugs were absorbed quickly from the upper part of the GI tract.

The area under the luminal concentration–time curve of drugs (AUC_{GI}) in each segment of the GI tract corresponds to the amount of drugs that passed through the segment after oral ingestion. Since, the amount of FD-4 that passed through each segment is always equal to the administered dose, the

Table 3 – The steady-state fluorescence polarization and steady-state fluorescence anisotropy at various regions in rat intestine

	Duodenum	Jejunum	Ileum	Colon
Polarization	0.423 ± 0.003	0.413 ± 0.004	0.405 ± 0.005	0.386 ± 0.001
Anisotropy	0.328 ± 0.003	0.319 ± 0.004	0.312 ± 0.004	0.295 ± 0.001

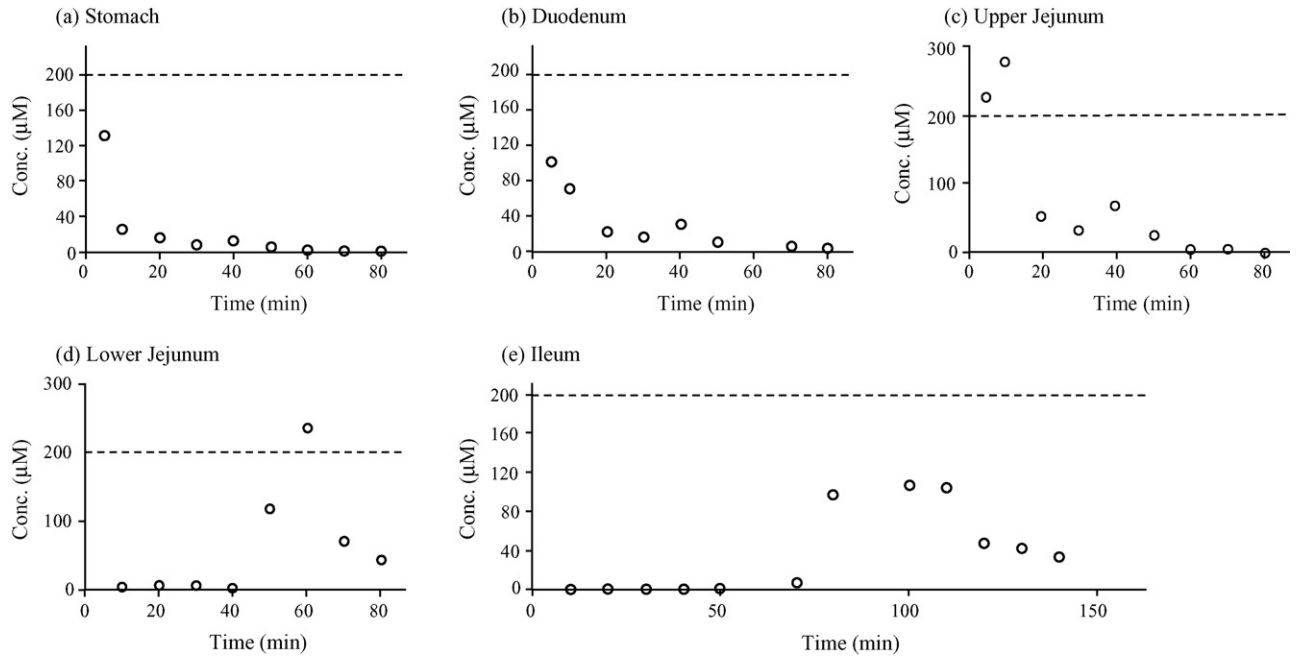

Fig. 4 – Time course of atenolol concentration in each segment of the GI tract. After oral administration of drug solution (dosage; 200 μM of each drugs, 1 mL), luminal residual water was sampled from each segment. The data are expressed as the mean of at least four experiments.

ratio of AUC_{GI} of each drug to that of FD-4 can represent the fraction of the drug remaining in each segment as

$$\text{fraction of drug remained in GI tract}\,(\%) = \frac{AUC_{GI(drug)}}{ACU_{GI(FD\text{-}4)}} \times 100 \quad (11)$$

Then the fraction of drugs absorbed from the GI tract until the time when it passed through each segment can be calculated as

$$\text{fraction of drug absorbed from GI tract}\,(\%) = \frac{AUC_{GI(drug)}}{ACU_{GI(FD\text{-}4)}} \times 100 \quad (12)$$

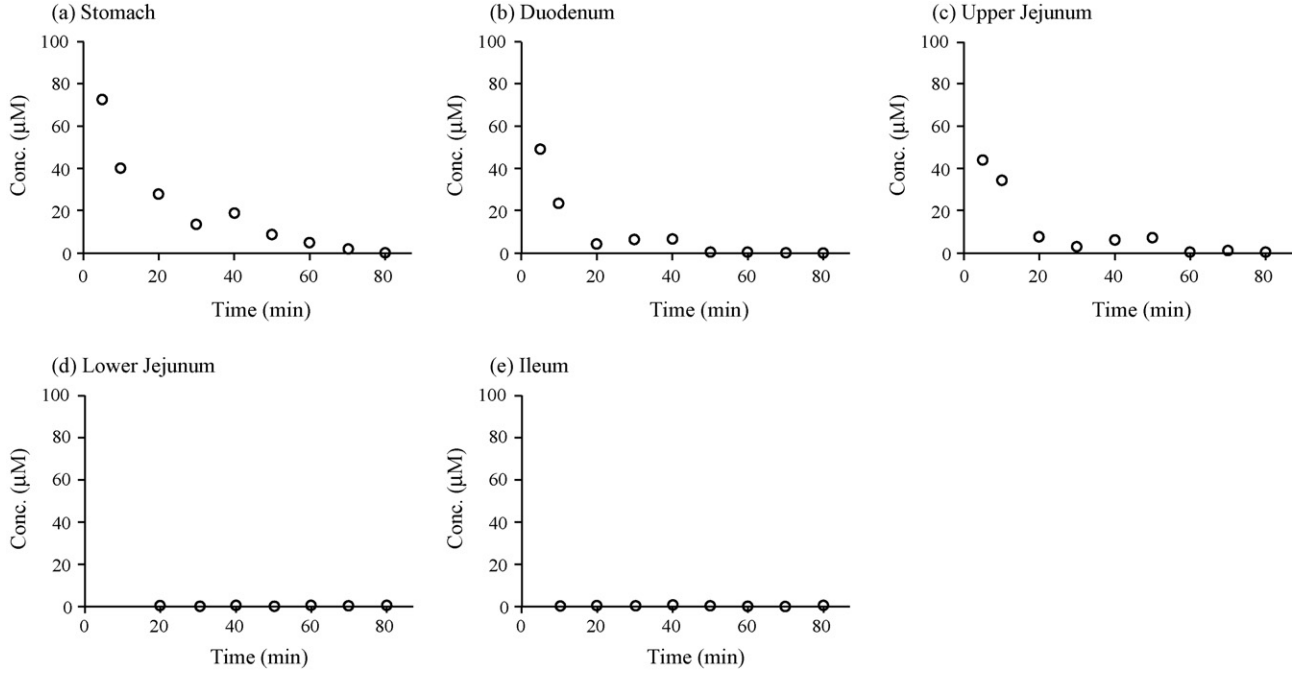

Fig. 5 – Time course of metoprolol concentration in each segment of the GI tract. After oral administration of drug solution (dosage; 200 μM of each drugs, 1 mL), luminal residual water was sampled from each segment. The data are expressed as the mean of at least four experiments.

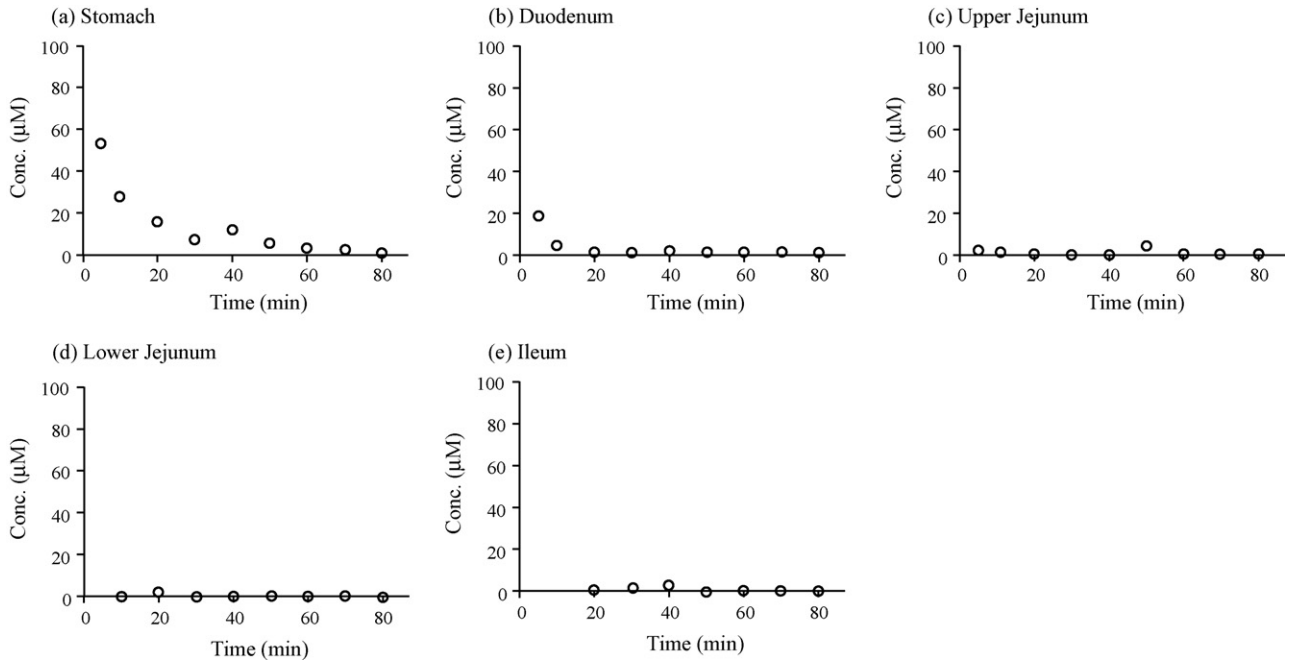

Fig. 6 – Time course of antipyrine concentration in each segment of the GI tract. After oral administration of drug solution (dosage; 200 μM of each drugs, 1 mL), luminal residual water was sampled from each segment. The data are expressed as the mean of at least four experiments.

The fraction of drugs that remained in the GI tract is summarized in Fig. 7. In the case of atenolol, almost 80% of the administered amount has passed through the upper jejunum and then, atenolol was absorbed in the jejunum and only 20% of drug has reached to the ileum. In contrast, both metoprolol and antipyrine were found to be absorbed quickly from the upper part of the GI tract and only a small fraction of drug remained in the GI tract when they have passed through the upper jejunum. Moreover, it was suggested that almost half of the administered dose of antipyrine was absorbed from stomach.

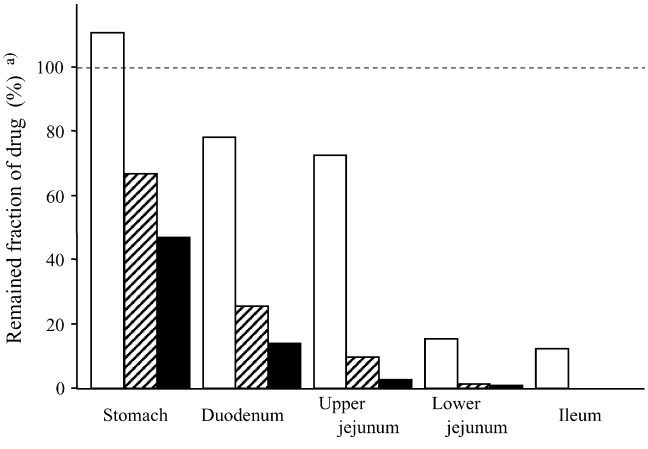

Fig. 7 – Remained amount (%) of three drugs in each segment of the GI tract. % remained = AUC_{drug}/AUC_{FD-4}, where AUC is area under the time curve of luminal drug concentration in each segment (Figs. 2–6). Open bars represent atenolol, hatched bars represent metoprolol, and filled bars represent antipyrine. The data are expressed as the mean of at least four experiments.

4. Discussion

The permeability to the membrane, luminal drug concentration and the residence time are the major determining factors for drug absorption after oral administration. Although some reports have investigated the transit of luminal contents in detail (Murata et al., 1987; Kimura and Higaki, 2002), there are few reports that demonstrate the relation between the permeability and the luminal concentration of drugs in each segment of the GI tract. In this study, in order to consider the site of drug absorption after oral administration, both the permeability and the luminal drug concentration were assessed *in situ* and *in vivo*.

It has been reported that the permeability of drugs is higher in the upper portion of the small intestine than in the lower one. Usually, the permeability of the colonic membrane is considered to be much lower than that of small intestine (Palm et al., 1996; Artursson et al., 1993). This has been proposed to be due to a decreased total surface area of the membrane and increased tightness of the epithelium (e.g. higher electrical resistance) in the colonic region (Thomson et al., 1986; Curran and Schwartz, 1960). In Table 4, the difference in villous surface area of three segments was calculated from reports of Mayhew (1988) and Collett et al. (1997). Villous structure of the jejunum amplifies the area for four-folds compared to the colon and two-folds to the ileum. Following the order of epithelial surface area, drug permeability in the colon must be markedly lower than that in upper small intestine. However in Table 1, only two drugs showed the significantly lower per-

Table 4 – Absorptive surface areas of various regions in rat intestine

	Jejunum	Ileum	Colon
Amplification ratio	7.84 ± 0.24[a]	3.55 ± 0.30[a], 3.9 ± 0.2[b]	1.9 ± 0.1[b]
Relative amplification	1	0.453	0.221

[a] Data from Mayhew (1988).
[b] Data from Collett et al. (1997).

meability in the colon and the differences of the permeability were only 1.4-fold. When comparing the permeability in the jejunum and ileum, no significant differences were observed between all drugs investigated. These findings have clearly demonstrated that the surface area is not a main factor to cause the regional differences in drug permeability. As one of the reasons to explain this discrepancy, it appears that the lipophilic drugs (highly permeable drugs) can permeate the intestinal membrane rapidly through the villous tips, thus the contribution of total surface area to the regional difference in drug permeability becomes smaller. A similar finding has previously been reported in which the absorptive surface area was compared between the intestinal membrane and Caco-2 monolayers (Artursson et al., 2001). In the case of hydrophilic drugs (poorly permeable drugs), they might diffuse farther toward the crypt regions before permeation and the differences in the surface area can be more significant. In our study, the permeability of mannitol and urea in the colon was markedly lower than that in the jejunum (Table 2).

From the ratio of the permeability of mannitol and urea, pore radii of the paracellular pathway in three different regions of the intestine were calculated, since both compounds are considered to permeate the epithelial cell layer predominantly through the paracellular pathway (Gan et al., 1993; Knipp et al., 1997). Again, although the permeability of mannitol and urea in the colon was markedly lower than that in the small intestine, pore radii of the membranes were similar in all segments. Therefore, the lower permeability of mannitol and urea in the colon should be attributable not to the tighter structure of the junctional pathway, but to the less number of pore associated with a smaller surface area of the epithelial membrane. In this meaning, colonic epithelium is regarded as relatively tight, having the higher electric resistance and low paracellular permeability (Curran and Schwartz, 1960; Ma et al., 1991, 1995).

In Table 1, naproxen and griseofulvin showed the relatively higher permeability in the colon than in the small intestine. This result is in good agreement with that in the previous studies (Fagerholm et al., 1997; Sandberg et al., 1988; Lindahl et al., 1996), although the exact reason of the favorable permeability in the colon has not been clarified yet.

It has been suggested that the mucus layer at the surface of the intestinal epithelium has only a minor function as a barrier (Winne and Verheyen, 1990), but others suggest that it could function as a rate limiting barrier against the absorption of highly permeable drugs (Nimmerfall and Rosenthaler, 1980; Smithson et al., 1981). The thickness of the mucus layer varies in different luminal segments. Szentkuti et al. have demonstrated that mucus layer is thicker in the upper intestine than in the lower or colon in fasted rat (Szentkuti and Lorenz, 1995). In this study, after removing the mucus layer by dithiothreitol, jejunal permeability of griseofulvin but not of naproxen was significantly enhanced to the same level with that in the ileum and colon, suggesting the significant role of mucus layer on the proximal-intestinal absorption of griseofulvin.

It has been suggested that the lipid composition in the intestinal membrane could be a factor to regulate the fluidity of the membrane and thereby affect the transcellular transport characteristics (Meddings and Theisen, 1989; Brasitus and Schachter, 1984). Many groups have previously demonstrated regional differences in the lipid composition and membrane fluidity. The study of Garriga et al. (2002) in chicken small intestine, Ibrahim and Balasubramanian (1995) in monkey small intestine and Dudeja et al. (1989) in human small intestine showed that the lower portion of the small intestine is more fluid than the upper. In our study, the same results were obtained in rat, therefore, it was considered that the difference in the membrane fluidity might contribute to the higher permeability of naproxen in the colon. However, some reports demonstrated controversial results in rat (Heubi and Fellows, 1985) and rabbit (Schwarz et al., 1984) where the BBMVs isolated from ileum were less fluid than those from duodenum and jejunum. For this point, more detailed studies concerning the regional differences in lipid composition of cell membranes should be necessary.

In order to simulate the oral drug absorption, kinetic models for drug absorption from the GI tract have been proposed in which the GI tract was divided into several compartments, such as stomach compartment, several compartments comprising the small intestine and the colonic compartment, and the drugs are absorbed from each compartment according to the permeability in each site (Sawamoto et al., 1997; Yu et al., 1996b). In those models, drugs are considered to transit through the GI compartment with a fluid flow. However, information about the fluid flow in the GI tract after oral ingestion of drugs with water is very poor and only indirect evidences are available so far. This situation also caused a difficulty to estimate the luminal drug concentration in each segment of the GI tract. In this study, therefore, we have tried to measure directly the fluid flow and the luminal concentration of drugs after oral administration.

Fluid volume in the GI tract was estimated from the concentration of non-absorbable marker, FD-4. From the time-profile of FD-4 concentration, it was confirmed that orally ingested water passed through the stomach very quickly with a half-life of 5–10 min, then reached to the distal part of the small intestine during 120–150 min. This time-profile of fluid movement in the GI tract is in good agreement with the report by Kimura and Higaki in rat (Kimura and Higaki, 2002). The luminal concentration of FD-4 can give the information on the fluid volume in each segment, and it was founded that the ingested water was rapidly absorbed at the upper part of

the small intestine and the peak concentration of FD-4 became four to five times higher than the initial dose concentration. This result simply means that only 20–25% of the ingested water remained in the lower part of the intestine.

Absorption of water from the intestine was considered to occur mainly by a passive process promoted by the osmotic gradients across the membrane (Leiper, 1998). Water has been presumed to pass the epithelial membrane through the paracellular pathway (Powell, 1981). However, recent studies have revealed that several subfamilies of aquaporins, water channels, are expressed on the apical membrane of the intestinal epithelial cells, and play an important role in the water absorption or secretion (Jung et al., 1994; Koyama et al., 1999). As a future work, by comparison of the water absorption rate and the expression levels of aquaporins in each segment of the GI tract, it may possible to estimate the contribution of aquaporins to the *in vivo* water absorption in the GI tract.

In addition to FD-4, luminal concentration of drugs having various permeability to the intestinal membrane was determined in each segment. Luminal concentration of poorly permeable drug becomes higher than the initial dose concentration at the lower part of small intestine as the same manner with FD-4, and highly permeable drugs are absorbed almost completely from the upper part of small intestine. This result clearly indicated that the luminal drug concentration is determined by the balance of the absorption rate of water and of drug itself. In case of poorly water-soluble drugs, the enhanced concentration in the GI tract might cause the precipitation that interrupts the absorption. From our findings, it can be concluded if the drug permeability is lower than that of atenolol, its luminal concentration is possible to exceed the dose concentration, but if the permeability is higher than that of metoprolol, the luminal concentration should always be lower than the dose concentration and has no chance for precipitation.

Furthermore, fraction of dose absorbed of drugs in each segment of the GI tract can be evaluated by comparing AUC of the luminal concentration of FD-4 and that of the drug. As is evident from Fig. 7, metoprolol and antipyrine are completely absorbed from the upper part of the small intestine. Therefore, if administered as a rapidly dissolving formulation, high permeability of these drugs at the lower part of the intestine is not notable for the absorption. The possible contribution of gastric absorption was also demonstrated for these drugs. Especially in the case of antipyrine, almost 50% of administered amount was absorbed from the stomach. In order to confirm this possibility, permeability of drugs to the gastric membrane should be determined. This study is now under investigation and will be presented at the next opportunity.

The pattern of site-dependent absorption of atenolol showed its higher absorbability in the middle portion of the jejunum. Since atenolol is a basic drug ($pK_a = 10.8$), higher luminal pH at the distal part of the GI tract may lead to less ionization of this drug and enhance its membrane permeability. In the intestinal tract, microclimate pH at the surface of the epithelial layer (unstirred water layer) is reported to be lower than that of the luminal fluid. To consider the effect of pH on drug permeability, the microclimate pH should be more critical than the luminal pH. Although the microclimate pH in each intestinal segment has not been determined, the result of site-dependent absorption of atenolol suggested that the microclimate pH is different in the different intestinal segments corresponding to the pH of the luminal fluid. Therefore, the regional difference in the luminal pH could be an important factor for site-dependent absorption of poorly permeable drugs.

5. Conclusions

In this study, in order to consider the site of drug absorption after oral administration, the permeability and the luminal concentration of drugs were determined. Both the physiological factors of the GI tract and the physicochemical factors of drugs were demonstrated to be important as main causes of the regional differences in drug permeability. The luminal drug concentration in each segment after oral administration was measured directly in fasted rats. This new experimental technique could make clear not only the time-profiles of luminal drug concentration but also the fraction of dose absorbed from each segment of the GI tract *in vivo*. These informations on site-dependent absorption of drugs are considered to be important for effective oral delivery systems.

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