Kinetic Analysis of the Drug Permeation Process Across the Intestinal Epithelium

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The rat intestinal lumen and the blood vessel were simultaneously perfused to study drug permeation across the intestinal epithelium. On the basis of drug disappearance from the intestinal lumen and its appearance into the vascular outflow, the mean time required for permeation across the intestinal membrane (MPT) and the permeation clearance (CLp) were calculated. MPT values of water, antipyrine, propranolol, imipramine and mannitol, varied from 0.45 min to 9.91 min depending on their physicochemical property. From both MPT and CLp, five drugs were classified as being (i) highly and rapidly absorbed (water, antipyrine), (ii) highly but slowly absorbed (propranolol, imipramine) and (iii) low and slowly absorbed (mannitol). Permeation profiles of these drugs were analyzed based on the diffusion model which defined the parameter for each permeation process, i.e. partitioning to and diffusion through the epithelium and clearance into the blood flow. Propranolol and imipramine partitioned into the membrane at a higher level than the other drugs. However, the clearance of both drugs from the epithelium was extremely slow, suggesting that this process is the rate-limiting step in their permeation. On the other hand, the rate-limiting step in the permeation of water and antipyrine was found to be the diffusion process in the epithelial layer.

KEY WORDS: intestinal absorption; vascular perfusion; mean permeation time; moment analysis; diffusion model.

INTRODUCTION

Most drugs are absorbed from the intestinal tract by passive diffusion after oral administration. Specific absorption mechanisms such as carrier-mediated transport of some cephalosporins, have been well characterized (1–3). However, the passive diffusion process used to be described simply using a parameter such as absorption rate constant, membrane permeability or absorption clearance. Though these parameters are useful in evaluating total bioavailability after oral administration (4), the mechanism of intestinal drug absorption needs to be studied in more detail. Using an *in vitro* electrophysiological technique, two possible routes for drug diffusion in the intestinal epithelium were demonstrated, i.e., the transcellular and paracellular routes (5,6). Further, the unstirred water layer at the surface of the intestinal epithelium plays a role as a significant barrier for the absorption of lipophilic drugs (7), and Ho et. al. estimated the effects of both the unstirred water layer and the aqueous pathway upon intestinal drug absorption (8).

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In this report, using vascular perfusion of the rat small intestine, the diffusion of several drugs across the intestinal membrane was studied. Since both drug disappearance from the lumen and its appearance into the vascular flow can be determined simultaneously, vascular perfusion of the intestinal segment can be utilized to investigate the intestinal drug absorption and metabolism (9–11). By this means, we calculated a new parameter which represents the mean time required for drug permeation across the intestinal epithelium. The notion of the mean organ transit time was discussed by Weiss (12), and Hori et al. have defined the parameter which represents the mean time necessary for drug secretion across the renal epithelial cells in the isolated perfused rat kidney (13). In addition, the permeation profiles of drugs were analyzed based on the diffusion model in order to clarify the roles of each permeation process in intestinal drug absorption.

MATERIALS AND METHODS

Materials

[^{3}H]Water (sp ac, 450 mCi/mole), [^{3}H]imipramine (63.9 Ci/mmole), [^{3}H]mannitol (30.0 Ci/mmole), [^{14}C]antipyrine (54.7 mCi/mmole) and [^{14}C]inulin (10.4 mCi/g) were purchased from New England Nuclear (Boston, MA). [^{3}H]Propranolol (20 Ci/mmole) was obtained from Amersham-Searl (Bucks, U.K.). Norepinephrine and dexamethasone were purchased from Sigma Chemical Co., (St. Louis, MO). Bovine serum albumin (BSA, powder fraction V) was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were of analytical grade commercially available.

Preparation of the Intestinal Segment

The small intestine (upper jejunum, about 25 cm) of male Wistar rats (B. W. 250–300 g) was perfused according to the method of Takahashi et al. (14). Briefly, polyethylene tubes (i.d. 0.5 mm) were cannulated to the superior mesenteric artery and the portal vein and used for vascular perfusion. Other polyethylene tubes (i.d. 3 mm) were placed in both ends of the intestinal segment and used for luminal perfusion. Then the intestinal segment with cannulated tubes was isolated from other portions and suspended in a serosal bath containing 100 ml of Krebs-Henseleit bicarbonate buffer (KHBB, pH 7.4) warmed to 37°C with a water jacket as illustrated in Fig. 1.

Vascular Single-Pass Perfusion

Single-pass perfusion of the blood vessel was started just after the intestine was isolated and continued throughout the experiment. KHBB that contains BSA (3%), D-glucose (10 mM), norepinephrine (6.4×10^{-5}%) and dexamethasone (6.4×10^{-5}%) was used as the vascular perfusate. The last two components were added to prevent excessive water secretion and mucosal inflammation (15). The rate of perfusion was usually 3 ml/min and changed from 0.5 ml/min to 5 ml/

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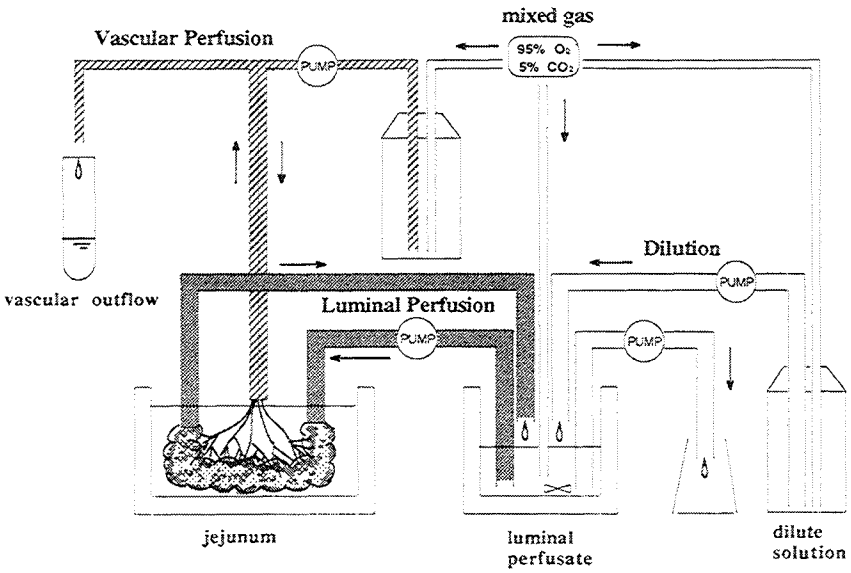

Fig. 1 Schematic diagram of experimental set-up.

min so that the blood flow rate dependency of drug absorption could be studied.

Luminal Recirculating Perfusion

The lumen of the isolated jejunum was perfused recirculatory with 30 ml of KHBB containing ^{3}H-water (0.7 μCi/ml initial concentration), ^{14}C-antipyrine (0.15 μCi/ml), ^{3}H-mannitol (5 μCi/ml), ^{3}H-propranolol (1 μCi/ml) or ^{3}H-imipramine (1 μCi/ml). When using ^{3}H-propranolol or ^{3}H-imipramine, 0.1 mM of non radio-labeled drug was added to the solution to prevent adsorption to polyethylene tubes. The perfusion rate was fixed at 2.5 ml/min in all experiments. In this study, the drug concentration in luminal perfusate was diluted using two pumps (as shown in Fig. 1) according to the first order rate (rate constant = 0.0667 min^{-1}). This compulsory dilution caused a sharp peak in the time-course of the drug appearance rate into the vascular outflow which enables to calculate the various parameters for drug permeation.

Drug Determination

Vascular outflow was collected at one minute intervals for 45 minutes. The rate of drug appearance into the vascular outflow was calculated from the drug concentration in each sample. Aliquots of the luminal perfusate (0.1 ml) were taken at 5-min intervals to measure the time-course of the luminal drug concentration. At the end of the experiment, drug concentration in the serosal bath fluid was also measured. The concentration of all drugs in each sample was calculated from the amount of radio-activity measured in a liquid scintillation counter (LSC 3500, Aloka, Tokyo, Japan).

Calculation of Moment Parameters

From the time-courses of both luminal concentration and vascular appearance rate of each drug, the mean permeation time (MPT) of a drug across the intestinal epithelium was calculated as the difference between the total mean transit time of permeation (MTT) and the mean residence time in the lumen (MRT) using the moment theory (Fig. 2) as follows:

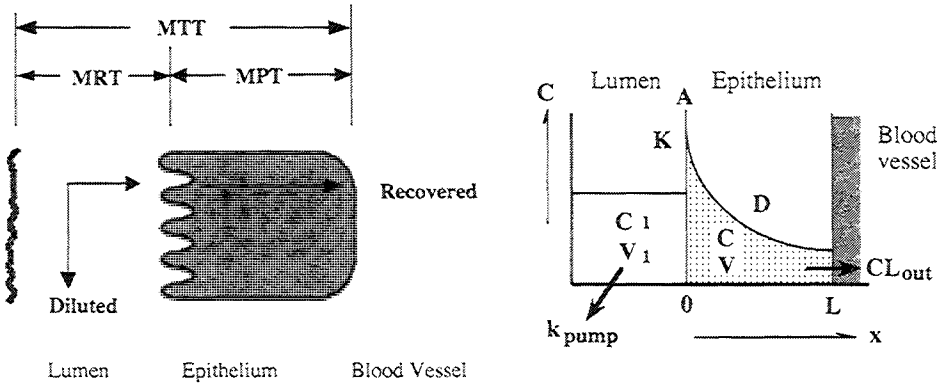

Fig. 2 Moment analysis (left) and diffusion model analysis (right) of drug permeation across the intestinal epithelium. The meaning of each parameter is defined in the text.

$$MTT = \frac{\int_0^\infty Q_{out(t)} * t \, dt}{\int_0^\infty Q_{out(t)} \, t \, dt} \quad (1)$$

$$MRT = \frac{\int_0^\infty C_{lum(t)} * t \, dt}{\int_0^\infty C_{lum(t)} \, dt} \quad (2)$$

$$MPT = MTT - MRT \quad (3)$$

where $Q_{out(t)}$ and $C_{lum(t)}$ represent the vascular appearance rate (% of dose/min) and luminal concentration (% of dose) at time t of each drug, respectively. Also, the membrane permeation clearance of the intestine (CLp) was calculated based on the mass balance of drugs between diluted amount from the lumen and the recovered amount in the vascular outflow (both amounts are defined as % of dose) using the equation;

$$CLp = \frac{\text{recovered amount}}{\text{diluted amount}} * CL_{dil} \quad (4)$$

where CL_{dil} was the clearance of a drug from the luminal perfusate by dilution pumps (product of dilution rate constant and volume of luminal perfusate = 2.0 ml/min). Diluted amount of drug was estimated as the difference between the total and the recovered amount.

Calculation of Diffusion Parameters

According to the diffusion model (Fig. 2), the partition coefficient of the drug between luminal perfusate and the surface of the intestinal epithelium (K), the diffusion constant in the epithelial layer (D) and the clearance from the epithelium to the blood vessel (CL_{out}) were calculated. In this model, L, A and V were defined as the effective thickness, the effective surface area and the effective volume of epithelium, C is the drug concentration in the epithelium, V_1 and C_1 are the volume and the drug concentration in the lumen, $\times$ is the distance, and k_{pump} is the dilution rate constant of the luminal perfusate. First, it was assumed that the drug diffused in the epithelial layer following Fick's second law of diffusion as;

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial X^2} \quad (5)$$

Boundary and initial conditions for this model are;

$$\text{at } t = 0, \; C|_{x=0} = KC_1, \; C|_{x>0} = 0 \quad (6)$$

$$x = 0, \; V1 \frac{\partial C_1}{\partial t} = \frac{V1}{K} \frac{\partial C}{\partial t}\bigg|_{x=0} = DA \frac{\partial C}{\partial X}\bigg|_{x=0} - k_{pump} \frac{V_1}{K} C \quad (7)$$

$$x = L, \; CL_{out} * C\big|_{x=L} = -DA \frac{\partial C}{\partial X}\bigg|_{x=L} \quad (8)$$

The Laplace transform for the drug appearance rate into the vascular outflow ($\overline{Q_{out}}$) is expressed as;

$$\overline{Q_{out}} = \frac{KVX_0}{KV(A \sinh B \, V/CL_{out} + \cosh B) + V_1B(1 + k_{pump}/s)(\sinh B + A \cosh B \, V/CL_{out})} \quad (9)$$

$$A = \sqrt{s \, D/L^2}, \; B = \sqrt{s \, L^2/D}$$

where X_0 is the initial amount of drug in the luminal perfusate (100%) and s is a Laplace variable with respect to time t. Since it is difficult to correctly determine L and V, hybrid parameters are defined as;

$$K' = KV, \; D' = \frac{D}{L^2}, \; k_{out} = \frac{CL_{out}}{V} \quad (10)$$

Using these parameters, Eq. (9) is written as;

$$\overline{Q_{out}} = \frac{K'X_0}{K'(A \sinh B/k_{out} + \cosh B) + V_1B(1 + k_{pump}/s)(\sinh B + A \cosh B/k_{out})} \quad (11)$$

$$A = \sqrt{s \, D'}, \; B = \sqrt{s/D'}$$

The vascular appearance rate profile of each drug was fitted to Eq. (11) using MULTI(FILT) (16) which is a nonlinear least squares computer program based on the fast inverse Laplace transform algorithm and run on personal computer (PC9801 FA, NEC, JAPAN).

RESULTS

Experimental Condition

When the intestinal lumen was perfused with a solution containing ^{14}C-inulin (0.2 μCi/ml, without dilution), only a small amount of radioactivity was detected in the vascular outflow and, at 45 min, cumulative amount of absorbed inulin was less than 0.5% of the total perfused amount. This means that the integrity of the membrane as a permeation barrier was retained well, at least during this experimental period. Also, the luminal concentration of inulin was kept constant, suggesting that the change in the luminal volume due to the water absorption or secretion was negligible. The viability of the membrane was also examined by observing the transamination of glutamic acid to alanine using the same method of Takahashi et al. (14). Cumulative amounts of glutamic acid and alanine in the vascular perfusate during 50 min were 9.09 ± 1.91 μmole and 4.57 ± 0.62 μmole, respectively. This result is similar to that reported previously (14).

Shown in Fig. 3 is the vascular flow rate-dependent permeation of ^{14}C-antipyrine and ^{3}H-water. When the vascular flow rate was under 2 ml/min, the absorption of both drugs was affected by changes in the vascular flow rate. At a higher flow rate (5 ml/min), the recovered amount of both drugs decreased, perhaps because of the high pressure inside the blood vessel. Since this study aims to detect the membrane permeation process itself, various parameters were calculated using 3.0 ml/min vascular flow rate. Under this condition, the time necessary for a drug to move along the blood

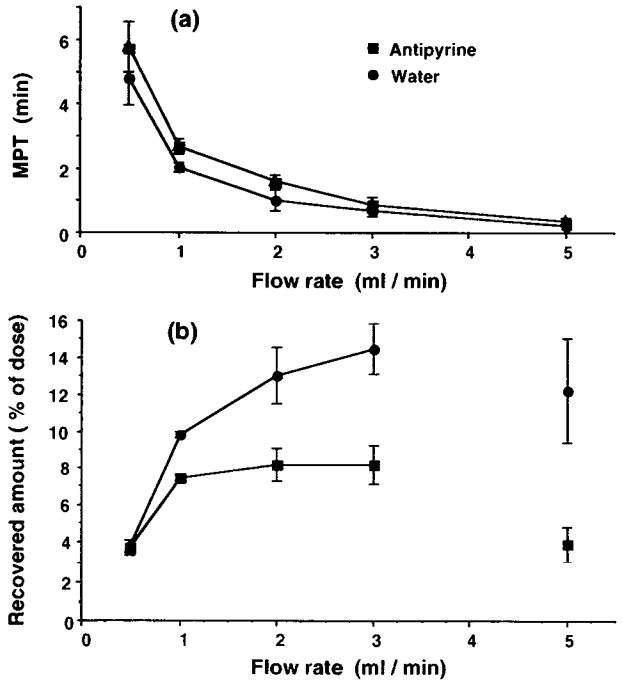

Fig. 3 Effects of the vascular perfusion rate on the absorption of ^{14}C-antipyrine and ^{3}H-water: (a) effect on MPT, (b) effect on recovered amount in the vascular outflow. Each point represents the mean ± SE of three experiments.

vessel after membrane permeation was less than 5 seconds (estimated from a bolus injection of drug into the vascular flow) and had no significant effect on the MPT calculation.

Moment Parameters for the Membrane Permeation of Various Drugs

Fig. 4 demonstrates the vascular outflow profiles of 5 drugs. The time course of the luminal drug concentration was also measured, which decreased in parallel to the dilution rate by fluid pumping because CL_{dil} was much higher than CL_p for all drugs (Table 2). The vascular appearance rates of water and antipyrine rapidly decreased after a sharp

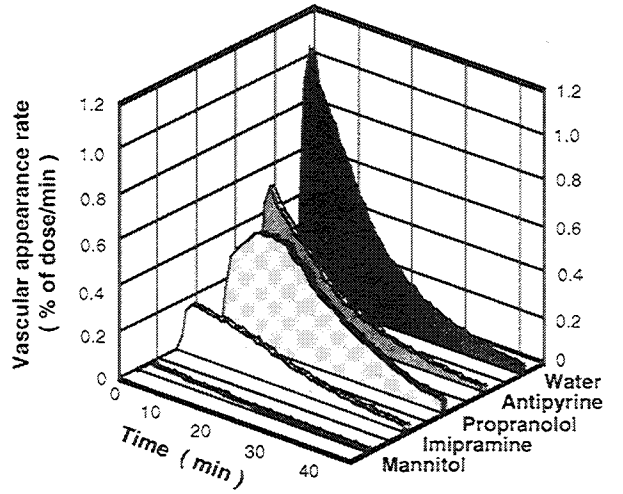

Fig. 4 Appearance rate of various drugs in the vascular outflow. Results are expressed as the mean of at least three experiments.

Table I. Recovery of Drugs in Vascular Outflow and Serosal Bath-Fluid

	Recovered amount (% of dose)	
	Vascular outflow	Serosal fluid
Water	14.5 ± 1.38	4.40 ± 1.14
Antipyrine	8.17 ± 1.05	0.58 ± 0.21
Propranolol	12.2 ± 1.30	0.29 ± 0.10
Imipramine	5.40 ± 0.43	0.74 ± 0.23
Mannitol	1.05 ± 0.22	0.037 ± 0.0053

Results are expressed as the mean ± S.E. of at least three experiments.

peak, whereas those of propranolol and imipramine decreased more gently. Only a little amount of mannitol was recovered in the vascular side. Drug amounts recovered in the vascular outflow, which was calculated from above outflow profiles are summarized in Table 1. Also, the amount in the serosal bath fluid at the end of experiments is shown. The amount of drugs that leaked to the serosal fluid was much less than that recovered in the vascular outflow.

Based upon the vascular outflow profile, MPT and CL_p of each drug were calculated according to equations (1)–(4), and are summarized in Table 2. Although the CL_p of water and propranolol are not significantly different, MPT of propranolol was 10-times longer than that of water. Mannitol showed the lowest and highest CL_p and MPT values, respectively.

Analysis of Drug Absorption Process Using the Diffusion Model

According to equation (11), parameters that represent each process of drug permeation were calculated in Table 3. Each parameter was obtained as a hybrid form (D′, K′ and k_{out}) with a thickness (L) or a volume of epithelium (V). However, since L and V were constant in our experiments, we evaluated the differences in each process among drugs by the hybrids. There were no distinct differences in the D′ of all drugs, while the other two parameters varied widely. Propranolol and imipramine, whose K′ values were higher than that of other drugs, showed distinctly low values of k_{out}. In contrast, the k_{out} of water and antipyrine were high, suggesting that both drugs were cleared quickly from the epithelium by the vascular flow. The K′ of mannitol was distinctly smaller than that of other drugs.

Using the parameters shown in Table 3, the MPT and

Table II. MPT and CLp Calculated from Moment Analysis

	MPT (min)	CLp (ml/min)
Water	0.65 ± 0.24	0.340 ± 0.037
Antipyrine	0.45 ± 0.20	0.179 ± 0.025
Propranolol	6.48 ± 0.80	0.286 ± 0.031
Imipramine	8.14 ± 1.00	0.114 ± 0.010
Mannitol	9.91 ± 3.03	0.018 ± 0.003

Results are expressed as the mean ± S.E. of at least three experiments.

Table III. Parameters for Membrane Permeation Identified by Diffusion Model Analysis

	$D' = \frac{D}{L^2}$	$K' = K \cdot V$	$k_{out} = \frac{CL_{out}}{V}$
Water	0.183 ± 0.021	2.92 ± 0.44	15.80 ± 1.34
Antipyrine	0.178 ± 0.026	1.20 ± 0.24	10.05 ± 1.02
Propranolol	0.097 ± 0.025	11.73 ± 2.34	0.046 ± 0.014
Imipramine	0.290 ± 0.062	16.56 ± 1.89	0.008 ± 0.001
Mannitol	0.289 ± 0.023	0.15 ± 0.04	0.279 ± 0.120

Results are expressed as the mean ± S.E. of at least three experiments.

CL_p of each drug can be calculated according to the following equations as:

$$\text{recovered amount} = \frac{K'/(1/D' + 1/k_{out})}{k_{pump} V_l + K'/(1/D' + 1/k_{out})} \quad (12)$$

$$MPT = \text{recovered amount } (1/2D' + 1/k_{out}) \quad (13)$$

$$CL_p = \frac{\text{recovered amount}}{\text{diluted amount}} CL_{dil} \quad (14)$$

The MPT and CLp of each drug obtained from the moment analysis (Table 2) and from the diffusion parameters are compared in Fig. 5. Except for mannitol, compatible values of MPT and CLp were obtained from both methods.

DISCUSSION

In the vascular perfusion studies, fresh blood taken from other animals or KHBB containing albumin and erythrocytes is often used as the vascular perfusate (15,17,18). Recently, perfluorochemical emulsion was utilized as a oxygen transporter instead of erythrocytes (11,14,19). In contrast, Levin et al. reported that after 1 hour of rat intestinal vascular perfusion with an erythrocyte-free solution, the tissue remained histologically intact and took up oxygen and glucose (20). In our preliminary study, the addition of erythrocytes to the vascular perfusate had no significant effect on the absorption of water and antipyrine. In addition, it was confirmed by the inulin perfusion and the transamination study that the barrier function and the viability of the intestinal epithelium were well retained during the experimental period. These results provide the validity of our method to estimate the drug permeation across the intestinal membrane.

From the CLp and MPT shown in Table 2, five drugs were classified as being (i) highly and quickly absorbed (water, antipyrine), (ii) highly but slowly absorbed (propranolol, imipramine) and (iii) low and slowly absorbed (mannitol). The analysis using the diffusion model clearly showed the reason for these difference. As defined in equations 12 and 14, K′ is the dominant parameter to determine CLp. The order of K′ followed the lipophilicity of drugs except water. Water molecules might diffuse easily into the water domain of the cell membrane and show a relatively high K′ value despite its low lipophilicity. Imipramine and propranolol partitioned to the membrane to a higher level than the other drugs, reflecting their high lipophilicity. Their electric charge (+ charge at neutral pH) possibly contributes also to the high affinity to the membrane. The low CLp of mannitol is thought to result from its low partitioning ability because of high hydrophilicity.

Equation 13 shows that MPT is determined by both D′ and k_{out}, other than recovered amount. The k_{out} values of imipramine and propranolol were two to three orders smaller than that of water or antipyrine, suggesting that the movement of both drugs from the epithelium to the vascular flow was slow enough to be the rate-limiting step of their absorption and caused the longer MPT. On the other hand, the rate-limiting step of water and antipyrine is considered to be the diffusion process. The small k_{out} of imipramine and propranolol would be due to their high affinity for components of the cell membrane or cytosol. Generally, basic drugs strongly interact with membrane lipids through electrostatic binding and are highly accumulated within the membrane. Therefore, other basic drugs are expected to show a similar profile of membrane permeation to imipramine or propranolol. The CLp of both drugs is also affected by this low clearance because, despite their higher partitioning ability, CLp was the same as that of water or antipyrine. Drugs

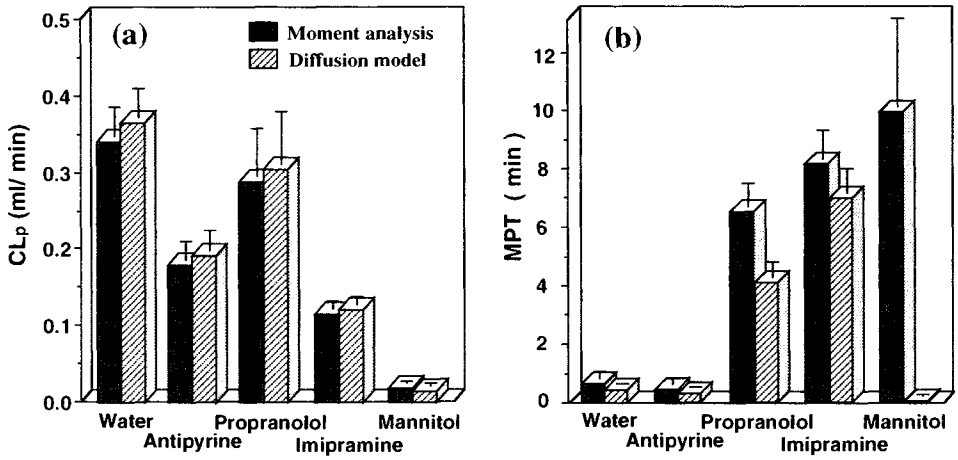

Fig. 5 A comparison of CLp (a) and MPT (b) obtained from moment and diffusion model analyses. Results are expressed as the mean ± SE of at least three experiments.

highly accumulated in the epithelium would reduce the concentration gradient within the epithelium and consequently lower their CLp.

The D′ of propranolol and imipramine should be smaller than those of water or antipyrine because of their interaction within the epithelium. However imipramine had a larger D′ value than other drugs (Table 3). Possibly, the clearance of imipramine from the epithelium strongly affected total permeation and could have obscured the estimation of D′. Thus, to obtain exact values of D′ for propranolol or imipramine, a more detailed study is necessary. However, for those drugs, diffusion process seems to be less important than other processes in regard to intestinal absorption.

As shown in Fig. 5, the values of MPT and CLp for each drug obtained by two methods, namely the moment analysis and the diffusion model, are compatible except for mannitol. Because the moment analysis is a model-independent calculation method (21,22), the results in Fig. 5 supported the applicability of the diffusion model to describe the membrane permeation process of four drugs. The MPT value of mannitol calculated using diffusion model parameters was extremely small compared with that from moment analysis. In our diffusion model, only one route was defined for drug permeation. We have already reported the significant contribution of the paracellular (tight-junctional) route to the permeation of water-soluble and low-molecular weight drugs, such as mannitol, across the intestinal membrane, besides the transcellular route (5,6). Therefore, to describe the permeation of mannitol, a more complex model, which includes the permeation through two routes might be necessary. The other drugs studied here are thought to permeate predominantly by one route, thus, a simple model should be sufficient for describing their permeation.

In conclusion, using the simultaneous perfusion technique, we identified a new parameter, MPT, for the drug permeation across the intestinal membrane and successfully demonstrated the different permeation profiles of drugs. In addition, by means of the diffusion model, the rate-limiting process in the permeation of each drug was clarified. The effect of such differences in the absorption pattern on the therapeutic efficacy of drugs in clinical applications has not yet been established. It may affect the rate of bioavailability of orally administered drugs, and/or it may affect the extent of the bioavailability if the drug undergoes extensive first-pass metabolism in the liver, because higher portal drug level as a result of rapid absorption might saturate the metabolic enzymes. Therefore, the results presented here are significant when considering not only the permeation process of drugs across the intestinal membrane but also the clinical efficacy of orally administered drugs.

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