Application of Fractal Kinetics for Carrier-Mediated Transport of Drugs Across Intestinal Epithelial Membrane

Takuo Ogihara,[1,2] Ikumi Tamai,[1] and Akira Tsuji[1,3]

Received July 24, 1997; accepted December 30, 1997

Purpose. Fractal kinetics was used for the analysis of the carrier-mediated transport for drugs across the intestinal epithelial cells.

Methods. The transport was examined under various agitation rates using a monolayer of Caco-2 cells and rabbit ileum sheets.

Results. The passive transport of antipyrine across Caco-2 cells was increased with the increasing rate of agitation and was supposed to be caused by a change in the thickness of the unstirred water layer. On the contrary, in the case of L-lactic acid transport, which follows a carrier-mediated transport mechanism, the more the agitation rate controlling the *fractal dimension* was increased, the more the permeability rate across the Caco-2 cells was decreased. Fractal kinetic analysis of L-lactic acid transport indicated that the permeability was caused by a single saturable process. Similar agitation effects with L-lactic acid transport were observed in the transport of phenylalanine and cephradine in Caco-2 cells. However, the permeability rates of benzoic acid and 3-O-methyl-D-glucose across Caco-2 cells and L-lactic acid transport across the rabbit ileum tissue indicated the maximum levels at a designated agitation rate. This phenomenon was likely to be caused by the agitation effects controlling not only the fractal environment but also the unstirred water layer.

Conclusions. Carrier-mediated transports are well defined by fractal kinetics rather than classical kinetic analysis. Fractal kinetics are one of the important areas for understanding and confirming the properties of a carrier-mediated transport process.

KEY WORDS: carrier-mediated transport; fractal kinetics; Caco-2 cells; rabbit ileum; diffusion chamber.

INTRODUCTION

It is well known that there are several carrier-mediated transport systems available for nutrients and drugs in the intestine (1). Recently, we demonstrated two types of carrier-mediated intestinal transport that are energized by an inwardly directed proton gradient and/or an outwardly directed bicarbonate gradient (2–8) for monocarboxylic acids, such as: acetic acid, L/D-lactic acids, nicotinic acid, benzoic acid, salicylic acid, and pravastatin using rabbit and rat jejunal brush-border membrane vesicles (BBMVs) and a human carcinoma cell line, Caco-2 cells. We have also demonstrated carrier-mediated transport mechanisms for peptidemimetic drugs such as β-lactam antibiotics (9) in the intestinal epithelial cells using animal jejunal BBMVs, Caco-2 cells and gene expression in *Xenopus laevis* oocytes. These carrier-mediated transports are likely to be superior to passive diffusion explained by a pH-partition theory (10) in the absorption of monocarboxylic acids and oligopeptides across the intestinal membrane.

Until recently, kinetic analysis of carrier-mediated transport has been based on classical kinetics for the transcellular transport rates being expressed by the sum of a Michaelis-Menten kinetic term and a passive diffusion term, as shown in Eq. (1),

$$J = J_{max} \times S/(K_t + S) + k_d \times S \quad (1)$$

where S is the concentration of the substrate, J_{max} is the maximum transport rate for the carrier-mediated process, K_t is the half-saturation concentration (Michaelis constant), and k_d is the first-order rate constant. When the passive diffusion was remarkably superior to the carrier-mediated process ($J_{max}/K_t \ll k_d$), the saturable component *via* some transport systems could not be found clearly from the concentration dependency on the total transport rates. Consequently, it has often been difficult to demonstrate kinetic evidence for carrier-mediated transport.

Most recently, Macheras has proposed a new and unique concept for kinetic analysis of carrier-mediated transport, where the fractal theory was applied as expressed in Eq. (2) (11),

$$J = J_{max}^{eff} \times (S)^{2-D}/(K_t^{eff} + S) \quad (2)$$

where J_{max}^{eff} is the effective maximum transport rate for the carrier-mediated process, K_t^{eff} is the effective half-saturation concentration, and D is a dimensionless number called the *fractal dimension.* This *fractal dimension* is a parameter for heterogeneous reactions when the reactants are spatially constrained on the microscopic level by either walls, phase boundaries, or force fields, such as the restricted movement of the carrier on the complex biological membrane. According to this theory, a couple of the new properties for the carrier-mediated transport mechanism has been understood as follows: 1) when the *fractal dimension* is increased by controlling the transport environment by agitation, for example. The permeability rate of the substrate is decreased as shown in Fig. 1 which represents the relationship between the concentration of substrate on the apical side of the intestinal epithelial cells and the permeability rate as a function of the fractal dimension; 2) the permeability rate can be accounted for only by a saturable process *via* a carrier-mediated transport system, in contrast with the classical kinetics as described in Eq. (1).

The purpose of the present study is to obtain experimental evidence for applying fractal kinetics to carrier-mediated transcellular transport data determined using Caco-2 cells and a diffusion chamber which was attached by rabbit ileum tissues. In the present study, L-lactic acid (8), phenylalanine (12), cephradine (13), benzoic acid (5), and 3-O-methyl-D-glucose (14) were used as substrates for the carrier-mediated transport. In addition, mannitol and antipyrine, which are transported by passive diffusion across paracellular or transcellular pathways in intestinal epithelial cells, were also used.

[1] Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920, Japan.

[2] Present affiliation; Fuji Central Research Laboratory, Mochida Pharmaceutical Co., Ltd., Jimba-aza-uenohara 722, Gotemba, Shizuoka 412, Japan.

[3] To whom correspondence should be addressed. (e-mail: tsuji@ kenroku.ipc.kanazawa-u.ac.jp)

ABBREVIATIONS: BBMVs, brush-border membrane vesicles; HBSS, Hank's balanced salt solution; HEPES, 2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethanesulfonic acid; MES, 2-(N-morpholino)ethanesulfonic acid; AIC, Akaike's information criterion.

0724-8741/98/0400-0620$15.00/0

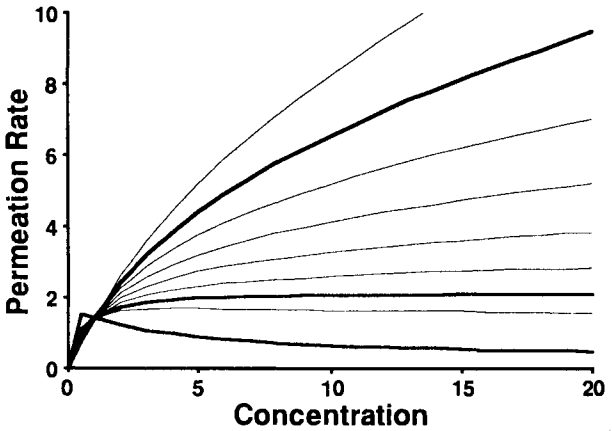

Fig. 1. Theoretical concentration dependency on the carrier mediated transport rate (J) as a function of the fractal dimension D in the range $0.4 \leq D \leq 1.5$ (Eq. 2, using $J_{max}^{eff} = 2.17$, and $K_t^{eff} = 0.5$). The values of D from top to bottom are: 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0,1.1, 1.5.

MATERIALS AND METHODS

Materials

The Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD). Polycarbonate membranes, Snapwell™ clusters, 11.2 mm in diameter and 3.0 μm pore size with an effective area of 1 cm^2 were purchased from Costar (Bedford, MA). L-[^{14}C]lactic acid (5.55 GBq/mmol) and [^{14}C]antipyrine (2.03 GBq/mmol) were purchased from American Radiolabeled Chemicals, Inc.(St. Louis, MO). L-[^{14}C]Phenylalanine (12.95 GBq/mmol), [^{3}H]mannitol (1110 GBq/mmol), [^{3}H]3-O-methyl-D-glucose (2848 GBq/mmol) and [^{14}C]benzoic acid (0.56 GBq/mmol) were purchased from New England Nuclear (Boston, MA). Cephradine was offered by Sankyo Co., Ltd., (Tokyo). Unlabeled L-lactic acid was obtained from Sigma Chemicals (St. Louis, MO). All other chemicals were of reagent grade and were commercially available.

Cell Culture and Ileum Membrane

The cultivation of Caco-2 cells was performed as described previously (5). All cells used in this study were between passages 33 and 59. The cells were grown for 21 to 23 days on a polycarbonate membrane. Membrane tissues were isolated from the ileum of male Japanese white rabbits weighing 2.2–2.8 kg (Nihon SLC). Isolated tissues were washed several times with Hank's balanced salt solution (1.2 mM $CaCl_2$, 0.6 mM KH_2PO_4, 1.2 mM $MgSO_4$, 119 mM NaCl, 2.4 mM K_2HPO_4, 10 mM D-glucose and 21 mM $NaHCO_3$, pH 7.4, and the osmolarity was 315 mOsm/kg).

Transcellular Transport Experiments

Details for the condition of each experiment are described in the legends to figures or table footnotes. A typical experiment for L-lactic acid transport was performed as follows. In the study of the transcellular transport across Caco-2, the cells grown on the polycarbonate membrane were washed twice with Hank's balanced salt solution without sodium ion (sodium-free HBSS: 0.952 mM $CaCl_2$, 5.36 mM KCl, 0.441 mM KH_2PO_4, 0.812 mM $MgSO_4$, 136.7 mM choline Cl, 0.385 mM K_2HPO_4, 30 mM D-glucose and 10 mM Hepes for pH 7.3 or 10 mM Mes for pH 6.0) with osmolarity of 315 mOsm/kg (8). The washed membrane was attached to the diffusion chamber (Costar, Bedford, MA). To initiate the transport experiments, 5 ml of sodium-free HBSS (pH 7.3, 37 C°) was put into the basolateral side (receiver side) and a test solution (sodium-free HBSS pH 6.0, 37°C) including L-[^{14}C]lactic acid as substrate was loaded into the apical side (donor side) of a cell insert. The cells were incubated at 37°C for the time indicated, and 5% CO_2/95% O_2 gas was bubbled into both sides of the cells at rates of 0, 15, 30 or 45 ml/min. At the designated time, either a 0.5 or 1.0 ml aliquot of solution was removed from the receiver side and replaced with an equal volume of fresh sodium-free HBSS (pH7.3, 37°C). To terminate the permeation, the solution in the both sides of the cells was removed by suction. In the case of the transport experiments of [^{14}C]benzoic acid across Caco-2 cells, the same solvents for L-[^{14}C]lactic acid were used. In the transport experiments of L-[^{14}C]phenylalanine, cephradine, [^{3}H]mannitol and [^{3}H]antipyrine by Caco-2 cells, sodium-involved HBSS solution (containing NaCl and K_2HPO_4 in place of choline Cl, and Na_2HPO_4) was used. In the case of the transport experiments of [^{3}H]3-O-methyl-D-glucose, sodium-involved and glucose-free HBSS solution (containing NaCl, K_2HPO_4 and mannitol in place of choline Cl, Na_2HPO_4 and D-glucose) was used. The amount of radio-labeled substrates transported across Caco-2 cells was estimated from the radioactivity in the sample and expressed as permeability (μl/mg protein) by dividing the transported amount by the initial concentration in the donor medium and correcting by the cellular protein amount. Radioactivity was determined using a liquid scintillation counter (LSC-1,000, Aloka Co., Ltd., Tokyo, Japan). Cellular protein was measured by the method of Lowry *et al.* (15) using bovine serum albumin as a standard. The amount of cephradine transported was determined by HPLC analysis. The HPLC system was as follows: the analytical column was Inertsil ODS-2™ 4.6 id. × 250 mm (GL Science Co., Ltd., Tokyo, Japan), and the mobile phase was a mixture of 10 mM acetate buffer (pH 6.0) and methanol (80/20) at a flow rate of 1.0 ml/min at the temperature of 40°C. Cephradine, detected at 220 nm, was determined by measuring the peak height in the HPLC chart. In the calibration, the correlation (R) between mass and peak height was more than 0.999.

To examine the permeability of L-[^{14}C]lactic acid across the rabbit ileum membrane attached to the diffusion chamber, a Cl-free solution (149 mM Na gluconate, 5.1 mM K gluconate, 1.4 mM Ca gluconate, 1.3 mM $MgSO_4$, 1.3 mM KH_2PO_4, 10 mM Na phosphate buffer, pH 6.0) for the mucosal side and a Cl-involved solution (128 mM NaCl, 5.1 mM KCl, 1.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.3 mM KH_2PO_4, 21 mM $NaHCO_3$, 10 mM Na phosphate buffer, pH 7.4) for the basolateral side were used. The amount of L-[^{14}C]lactic acid transported across the rabbit ileum membrane was estimated from the radioactivity in the sample and expressed as permeability (μl/cm^2 membrane).

Data Analysis

The permeability rate corrected by protein content or membrane surface area was evaluated from the slope of the initial linear portion of permeability amount (nmol/mg protein or nmol/cm^2 membrane) versus time (min) curves by linear regres-

sion analysis. In order to estimate the kinetic parameters for the saturable transport across the Caco-2 monolayers, Eq. (1) for classical kinetics and Eq. (2) for fractal kinetics were fitted to the transport rate (J) by using the nonlinear least-square regression analysis program, MULTI (16). Observed transport data were expressed as mean ± S.E.M. (n = 3). Statistical analysis was performed by a student's two-tailed t-test. The difference between means was considered to be significant when P-values were less than 0.05. The AIC, which is equal to $N \cdot \ln(SS) + 2p$, was also used to compare the models, where N is the number of observations, p is the number of model parameters, and SS is the residual sum of squares. A model with lower AIC value is considered more efficient.

RESULTS

Transcellular Transport

(1) L-Lactic Acid

In order to examine the relationship between the rate (ml/min) of agitation of the transport medium and the permeability of L-lactic acid across Caco-2 cells where the concentration ranged from 1 to 20 mM, the concentration-dependent transport of L-lactic acid was determined. As shown in Fig. 2, when no agitation was used, the result indicated that the permeability of L-lactic acid consisted of a saturable process at lower concentrations and a nonsaturable process at higher concentrations. According to the classical kinetic analysis of concentration-dependence obeying Eq. (1), the kinetic parameters obtained for L-lactic acid transport were: J_{max} of 2.21 ± 0.22 nmol/min/mg protein, K_t of 3.75 ± 0.40 mM, and k_d of 0.09 ± 0.01 μl/min/mg protein, which were similar to values obtained previously (8); the AIC value was 37.1. When the gas was bubbled through the chamber at the rate of 15 ml/min, the permeability rate of L-lactic acid was significantly reduced. On increasing the agitation rate to 45 ml/min, a reduced permeability rate was observed. By fractal kinetic analysis using Eq. (2) for the concentration-dependent L-lactic acid transport under all agitation conditions, the kinetic parameters estimated were: J_{max}^{eff} of 2.17 nmol/min/mg protein and K_t^{eff} of 0.50 mM. D-values, which represent the fractal dimensions, were 0.57, 0.64, and 0.77, when the agitation rates were 0, 15, and 45 ml/min, respectively. The AIC value was 7.9.

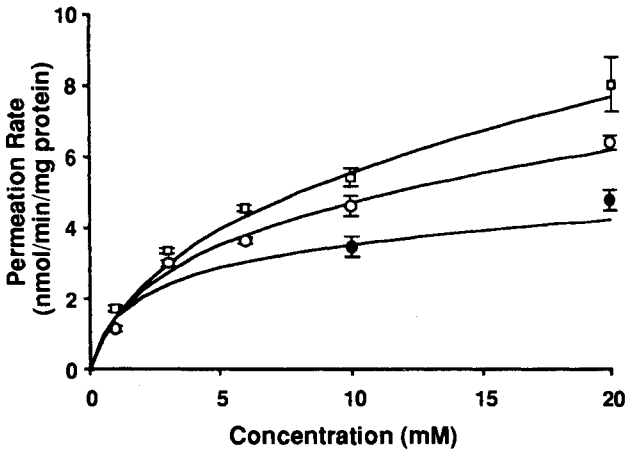

Fig. 2. Concentration dependence of L-lactic acid transport across Caco-2 monolayers in several agitation conditions of 0 (□), 15 (○), and 45 ml/min (●). Permeability of L-[^{14}C]lactic acid (from 1 to 20 mM) was measured at 37°C and by incubating Caco-2 monolayers in sodium-free HBSS buffer (at apical pH 6.0 and at basolateral pH 7.3). An aliquot of sample was withdrawn from the basolatral side at the times indicated. Each point represents the mean ± S.E.M. of three experiments. The solid lines were fitted by Eq. (2) using J_{max}^{eff} = 2.17 nmol/min/mg protein and K_t^{eff} = 0.5 mM, the values of D from top to bottom are 0.57, 0.64, and 0.77 at the agitation rates of 0, 15, and 45 ml/min, respectively.

(2) Phenylalanine and Cephradine

Figure 3 represents the relationship between the agitation rate and the permeability rates of L-phenylalanine (5 mM) and cephradine (10 mM) across Caco-2 monolayers. In the case of L-phenylalanine, when the agitation rates in the chamber were 0, 15, and 30 ml/min, the permeability rates were 0.639 ± 0.047, 0.534 ± 0.031, and 0.521 ± 0.076 nmol/min/mg protein, respectively. The fractal kinetic analysis for the concentration-dependent L-phenylalanine transports provided the following parameters: J_{max}^{eff} of 0.698 nmol/min/mg protein and K_t^{eff} of 4.50 mM. D-values were 0.62, 0.71, and 0.76, when the agitation rates were 0, 15, and 30 ml/min, respectively (data not shown). In the experiment with cephradine, when the agitation rates in the chamber were 0, 15, and 45 ml/min, the permeability rates were 2.655 ± 0.086, 2.183 ± 0.106, and 2.023 ± 0.120 nmol/min/mg protein, respectively.

(3) Mannitol and Antipyrine

Figure 4 represents the relationship between the agitation rate and the permeability rates of mannitol and antipyrine at the concentration of 10 mM across Caco-2 monolayers. When the bubbling rates in the chamber were 0, 15, 30, and 45 ml/min, the permeability rates of mannitol were 2.107 ± 0.349, 2.050 ± 0.133, 2.221 ± 0.186, and 2.048 ± 0.067 nmol/min/mg protein, respectively, indicating no significant change in the permeability rates in spite of increasing the agitation rate. In the case of antipyrine, when the bubbling rates in the chamber were 0, 15, and 30 ml/min, the permeability rates were 58.5

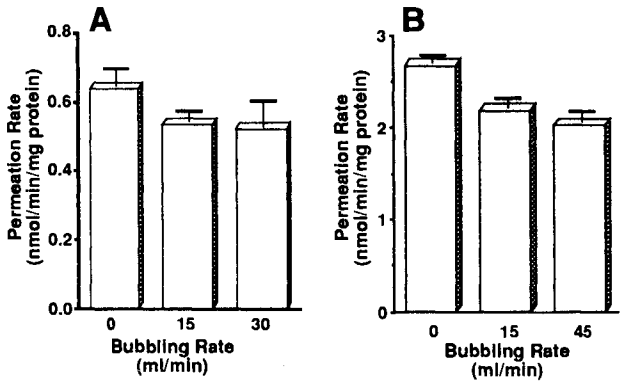

Fig. 3. Relationship between permeability rates of L-phenylalanine (5 mM Panel A) and cephradine (10 mM, Panel B) across monolayers of Caco-2 cells and the agitation rates. Permeabilities of L-[^{14}C]phenylalanine and cephradine were measured at 37°C and by incubating Caco-2 monolayers in sodium-involved HBSS solution containing NaCl and K_2HPO_4 in the place of choline Cl, and Na_2HPO_4. Each value represents the mean ± S.E.M. of three experiments.

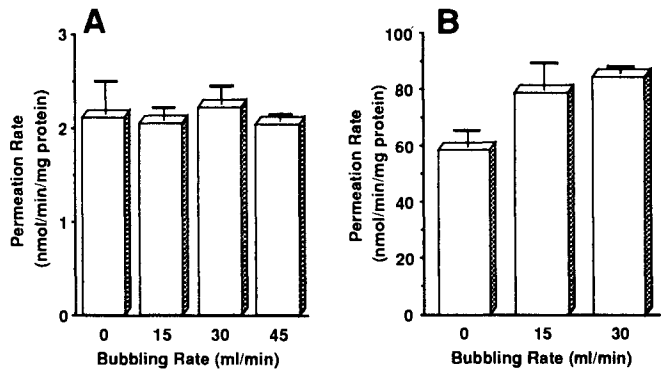

Fig. 4. Relationship between permeability rates of mannitol (Panel A) and antipyrine (Panel B) at the concentration of 10 mM across monolayers of Caco-2 cells and agitation rates. The incubation condition was the same as described in the legend to Fig. 3. Each value represents the mean + S.E.M. of three experiments.

± 5.7, 79.1 ± 9.2, and 84.7 ± 1.2 nmol/min/mg protein, respectively, resulting in a significant increase in the permeability with increasing agitation rates.

(4) Benzoic Acid and 3-O-Methyl-D-Glucose

Figure 5 shows the concentration dependence of benzoic acid transport across Caco-2 monolayers under several agitation conditions. Without agitation, the classical kinetic analysis provided the parameters as follows: J_{max} of 2.21 ± 0.22 nmol/min/mg protein, K_t of 3.75 ± 0.40 mM, and k_d of 0.09 ± 0.01 μl/min/mg protein, which are comparable to the values reported previously (5); the AIC value was 6.2. When the gas was bubbled through the chamber at the rate of 15 ml/min, the permeability rate of benzoic acid was increased. However, when the agitation rate was increased to 45 ml/min, the permeability rate was reduced. Fractal kinetic analysis of the concentration-dependent benzoic acid transport at any agitation provided the following parameters: J_{max}^{eff} of 279 nmol/min/mg protein and J_t^{eff} of 19.3 mM. D-values were 1.24, 1.07, and 1.10 at the agitation rates of 0, 30, and 45 ml/min, respectively. The AIC value was −28.4.

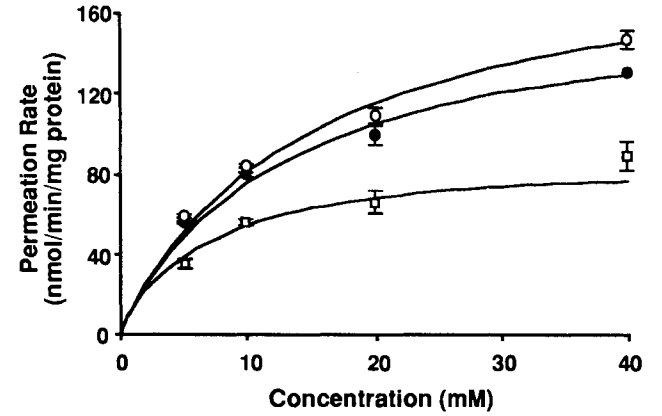

Fig. 5. Concentration dependence of benzoic acid transport across Caco-2 monolayers at the agitation rates of 0 (□),15 (○), and 45 ml/min (●). The incubation condition was the same as described in the legend to Fig. 2. Each point represents the mean ± S.E.M. of three experiments. The solid lines were fitted by Eq.(2) using J_{max}^{eff} = 279 nmol/min/mg protein and K_t^{eff} = 19.3 mM, the values of D from top to bottom are 1.07, 1.10 and 1.24 at the agitation rates of 30, 45, and 0 ml/min, respectively.

The permeability rate of 3-O-methyl-D-glucose at 10 mM concentration across Caco-2 monolayers indicated results very similar to those with benzoic acid, showing that the maximum transport at the agitation rate was 15 ml/min. When the bubbling rates in chamber were 0, 15, and 30 ml/min, the permeability rates were 18.1 ± 0.4, 27.1 ± 3.1, and 24.7 ± 0.6 nmol/min/mg protein, respectively.

(5) Transmembrane Transport of L-Lactic Acid

Figure 6 represents the relationship between the permeability rate of L-lactic acid transport at the concentration of 20 mM across the rabbit ileum tissue and the agitation rate. The result was consistent with the case of benzoic acid and 3-O-methyl-D-glucose across Caco-2 cells. Therefore, when the bubbling rates in the chamber were 0, 15, 30, and 45 ml/min, the permeability rates were 8.78 ± 1.11, 14.31 ± 1.02, 18.44 ± 2.68, and 13.57 ± 0.34 nmol/min/mg protein, respectively. The experiment using the same ileum tissues showed that when the bubbling rate was lowered to 30 ml/min from 45 ml/min, the permeability rate was again at a maximum (data not shown).

DISCUSSION

In the case of L-lactic acid, which is transported *via* monocarboxylic acid transporters (1,2–8), the more the agitation rate was increased, the more the permeability rate from the apical side to the basolateral side of the monolayer of Caco-2 cells was decreased. In the experiments with phenylalanine, which is transported *via* an amino acid transporter (1,12), and with

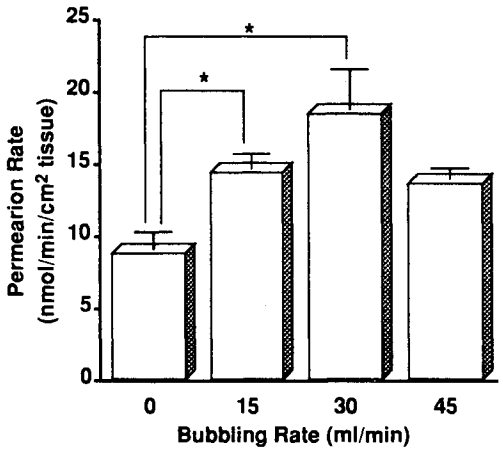

Fig. 6. Relationship between the permeability rates of lactic acid at the concentration of 20 mM across the rabbit ileum tissue and the agitation rates. Permeability of L-[^{14}C]lactic acid was measured at 37°C and by incubating the rabbit ileum tissue in Cl-free solution containing 149 mM Na gluconate, 5.1 mM K gluconate, 1.4 mM Ca gluconate, 1.3 mM $MgSO_4$, 1.3 mM KH_2PO_4, 10 mM Na Phosphate buffer, pH 6.0 for mucosal side and a Cl-involved solution (128 mM NaCl, 5.1 mM KCl, 1.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.3 mM KH_2PO_4, 21 mM $NaHCO_3$, 10 mM Na Phosphate buffer, pH 7.4) for basolateral side. Each value represents the mean ± S.E.M. of three experiments. *Significantly different between bubbling rates ($p < 0.05$).

cephradine, one of the β-lactam antibiotics, which is transported *via* an oligopeptide transporter in the intestinal epithelial cells (1,9), the results were comparable with that of L-lactic acid. The transport phenomena for these compounds were determined by fractal kinetics to be carrier-mediated. On the contrary, in the experiment with mannitol as a substrate, which is transported by passive diffusion *via* the paracellular route of the intestinal epithelial cells, the permeability rate was not significantly changed in spite of increasing the agitation rate. For antipyrine, which was another substrate for passive diffusion *via* the transcellular route of the intestinal epithelial cells, the more the agitation rate was increased, the more the permeability rate was increased. It is likely that the rate-limiting process for mannitol exhibiting very low permeability was paracellular diffusion rather than that across the unstirred water layer existing in front of the cell membrane surface, resulting in permeability rates independent of agitation rate. On the other hand, in the case of the permeation exhibiting higher rates for the substrates such as antipyrine, the transport across the unstirred water layer becomes the rate-limiting process. Therefore, when the agitation rate was increased, the unstirred water layer thickness was decreased, consequently, the permeability rate of antipyrine was increased.

Interestingly, in the case of benzoic acid, which is one of the substrates *via* a monocarboxylic acid transporter, and 3-O-methyl-D-glucose, which is a substrate *via* a D-glucose transporter, when the agitation rate was increased, the permeability rates reached maximum levels at designated agitation rates. Because the permeability rates of these substrates across the membrane by transporters are much faster than those across the unstirred water layer on the Caco-2 monolayer, the agitation influenced not only the fractal dimensions but also the diffusion rate across the unstirred water layer.

When L-lactic acid is transported across the rabbit ileum tissue, the result exhibited a different transport mechanism observed in Caco-2 cells but was consistent with the transport mechanism of benzoic acid and 3-O-methyl-D-glucose across Caco-2 cells. It is most likely that this phenomenon observed in the isolated ileum tissue with an effective large surface area available for carrier-mediated transport is the result of agitation effects controlling the fractal environment and the thickness of the unstirred water layer.

The present study was intended to demonstrate the fractal kinetic analysis of the carrier-mediated transcellular transport data for several drugs across the intestinal epithelial cells. Actually, the transcellular transport rates might be expressed by the sum of the Michaelis-Menten kinetic term modified by fractal kinetics and a passive diffusion term. We have reported carrier-mediated transport of several drugs such as oligopeptides (1,9,21–23), monocarboxylic acids (2–8,17–20) and organic phosphates (1,24) in the intestinal epithelial cells using animal jejunal BBMV and Caco-2 cells, and by gene expression in *Xenopus laevis* oocytes. In these studies, we have performed careful testing to acquire substantial evidence in order to satisfy the criteria for the carrier-mediated transport mechanism from several viewpoints, such as: temperature and concentration dependence, metabolic energy dependence, significant inhibitory effects caused by structural analogues, counter transport effect, effects of agents against proteinase, amino acid modifications, and stereoselective permeability between enantiomers. From the results of the present study, it was indicated that fractal kinetics have become a tool to provide additional evidence for the carrier-mediated transport of substrates. Fractal kinetics are one of the important areas for understanding and confirming the properties of carrier-mediated drug transport.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Hideki Takayasu, Graduate School of Information Sciences, Tohoku University, for his discussions regarding fractal kinetics and useful advice in preparing this manuscript. This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan, the Japan Health Science Foundation, Drug Innovation Project and a grant from the Kanae Foundation of Research for New Medicine.

REFERENCES

1. A. Tsuji and I. Tamai. Carrier-mediated intestinal transport of drugs. *Pharm. Res.* **13**:963–977 (1996).
2. A. Tsuji, M. T. Simanjuntak, I. Tamai, and T. Terasaki. pH-dependent intestinal transport of monocarboxylic acids: Carrier-mediated and H^+-cotransport mechanism versus pH-partition hypothesis. *J. Pharm. Sci.* **79**:1123–1124 (1990).
3. M. T. Simanjuntak, I. Tamai, T. Terasaki, and A. Tsuji. Carrier-mediated uptake of nicotinic acid by rat intestinal brush-border membrane vesicles and relation to monocarboxylic acid transport. *J. Pharmacobio-Dyn.* **13**:301–309 (1990).
4. M. T. Simanjuntak, T. Terasaki, I. Tamai, and A. Tsuji. Participation of monocarboxylic anion and bicarbonate exchange system for the transport of acetic acid and monocarboxylic acid drugs in the small intestinal brush-border membrane vesicles. *J. Pharmacobio-Dyn.* **14**:501–508 (1991).
5. A. Tsuji, H. Takanaga, I. Tamai, and T. Terasaki. Transcellular transport of benzoic acid across Caco-2 cells by a pH-dependent and carrier-mediated transport mechanism. *Pharm. Res.* **11**:30–37 (1994).
6. H. Takanaga, I. Tamai, and A. Tsuji. pH-Dependent and carrier-mediated transport of salicylic acid across Caco-2 cells. *J. Pharm. Pharmacol.* **46**:567–570 (1994).
7. I. Tamai, H. Takanaga, T. Ogihara, M. Yoneda, and A. Tsuji. Proton-cotransport of pravastatin across intestinal brush-border membrane. *Pharm. Res.* **12**:1727–1732 (1995).
8. T. Ogihara, I. Tamai, H. Takanaga, Y. Sai, and A. Tsuji. Stereoselective and carrier-mediated transport of monocarboxylic acids across Caco-2 cells. *Pharm. Res.* **13**:1828–1832 (1996).
9. A. Tsuji. Peptide-Based Drug Design, Chapter 5: Intestinal Absorption of β-Lactam Antibiotics, M. D. Taylar and G. L. Amidon, ed., American Chemical Society (Washington, DC, 1995).
10. B. B. Brodie and C. A. M. Hogben. Some physico-chemical factors in drug action. *J. Pharm. Pharmacol.* **9**:345–380 (1957). *Res.* **12**:541–548 (1995).
11. P. Macheras, Carrier-mediated transport can obey fractcal kinetics. *Pharm. Res.* **12**:541–548 (1995).
12. I. J. Hidalgo and R. T. Borchardt. Transport of a large neutral amino acid (phenylalanine) in a human intestinal epithelial cell line: Caco-2. *Biochem. Biophys. Acta* **1028**:25–30 (1990).
13. S. Matsumoto, H. Saito, and K. Inui. Transcellular transport of oral cephalosporins in human intestinal epithelial cells, Caco-2: Interaction with dipeptide transport systems in apical and basolateral membranes. *J. Pharmacol. Exp. Ther.* **270**:498–504 (1994).
14. S. T. Riley, G. Warhurst, P. T Crowe, and L. A. Turnberg. Active hexose transport across cultured human Caco-2 cells: characterization and influence of culture conditions. *Biochem. Biophys. Acta* **1066**:175–182 (1991).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
16. K. Yamaoka, Y. Tanigawara, T. Nakagawa, and T. Uno. A pharma-

cokinetic analysis program (MULTI) for microcomputer. *J. Pharmacobiodyn.* **4**:879–885 (1981).
17. H. Takanaga, I. Tamai, S. Inaba, Y. Sai, H. Higashida, H. Yamamoto, and A. Tsuji. cDNA cloning and functional characterization of rat intestinal monocarboxylate transporter. *Biochem. Biophys. Res. Commun.* **217**:370–377 (1995).
18. I. Tamai, H. Takanaga, H. Maeda, Y. Sai, T. Ogihara, H. Higashida, and A. Tsuji. Participation of a proton-cotransporter, MCT1, in the intestinal transport of monocarboxylic acids. *Biochem. Biophys. Res. Commun.* **214**:482–489 (1995).
19. H. Takanaga, H. Maeda, H. Yabuuchi, I. Tamai, H. Higashida, and A. Tsuji. Nicotinic acid transport mediated by pH-dependent anion antiporter and proton cotransporter in rabbit intestinal brush-border membrane. *J. Pharm. Pharmacol.* **48**:1073–1077 (1996).
20. I. Tamai, H. Takanaga, H. Maeda, H. Yabu-uchi, Y. Sai, Y. Suzuki, and A. Tsuji. Intestinal brush-border membrane transport of monocarboxylic acids mediated by proton-coupled transport and anion anitiport mechanisms. *J. Pharm. Pharmacol.* **49**:108–112 (1997).
21. I. Tamai, N. Tomizawa, A. Kadowaki, T. Terasaki, K. Nakayama, H. Higashida, and A. Tsuji. Functional expression of intestinal small peptide/β-lactam antibiotic transporter in *Xenopus laevis* Oocytes, Biochem. Pharmacol. **48**:881–888 (1994).
22. I. Tamai, N. Tomizawa, T. Takeuchi, K. Nakayama, H. Higashida, and A. Tsuji. Functional expression of transporter for β-lactam antibiotics and dipeptides in *Xenopus laevis* oocytes injected with messenger RNA from human, rat and rabbit small intestines. *J. Pharm. Exp. Ther.* **273**:26–31 (1995).
23. I. Tamai, K. Hayashi, T. Terao, Y. Sai, T. Shiraga, K. Miyamoto, E. Takeda, H. Higashida, and A. Tsuji. The predominant contribution of oligopeptide transporter PepT1 to intestinal absorption of β-lactam antibiotics in the rat small intestine. *J. Pharm. Pharmacol.* **49**:796–801 (1997).
24. T. Ishizawa, S. Sadahiro, K. Hosoi, I. Tamai, T. Terasaki, and A. Tsuji Mechanisms of intestinal absorption of the antibiotic, fosfomycin, in brush-border membrane vesicles in rabbits and humans *J. Pharmacobio-Dyn.* **15**:481–489 (1992).