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Oral absorption of cephalosporins is quantitatively predicted from in vitro uptake into intestinal brush border membrane vesicles

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Abstract

In order to establish a method to predict oral absorption of drugs, which are absorbed by the oligopeptide transporter (PepT1), fraction absorbed (F) of cephalosporin antibiotics was predicted from in vitro uptake into rat intestinal brush border membrane vesicles (BBMV). Using in vitro uptake data, F values of cephalosporins in humans were predicted using the equation derived from the complete radial mixing (CRM) model, which was proposed by Amidon et al. (Amidon et al., J. Pharm. Sci. 69 (1980) 1369). In the present study, uptake into BBMV was measured at 25 and 4°C in the presence of an H^+-gradient, and the uptake clearance (CL_{uptake}) was calculated. Clearance for the uptake mediated by PepT1 (ΔCL_{uptake}) was then calculated as CL_{uptake} at 25°C minus that at 4°C. When ΔCL_{uptake} and F values were analyzed according to the present equation, fairly good correlation between ΔCL_{uptake} and F was observed. It was further demonstrated that the present method may be able to quantitatively predict F values of cephalosporins by using several cephalosporins as standards.

Keywords: Cephalosporin antibiotics; Oligopeptide transoporter; PepT1; Prediction; Absorption; Rat intestinal BBMV

Abbreviations: AIC, Akaike's information criterion; BBMV, brush border membrane vesicles; CRM model, complete radial mixing model; CAT model, compartmental absorption and transit model; CLuptake, uptake clearance; F, fraction absorbed; CCL, cefaclor; CETB, ceftibuten; CFIX, cefixime; CTM, cefotiam; CEZ, cefazolin; CEX, cephalexin; CED, cephradine; GLY-SAR, glycyl-sarcosine; GLU-ALA, glutamyl-alanine; GLY-PRO, glycyl-proline; Hepes, 2-[4-(2-hydroxy ethyl)-1-piperazinyl] ethane sulfonic acid; Tris, 2-amino-2-hydroxymethyl-1, 3-propanediol; Mes, 2-morpholinoethanesulfonic acid, monohydrate.

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1. Introduction

It has been demonstrated that transporters play an important role in the intestinal absorption of drugs (Tsuji and Tamai, 1996). For example, the oligopeptide transporter in the small intestine (PepT1) is responsible for oral absorption of some β-lactam antibiotics as well as di/tripeptides (Dantzig and Bergin, 1990; Tsuji and Tamai, 1996). The folic acid transporter is responsible for absorption of folate and its analogs, e.g., methotrexate, and the monocarboxylic acid transport system(s) is involved in absorption of various monoanionic drugs with a carboxylic acid moiety (Hamid et al., 1987; Tsuji and Tamai 1996). Therefore, not only passive diffusion but also carrier-mediated transport contributes to drug absorption from the small intestine.

Extent of oral absorption (fraction absorbed, F) of certain drugs in humans may be predicted from in vitro studies. It is reported that F values of drugs that are absorbed by passive diffusion may be predicted from transcellular transport studies using Caco-2 cell monolayer (Artursson and Karlsson, 1991; Chong et al., 1996; Gres et al., 1998). In contrast, prediction of F using Caco-2 cell monolayer is poor for the drugs that are absorbed by a carrier-mediated process, which is probably because of low expression of transporters in Caco-2 cells (Inui et al., 1992; Chong et al., 1996). It is shown that orally active cephalosporins are absorbed from the small intestine mainly by PepT1 (Gochoco et al., 1994; Tsuji and Tamai, 1996) and that prediction of their F values from transcellular transport studies using Caco-2 cell monolayer is poor (Chong et al., 1996; Gres et al., 1998).

Several other methods are also proposed to predict F values in humans. The complete radial mixing (CRM) and the compartmental absorption and transit (CAT) models have been proposed to predict F in humans from in situ perfusion experiments in rats or humans (Amidon et al., 1980; Yu et al., 1996). These methods have an advantage that F can be predicted regardless of the mechanisms of absorption, i.e., F values of the drugs that are absorbed by transporters as well as passive diffusion can be equally predicted. However, in situ perfusion experiments are time and labor consuming, and costly especially when human subjects are used. Although in vitro methods are desirable, no in vitro method has been reported, which can quantitatively predict F of the drugs that are absorbed by transporter(s). In the present study, uptake of cephalosporins into rat intestinal brush border membrane vesicles (BBMV) was measured for cephalosporins with high, medium and low F values (Table 1), and a method was proposed to predict their F values in humans from in vitro uptake studies.

2. Materials and methods

2.1. Materials

Cefaclor (CCL) and ceftibuten (CETB) were kindly donated from Shionogi Pharmaceutical Co. (Osaka, Japan). Cefixime (CFIX) and cefotiam (CTM) were kindly donated from Fujisawa Pharmaceutical Co. (Osaka, Japan) and Takeda Pharmaceutical Industries (Osaka, Japan), respectively. Cefazolin (CEZ), cephalexin (CEX), cephradine (CED), glycyl-sarcosine (GLY-SAR), glutamyl-alanine (GLU-ALA) and glycyl-proline (GLY-PRO) were purchased from Sigma Chemical Co. (St. Louis, MO). 2-[4-(2-hydroxy ethyl)-1-piperazinyl] ethane sulfonic acid (Hepes), 2-amino-2-hydroxymethyl-1, 3-propanediol (Tris) and 2-morpholinoethanesulfonic acid, monohydrate (Mes) were purchased from Wako Pure Chemicals (Osaka, Japan). All other chemicals were of the highest grade available.

Table 1
Extent of oral absorption (F) of cephalosporins

	F	References
CED	0.94[a]	Hardman and Limbird (1996)
CCL	0.90[a]	Hardman and Limbird (1996)
CEX	0.90[a]	Hardman and Limbird (1996)
CETB	0.70[a]	Unpublished observation
CFIX	0.47[a]	Faulkner et al. (1988)
CEZ	0.063[b]	Yoshimura et al., (1985)
CTM	0.031[b]	Nishimura et al., (1986)

[a] F in humans.
[b] F in mice since F values in humans are unavailable.

2.2. Preparation of rat intestinal brush border membrane vesicles

BBMV was prepared by the calcium precipitation method as described previously (Itoh et al., 1998). Prepared BBMV was suspended in 100 mM D-mannitol, 100 mM KCl and 10 mM Hepes/Tris (pH 7.5) and stored at −80°C. It was used for uptake experiments within 7 days. Purity of BBMV was evaluated by comparison of the activities of alkaline phosphatase (a marker enzyme of the brush border membrane) and Na^+/K^+ ATPase (a marker enzyme of the basolateral membrane) with those of the initial homogenate. Enzyme activities were measured according to the methods by Parkinson et al. (Parkinson et al., 1972) with slight modification. Protein concentrations of BBMV were determined using a Bio-Rad Protein assay kit (Bio-Rad Laboratories, Richmond, CA).

2.3. Uptake experiment

Uptake of a drug into BBMV was measured at 25 or 4°C by the rapid filtration method. The uptake was initiated by adding 180 μl of the incubation medium to 20 μl of BBMV suspension. The incubation medium consists of 100 mM D-mannitol, 100 mM KCl, 20 mM Mes/Tris (pH 5.5) and an appropriate concentration of a drug (see legend of each figure). At appropriate time, the reaction was stopped by the addition of 1 ml of ice-cold stop solution consisting of 100 mM D-mannitol, 100 mM KCl and 10 mM Mes/Tris (pH 5.5). The mixture was immediately filtered (Millipore filter, HAWP, 0.45 μm of pore size, 25 mm (i.d.)) followed by washing with 5 ml of ice-cold stop solution. The filter was transferred into a glass tube and the drug retained in the vesicles was extracted with 500 μl of distilled water using a vortex mixer. A 100 μl aliquot was injected onto HPLC.

Adsorption of the drug to BBMV was measured by adding the drug after the stop solution was added to the vesicle suspension. Adsorbed drug was assayed in the same manner as described above. Adsorbed amount was subtracted and the corrected value was considered as the uptake amount.

For calculation of ΔCL_{uptake}, uptake of cephalosporin at 25 and 4°C were measured using the same lot of BBMV preparation. On the other hand, time course and inhibition studies were conducted using different lots of BBMV preparations, which are reflected in different protein concentrations.

2.4. HPLC conditions for determination of cephalosporins

High-performance liquid chromatograph was used to determine the concentrations of cephalosporins. Mightysil-18® (4.6 mm (i.d.) × 150 mm; Kanto Chemical, Tokyo, Japan) was used as an analytical column. The mobile phase for determination of CED, CCL, CEX, CEZ and CTM was 20 mM ammonium acetate–methanol (80:20 (vol/vol)). These cephalosporins were detected at 220 nm. The mobile phase for CFIX was an aqueous solution consisting of 10 mM tetra-*N*-butylammonium bromide and 20 mM ammonium acetate, which was mixed with acetonitrile (72.5:27.5 (vol/vol)). The mobile phase for CETB was 50 mM phosphate buffer (pH 3.0)–methanol (87.5:12.5 (vol/vol)). CFIX and CETB were detected at 290 and 262 nm, respectively. Flow rate was 0.8 ml/min for all cephalosporins.

2.5. Data analysis

In order to evaluate uptake efficiency, the uptake clearance (CL_{uptake}) was calculated according to Eq. (1)

$$CL_{uptake} = v/C_0, \quad (1)$$

where v and C_0 are the initial uptake rate (uptake in 5 s) and the initial medium concentration of the drug, respectively. We also calculated the difference between CL_{uptake} at 25°C and that at 4°C, which was defined as ΔCL_{uptake}. This ΔCL_{uptake} was considered as the uptake clearance solely by the transporter.

According to the CRM model (Amidon et al., 1980, 1988), F value in humans following oral administration can be described with Eq. (2)

$$F = 1 - \exp(-2 \times P^*_w), \quad (2)$$

where P_w^* is the dimensionless intestinal wall permeability obtained from rat in situ perfusion experiments. In order to calculate F from the present uptake data, Eq. (2) was transformed to Eq. (3)

$$F = 1 - \exp(-A \times CL_{uptake}), \quad (3)$$

where A is a constant, which can be considered a scaling factor, and CL_{uptake} is the initial uptake clearance into BBMV. The A value was obtained by a non-linear regression method (MULTI, Yamaoka et al., 1981).

2.6. Statistical analysis

Statistical analysis was performed using Student's t-test with P values of < 0.05 being considered statistically significant.

3. Results

3.1. Purity of brush border membrane vesicles

In the present BBMV preparations, enrichment factors of alkaline phosphatase and Na^+/K^+ ATPase were 12.1 ± 3.7 (mean $\pm$ S.D., $n = 10$) and 0.92 ± 0.25 (mean $\pm$ S.D., $n = 10$), respectively. These values indicated that the present BBMVs were prepared with negligible contamination of basolateral membranes.

3.2. Time courses of uptake of cephalosporins

Time courses of the uptake of cephalosporins in the presence of an H^+-gradient ($pH_{in} = 7.5$, $pH_{out} = 5.5$) are shown in Fig. 1. Uptake of CETB showed an overshoot phenomenon, whereas uptake of CCL, CED, CEX, CEZ, CFIX and CTM into BBMV did not show an overshoot. Since uptake of each cephalosporin was linear up to 10 s, uptake in 5 s was considered as the initial uptake. It should be noted that uptake of each drug was measured using different lots of BBMV. Because of significant lot-to-lot variability, the slightly greater initial uptake of CETB than other cephalosporins does not necessarily indicate the greater absorption of this drug.

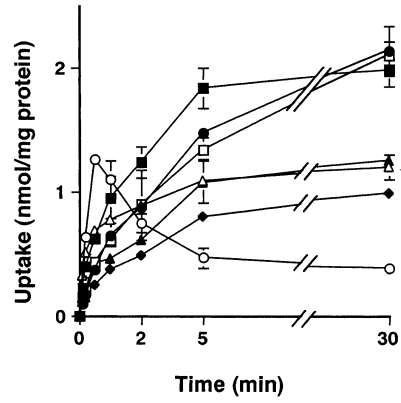

Fig. 1. Time courses of the uptake of cephalosporins into BBMV. Vesicles (20 μl, 74.6–194 μg protein) were incubated at 25°C in the incubation medium (180 μl) containing 100 mM D-mannitol, 100 mM KCl, 20 mM Mes/Tris (pH 5.5) and 1.0 mM of each cephalosporin, except for CETB. The concentration of CETB was 0.5 mM. Each point represents the mean ± S.D. of three to four determinations. (■) CCL, (●) CED, (○) CETB, (△) CEX, (□) CEZ, (▲) CFIX, (◆) CTM. Inset: magnified initial uptake.

3.3. Inhibition studies

Uptake of CETB was measured with an H^+-gradient in the absence or presence of 20 mM GLY-SAR, GLU-ALA, GLY-PRO, CTM, CEZ or 10 mM CCL. Dipeptides and CCL significantly inhibited the uptake of 0.5 mM CETB (Table 2). In contrast, 20 mM CEZ and CTM showed no inhibitory effect on CETB uptake.

Uptake of 1 mM CTM or CEZ was measured with an H^+-gradient in the absence or presence of 20 mM GLY-SAR, GLY-PRO, GLU-ALA or 10 mM CCL. As summarized in Table 2, uptake of CTM or CEZ was inhibited by none of these PepT1 substrates.

3.4. Correlation between CL_{uptake} and F

Uptake clearance (CL_{uptake}) at 25°C was calculated according to Eq. (1) and the results are summarized in Table 3. It should be emphasized that uptake of all drugs was measured using an identical lot of BBMV. However, this lot was different from any of those used in the time course studies (Fig. 1). Reported F values in

Table 2
Effects of various compounds on uptake of CETB, CEZ and CTM into BBMV in 5 s[a]

Inhibitor	0.5 mM CETB	1 mM CEZ	1 mM CTM
Control	100	100	100
10 mM CCL	68.3 ± 3.9**	96.3 ± 11.4	95.2 ± 10.1
20 mM GLY-SAR	51.2 ± 4.2***	91.4 ± 19.0	106 ± 8.0
20 mM GLU-ALA	63.1 ± 4.5**	93.6 ± 8.8	99.1 ± 10.4
20 mM GLY-PRO	73.9 ± 7.8*	101 ± 12.0	99.8 ± 5.8
20 mM CTM	96.4 ± 7.7	–	–
20 mM CEZ	105 ± 4.0	–	–

[a] Values are expressed as % of the control (mean ± S.D., $n = 3$). –, not measured.
* Significantly different from the control ($P < 0.05$).
** Significantly different from the control ($P < 0.01$).
*** Significantly different from the control ($P < 0.001$).

humans were plotted against CL_{uptake} values in Fig. 2. The data were analyzed according to Eq. (3), and the A value of 2.12 (mg protein × 5 s/μl) was obtained. The curve fit is also shown in Fig. 2 as a solid line. Calculated F value (solid line) was greater than the reported F value for CTM and CEZ, whereas calculated value was smaller than the reported value for CCL, CED and CEX. It was obvious that prediction of F values using CL_{uptake} at 25°C was poor for cephalosporins with high as well as low F values.

Table 3
CL_{uptake} at 25°C, 4°C and ΔCL_{uptake}

	CL_{uptake} at 25°C[a]	CL_{uptake} at 4°C[a]	ΔCL_{uptake}[a,b]
CED	1.10 ± 0.16	0.19 ± 0.06***	0.91 ± 0.17
CCL	1.67 ± 0.30	0.39 ± 0.08**	1.28 ± 0.31
CEX	1.25 ± 0.35	0.34 ± 0.10*	0.92 ± 0.37
CETB	1.05 ± 0.15	0.28 ± 0.05**	0.77 ± 0.16
CFIX	0.79 ± 0.11	0.52 ± 0.08**	0.27 ± 0.13
CEZ	0.29 ± 0.13	0.28 ± 0.07	0.02 ± 0.11
CTM	0.35 ± 0.05	0.28 ± 0.05	0.06 ± 0.07

[a] Values are expressed as $\times 10^{-1}$ μl/mg protein/5 s (mean ± S.D., $n = 3$).
[b] ΔCL_{uptake} was calculated as CL_{uptake} at 25°C minus that at 4°C.
* Significantly different from the corresponding value at 25°C ($P < 0.05$).
** Significantly different from the corresponding value at 25°C ($P < 0.01$).
*** Significantly different from the corresponding value at 25°C ($P < 0.0001$).

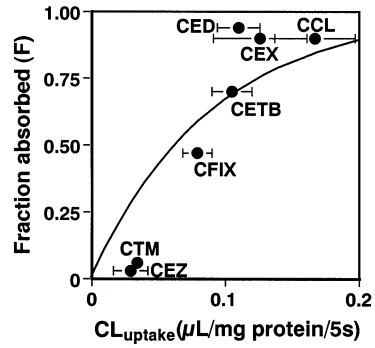

Fig. 2. Plot of the reported F versus CL_{uptake}. Vesicles (20 μl, 78.2 μg protein) were incubated at 25°C in the incubation medium (180 μl) for 5 s. The medium compositions were the same as those described in the legend of Fig. 1. Reported F values are listed in Table 1. A solid line shows curve fit obtained by non-linear regression analysis. Each point represents the mean ± S.E. of three determinations.

3.5. Correlation between ΔCL_{uptake} and F

Uptake of cephalosporins into BBMV was also measured at 4°C and the CL_{uptake} values calculated according to Eq. (1) are summarized in Table 3. It should be noted that uptake at 4°C was measured using the same lot of BBMV as that used for the uptake studies at 25°C. CL_{uptake} value at 4°C was significantly smaller than that at 25°C for CCL, CED, CETB, CEX and CFIX, whereas CL_{uptake} values of CEZ and CTM at 4°C were similar to those at 25°C.

In order to estimate the uptake solely by PepT1, ΔCL_{uptake} was calculated as the CL_{uptake} at 25°C minus that at 4°C. Reported F values were plotted against ΔCL_{uptake} in Fig. 3 and the data were analyzed according to Eq. (3), where CL_{uptake} was replaced with ΔCL_{uptake}. The A value obtained by non-linear regression analysis was 21.1 (mg protein × 5 s/μl), and the curve fit is shown as a solid line in Fig. 3. The curve fit in Fig. 3 was significantly better than that in Fig. 2, i.e., predicted F values of both poorly absorbed

(CEZ and CTM) and well absorbed cephalosporins (CCL, CED and CEX) were similar to the reported F values. Better prediction was also reflected in the smaller Akaike's Information Criterion (AIC) value, which were -6.76 and -24.2 for the fitting Figs. 2 and 3, respectively.

4. Discussion

In the present study, we attempted to predict oral absorption of PepT1 substrates in humans from the in vitro uptake study using rat intestinal BBMV. Cephalosporin antibiotics with high (CED, CCL, CEX and CETB), medium (CFIX) and low (CEZ and CTM) F values were used as model compounds (Table 1). Since F values in humans were unavailable for CEZ and CTM, reported F values in mice were used. In general, drugs that are poorly absorbed in humans are also poorly absorbed in other animal species following oral administration (Chiou and Barve, 1998). Therefore, CEZ and CTM must be poorly absorbed in humans although the F values in humans may be slightly different from those listed in Table 1. In fact, CEZ and CTM are clinically used parenterally.

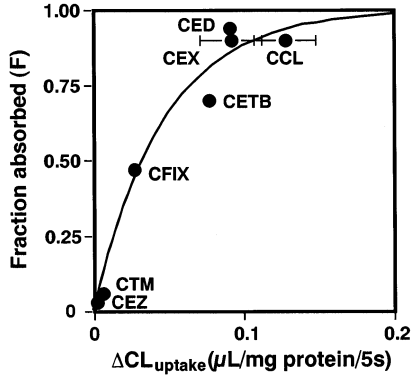

Fig. 3. Plot of the reported F versus ΔCL_{uptake}. Vesicles (20 μl, 78.2 μg protein) were incubated at 25 or 4°C in the incubation medium (180 μl) for 5 s. The medium compositions were the same as those described in the legend of Fig. 1. ΔCL_{uptake} was calculated as the CL_{uptake} at 25°C minus that at 4°C (Table 3). A solid line shows curve fit obtained by non-linear regression analysis. Each point represents the mean ± S.E. of three determinations.

Enrichment factors of the marker enzymes were comparable to those reported previously (Yoshikawa et al., 1989). Uptake characteristics of cephalosporins into BBMV were also similar to those reported previously. For example, overshoot phenomenon observed for CETB in the present study was similar to that reported by Yoshikawa et al. (1989). Lack of overshoot for CCL, CED, CEX, CEZ and CTM was also consistent with those reported previously (Okano et al., 1986; Sugawara et al., 1992). The only exception was the uptake of CFIX. Although no overshoot was observed in the present study at the extravesicular pH of 5.5, an overshoot phenomenon has been reported for the uptake of CFIX at pH 5.0 (Tsuji et al., 1987). The higher extravesicular pH in the present study may have resulted in lack of overshoot since the uptake is smaller at pH 5.5 than that at pH 5.0 (Tsuji et al., 1987). Overall, the observations in this study indicate that the PepT1 was functional in the present BBMV preparations.

Uptake was measured at 25°C with the extravesicular pH being 5.5 in the present study. Many uptake studies with BBMV have been conducted under the same conditions. The microclimate pH at the surface of rat intestinal epithelial cells is 5.84–6.10 (Said et al., 1986), indicating that the present pH may not be significantly different from that in vivo. Also, uptake experiments are often conducted at 25°C partly because of instability of some β-lactam antibiotics. The optimum pH and temperature for transport by PepT1 may have to be clarified in the future.

We used CETB as a model substrate to evaluate affinity of cephalosporins to PepT1. Since dipeptides (GLY-SAR, GLU-ALA, GLY-PRO) and CCL inhibited the uptake of CETB (Table 2), it was confirmed that these compounds possess affinity to PepT1 as previously reported (Muranushi et al., 1989). CL_{uptake} was significantly greater at 25°C than at 4°C for CCL, CED, CETB and CFIX (Table 3), which agrees with the previous observations that these cephalosporins are the substrates of PepT1. Since carrier-mediated transport is significantly reduced at low temperature, it may be assumed that CCL, CED, CETB, CEX and CFIX are taken up into BBMV

by both passive and carrier-mediated processes at 25°C and that they are taken up only by passive diffusion at 4°C. Indeed, it is shown that the uptake of CFIX into BBMV at 4°C is very similar to that by passive diffusion at 37°C (Tsuji et al., 1987).

CEZ and CTM did not inhibit the uptake of CETB, and dipeptides or CCL did not inhibit the uptake of CEZ and CTM (Table 2). Moreover, CL_{uptake} values of CEZ and CTM at 25°C were almost equal to those at 4°C. These observations are consistent with the fact that CEZ and CTM are not PepT1 substrates (Matsumoto et al., 1995). Therefore, CEZ and CTM are probably taken up into BBMV only by passive diffusion both at 25 and 4°C.

The present observations for CEZ and CTM indicate that uptake by passive diffusion may differ to an insignificant extent between 4 and 25°C. Therefore, it was assumed that CL_{uptake} at 4°C reflect the uptake solely by passive diffusion and that the uptake clearance mediated solely by PepT1 can be represented with ΔCL_{uptake}.

When ΔCL_{uptake} was substituted for CL_{uptake} in Eq. (3), ΔCL_{uptake} and F were much better fit to Eq. (3) than that for CL_{uptake} and F (Figs. 2 and 3), suggesting that F may be predicted if the uptake solely by PepT1 is appropriately estimated. In order to establish a method to predict F, CED, CFIX and CTM were chosen as standard compounds with excellent, medium and poor absorption, respectively. Reported F values of these three cephalosporins were plotted against the ΔCL_{uptake} values, analyzed according to Eq. (3), and the A value of 24.0 (mg protein $\times$ 5 s/μl) was obtained. Using this A value, F value of each cephalosporin was calculated from ΔCL_{uptake}. When the F values thus calculated were plotted against the reported F values (Fig. 4), the plots of CEZ, CETB, CEX and CCL were close to the 1:1 correlation line, demonstrating that F values of these cephalosporins may be fairly well predicted according to the present method.

Since the cephalosporins used in the present study are highly hydrophilic, the contribution of the passive diffusion may be negligible in the in vivo absorption. In fact, the contribution of passive diffusion in in situ perfusion study was negligible for CCL and CEX (Sinko and Amidon, 1988; Bai and Amidon, 1992). Although the contribution of passive diffusion was not negligible for CED in the same in situ perfusion study, the carrier-mediated absorption was still predominant (Bai and Amidon, 1992). This may be the reason why the F values can be fairly well predicted using the uptake clearance solely by the transporter, i.e., ΔCL_{uptake}.

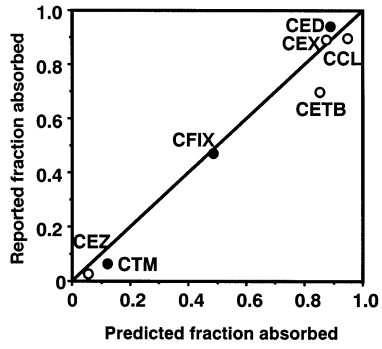

Fig. 4. Correlation between the reported and predicted F. Predicted values of CCL, CETB, CEX and CEZ (compounds symbolized with ○) were calculated according to Eq. (3) using the A value obtained from the data of CED, CFIX and CTM (compounds symbolized with ●) and ΔCL_{uptake} value of each cephalosporin.

In the present uptake experiments, the concentration (1 mM) of CCL, CED and CEX were smaller than the reported K_m values. The reported K_m values are 3.0 mM at pH 5.5 for CCL and 9.4 mM at pH 6.0 for CED, which were obtained with rat intestinal BBMV (Okano et al., 1986; Yoshikawa et al., 1989). The K_m values of CEX reported in Caco-2 cells are 2.9–7.5 mM at pH 6.0 (Dantzig and Bergin, 1990; Gochoco et al., 1994). CFIX concentration (1 mM) also appeared to be smaller than the K_m value since the K_m values in rat intestinal BBMV are 0.83 and 5.63 mM at pH 5.0 and 6.0, respectively (Tsuji et al., 1987). On the other hand, CETB concentration (0.5 mM) was slightly greater than the K_m value (0.17–0.43 mM at pH 5.5) (Yoshikawa et al., 1989; Naasani et al., 1995). It is desirable to obtain the clearance at the concentration lower than the K_m value in order to estimate the maximum uptake clearance. However, we were unable to lower the concentration of CETB because of

insufficient sensitivity of the present analytical method.

When oral dose of each cephalosporin is ingested with 200 ml of water, the concentrations are 3.4, 0.89, 0.90, 1.22 and 0.56 mM for CCL, CED, CEX, CETB and CFIX, respectively. The concentrations of CED and CEX are much smaller than their K_m values (see above for K_m values), indicating that the present ΔCL_{uptake} obtained at 1 mM may represent the in vivo uptake clearance by PepT1. On the other hand, the concentrations of CCL and CFIX are slightly smaller or close to their K_m values. Since the drug concentration may decrease due to dilution by gastrointestinal fluids and to subsequent absorption in the small intestine, the concentrations of CCL and CFIX may be lower than their K_m values at the absorption site of the small intestine. Therefore, the present ΔCL_{uptake} values obtained at 1 mM probably represent the in vivo uptake clearance values of CCL and CFIX by PepT1. The concentration of CETB immediately after ingestion (1.22 mM) and that used in the present uptake study (0.5 mM) are much greater than the K_m value. The lower concentration of CETB used in the present study than that after ingestion in vivo may have resulted in the greater predicted F value than the reported value. Since it is difficult to estimate the drug concentration at the absorption site, we would like to use the concentration that is lower than the K_m value, in order to predict the potentially maximum F of the drug. If the drug concentration at the absorption site is greater than the K_m value, we may have to apply some correction that should be established in the future.

It has been reported using rat intestinal segments that CEZ may be transported in a secretory direction with an energy dependent process (Saitoh et al., 1996). On the other hand, it is shown that uptake of CEZ into BBMV is smaller than PepT1 substrates in the present study (Table 3). Moreover, considering the low lipophilicity of CEZ, it is unlikely that this drug is significantly absorbed by passive diffusion. Therefore, the contribution of secretory process in the absorption of CEZ may be insignificant because of very poor uptake into intestinal epithelial cells. In any case, effect of secretory process on the present prediction method has to be taken into account in future studies. In contrast, no secretory process is observed for CTM in Caco-2 cells (Inui et al., 1992), indicating that the present method can be applied to CTM.

As described above, orally active cephalosporins used in the present study are absorbed from the small intestine predominantly by PepT1 (Hori et al., 1988). Therefore, it was unnecessary to take into consideration the contribution of passive diffusion or of other transporters in the present study. However, for PepT1 substrates with greater lipophilicity, contribution of passive diffusion may not be negligible. Moreover, for some PepT1 substrates the contribution of other transport systems may have to be taken into account. In fact, one of the orally active cephalosporins, cefdinir, is reported to be absorbed by the monocarboxylic acid as well as the oligopeptide transport system (Tsuji et al., 1993). Apparently, the present method has to be refined to predict oral absorption of drugs that are absorbed via multiple pathways.

Lot-to-lot variability of BBMV preparations as well as the time and labor required for preparation of BBMV may limit the use of the present method for the screening of a number of drug candidates for oral absorbability. However, to our knowledge, the present method is the first in vitro method that may be able to quantitatively predict oral absorption of the drugs that are absorbed by a transporter. In our preliminary study, it is shown that the present approach may be applied to Caco-2 cell system, which is probably more useful for the purpose of screening. Moreover, studies using PepT1 cDNA transfected cells may also be useful to accurately estimate the transport by PepT1.

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