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An in vitro system for prediction of oral absorption of relatively water-soluble drugs and ester prodrugs

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Abstract

We developed an in vitro system simulating the physiological condition in the gastrointestinal (GI) tract for prediction of oral absorption of relatively water-soluble drugs and ester prodrug pivampicillin. This evaluation system includes a drug-dissolving vessel (DDV, assumed stomach), a pH adjustment vessel (PAV, assumed intestine) and a side-by-side diffusion chamber that is mounted by a Caco-2 monolayer, which is grown on a polycarbonate filter, or by a rat intestine between the donor and receiver compartments. Our proposed system can accommodate large amounts of solid drugs, simulating a drastic pH change process in GI tract, that is, an orally administered solid drug is dissolved in the stomach (pH 1–2) and transferred to the intestine (pH 6), and that dissolution process can also be monitored. The optimal flow rates for our system are 0.35–1.10 ml/min. Using this system, cumulative permeations of eight relatively water-soluble drugs were compared, and these cumulative permeations indicated the ability of drug absorption in humans. Drugs that permeated across a Caco-2 monolayer at cumulative permeation of more than 0.03% or over 0.04% in rat intestine can be almost completely absorbed in humans. If the cumulative permeation across a Caco-2 monolayer is lower than 0.03% or below 0.04% in the rat intestine, there was a good linear correlation between cumulative permeation across a Caco-2 monolayer and oral absorption in humans, or between cumulative permeation across a rat intestine and oral absorption in humans. In the case of relatively water-soluble drugs, a good linear correlation was obtained between cumulative permeation across a Caco-2 monolayer and cumulative permeation across a rat intestine. This result indicates that it is possible to predict the oral absorption of a relatively water-soluble drug in humans based on the cumulative permeation of the drug across a Caco-2 monolayer and/or a rat intestine. The time course of permeation of the ester prodrug pivampicillin, which is metabolized in a Caco-2 monolayer or in a rat intestine, was also evaluated. It stated clearly that it is also possible to predict the oral absorption of pivampicillin in humans based on the cumulative permeation across a Caco-2 monolayer or rat intestine. Our newly developed system enables more kinds of oral preparations and also pH-dependent soluble drugs to be evaluated.

Keywords: Caco-2 cells; Rat intestine; In vitro model; Oral absorption; Permeability; Ester prodrugs

1. Introduction

Oral administration is the easiest and most useful method for drug delivery, and prediction of drug absorption is therefore very important for the design of an oral preparation. Only a few experimental in vitro methods have so far been established for prediction of drug absorption capability in vivo. Ginski and coworkers have also reported a continuous dissolution/Caco-2 system (Ginski and Polli, 1999; Ginski et al., 1999). However, these methods do not take into account the drastic pH change in the gastrointestinal (GI) tract in

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the case of an oral preparation (i.e. the drastic change between an acidic condition in the stomach and a neutral or basic condition in the intestine). In our laboratory, a system for predicting drug absorption that takes into account dissolution of solid drugs and changes in pH in GI tract in vitro has been developed (Kobayashi et al., 2001). However, this system cannot be used for models other than Caco-2 cell monolayers, for example, isolated tissues or artificial membranes. Moreover, the volume of the drug-dissolving vessel (DDV) is only 3 ml; thus, a product that is more than 30 mg cannot be used for this system. It is therefore difficult for this system to predict absorption if administration dose is increased. Modifications to the system that enable more kinds of oral preparations and also drug–drug interactions to be evaluated were therefore needed. We therefore developed a system that can accommodate large amounts of solid drugs, simulating the physiological condition in GI tract, and that dissolution process can also be monitored. In this new system, not only Caco-2 monolayers but also other membranes, such as rat intestine, can be mounted on the chamber, and the volumes of the DDV and the pH adjustment vessel (PAV) are both increased to 10 ml. Using this system, cumulative permeations of eight relatively water-soluble drugs were compared.

In oral drug delivery programs, ester prodrugs are commonly used to enhance membrane permeation and transepithelial transport of hydrophilic drugs by increasing the lipophilicity of the parent compound, resulting in enhanced transmembrane transport by passive diffusion (Balant et al., 1990; Taylor, 1996). Pivampicillin is the pivaloyloxymethyl ester of ampicillin, which on hydrolysis yields active ampicillin. This ester is rapidly hydrolyzed in the body by nonspecific esterases, which are present in the gastric mucosa and other tissues. Pivampicillin has no antibacterial activity until it is transformed into ampicillin (Foltz et al., 1970). The use of the prodrug pivampicillin markedly increases the plasma levels of ampicillin compared to the levels in the case of oral ampicillin. However, enzymes and enzymatic activities of rat intestine and Caco-2 cells, a human colon carcinoma cell line, must be different due to species differences. Therefore, to determine whether this new system enables accurate evaluation of intestinal metabolism, permeation across a Caco-2 cell monolayer and that across rat intestine of ampicillin and its ester prodrug pivampicillin, which would be metabolized during permeation, were also studied.

2. Materials and methods

2.1. Materials

Ozagrel hydrochloride, levofloxacin, acebutolol hydrochloride, ampicillin, and pivampicillin were kindly donated by Ono Pharmaceutical Co., Ltd. (Osaka, Japan), Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan), Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan), Sankyo Pharmaceutical Co., Ltd. (Tokyo, Japan), and Takeda Pharmaceutical Co., Ltd. (Osaka, Japan), respectively. Tryptamine hydrochloride was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Metoprolol tartrate and ritodrine were purchased from Sigma (St. Louis, MO). Caffeine anhydrous and atenolol were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Theophylline was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Other chemicals were of the highest grade available and were used without further purification.

2.2. Animals

Male Wistar rats weighing 250–350 g (NRC Haruna, Gunma, Japan) were fasted for 12 h before the experiments but were allowed free access to water. During the experiments, rats were anesthetized with ether and the jejunum of each rat (2.0–2.5 cm) was removed.

2.3. Drug absorption prediction system

As shown in Fig. 1, our system for predicting drug absorption takes into account drug dissolution and pH change in GI tract. In this system, a drug (60 mg, solid form) is added to a DDV (assumed stomach, pH 1.0, 10 ml) and the dissolved drug is transferred to a PAV (assumed intestine, pH 6.0, 10 ml). Each of these vessels is a plastic vial. The compositions of the drug-dissolving solution (pH 1.0), pH adjustment solution (pH 12.0), and receiver solution (pH 7.4) are shown in Table 1. The flow rate (0.5 ml/min) of each solution is controlled by a peristaltic pump. The drug solution is transferred to the donor compartment of

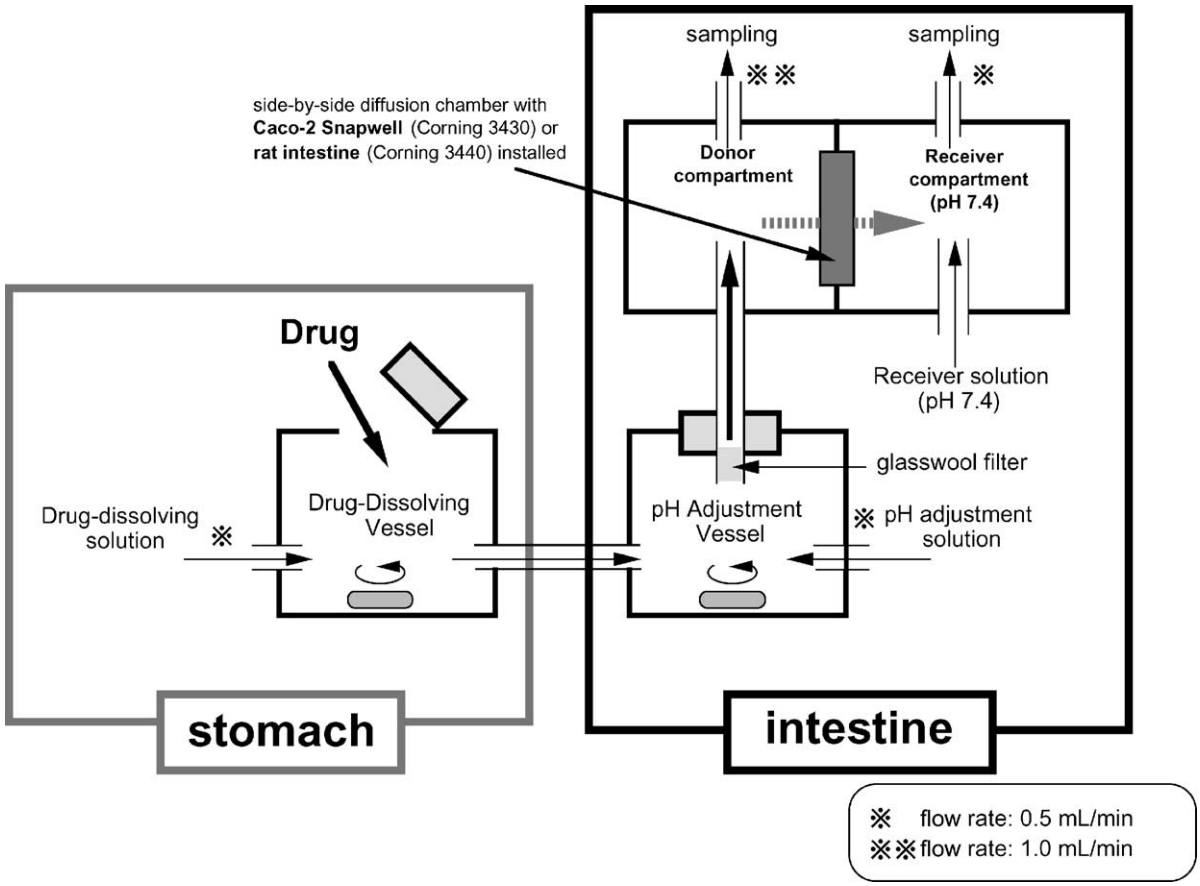

Fig. 1. Scheme of the drug absorption prediction system.

Table 1
Compositions of flowing solutions (mM)

	Drug-dissolving solution (pH 1.0)	pH adjustment solution[a] (pH 12.0)	Receiver solution[b] (pH 7.4)
KCl		10.7	5.37
KH_2PO_4		0.88	0.44
NaCl	34.2	172	137
Na_2HPO_4		0.68	0.34
D-Glucose	50		25
$CaCl_2$	2.52		1.26
$MgSO_4$	0.81		0.41
MES		20	
HEPES			10
HCl	68		
NaOH		68	

[a] pH was adjusted to 6.0 with Tris before addition of NaOH.
[b] pH was adjusted to 7.4 with Tris.

side-by-side diffusion chamber (Corning Costar Co.). Mounted between the donor and receiver compartments is a Caco-2 monolayer grown on a Snapwell (0.4 μm in pore size, 1 cm^2 in growth area, Corning Castar Co.) or a jejunum removed from a rat. The drug permeates to the receiver compartment of side-by-side diffusion chamber and is collected by a fraction collector every 5 min over a period of 200 min. When pivampicillin was used, in order to prevent the hydrolysis of pivampicillin, the samples obtained were frozen immediately. A silicon tube (i.d. 0.5 mm) was used to connect each vessel and the compartment. All the solutions and both vessels and both compartments were preheated to 37 °C and maintained at that temperature. When the jejunum of a rat was used, the buffer was preoxygenated with O_2/CO_2 (95:5) mixture gas. Under the condition of bubbling with mixture

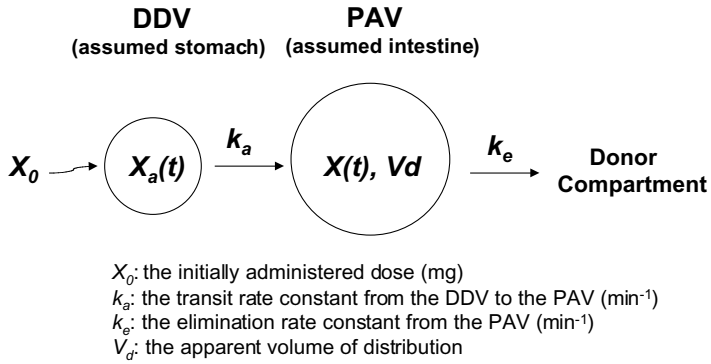

Fig. 2. Visual representation of one-compartment drug-distribution model.

gas, transport of drugs from the donor to the receiver compartment across the rat intestine was measured.

2.4. Pharmacokinetic model

The efflux of relatively water-soluble drug to the donor compartment of side-by-side diffusion chamber in our system is expected to follow a one-compartment drug-distribution model such as that shown in Fig. 2. The outflow from the PAV is two-fold faster than the inflow to the PAV from the DDV in this one-compartment model, which result to the reversed assignment of k_a and k_e (flip–flop phenomenon), therefore, the concentration of drug in the donor phase was quantified by using the first-order rate constants according to the classical expression:

$$C = \frac{k_e X_0}{(k_e - k_a)V_d}(e^{-k_a t} - e^{-k_e t}) \quad (1)$$

$$\mathrm{AUC} = \int_0^\infty C\,dt = \frac{k_e X_0}{(k_e - k_a)V_d}\left(\frac{1}{k_a} - \frac{1}{k_e}\right) = \frac{X_0}{k_a V_d} \quad (2)$$

where C values represent the concentration of drugs remaining in the donor phase at each sampling time, AUC is area under the curve, X_0 is the initial administered dose in the DDV, k_a is the transit rate constant from the DDV to the PAV, k_e is the elimination rate constant from the PAV, and V_d is the apparent volume of distribution.

2.5. Cell culture

Caco-2 cells were purchased from American Type Culture Collection (Rockville, ML). The cells were routinely maintained in plastic culture flasks (Falcon, Becton Dickinson and Co., Lincoln Park, NJ). These stock cells were subcultivated before reaching confluence. The growth medium was Dulbecco's modified Eagle's medium (DMEM) (Sigma) supplemented with 10% fetal bovine serum (ICN Biomedicals, Inc., Aurora, OH), 1% nonessential amino acids (Gibco), and 4 mM glutamine without antibiotics. The monolayer cultures were grown in a CO_2 incubator (5% CO_2) at 37 °C. The cells were harvested with 0.25% trypsin and 0.2% EDTA (0.5–1 min at 37 °C), resuspended, and seeded into a new flask. Cells between the 35th and 50th passages were used in this study.

For the transport studies, Caco-2 cells were seeded on Snapwell at a cell density of 8×10^4 cells per filter. The cell monolayers were fed a fresh growth medium every 2 days and were then used on the 20th to 28th day for the transport experiments. To evaluate the integrity of the monolayer, transepithelia electrical resistance (TEER) was measured using a Millicell-ERS (Millipore Co., Bedford, MA). TEER of the filter was subtracted from the total TEER measurements of Caco-2 cell epithelia. The Caco-2 monolayers were used when their TEERs were >600 $\Omega\,cm^2$. When a Caco-2 monolayer was used, after the permeation experiment was finished, the permeation rate of 100 μM fluorescein isothiocyanate (FITC)–dextran (molecular weight, 4400) was measured to check that the barrier

Table 2
HPLC conditions used to determine the concentration of each drug

Drug	Column	Wavelength	Mobile phase
Tryptamine	A	281	50 mM H_3PO_4 in 40% CH_3CN, pH 3.0
Caffeine	A	273	50 mM KH_2PO_4 in 10% CH_3CN
Theophylline	A	275	50 mM KH_2PO_4 in 15% CH_3CN
Levofloxacin	B	265	50 mM KH_2PO_4 in 20% CH_3CN
Ozagrel	A	270	50 mM KH_2PO_4 in 15% CH_3CN
Acebutolol	A	260	50 mM KH_2PO_4 in 40% CH_3CN
Ampicillin	A	215	50 mM KH_2PO_4 in 10% CH_3CN
Pivampicillin	A	215	50 mM KH_2PO_4 in 50% CH_3CN
Metoprolol	C	225/320[a]	20 mM KH_2PO_4, 2 mM 1-octanesulfonic acid in 45% CH_3CN
Ritodrine	C	280/305[a]	20 mM KH_2PO_4, 0.3 Mm 1-octanesulfonic acid in 30% CH_3CN
Atenolol	A	272/305[a]	50 mM KH_2PO_4 in 25% CH_3CN

Column temperature and flow rate were 55 °C and 0.7 ml/min, respectively. A: Hitachi 3053; length, 250 mm; i.d. 4 mm (Hitachi Co., Ltd.). B: Inertsil ODS-3; length, 250 mm; i.d. 4.6 mm (GL Sciences Inc.). C: ERC-ODS-1161; length, 100 mm; i.d. 6.0 mm (ERC Inc.).
[a] Excitation/emission wavelength.

function had been maintained during the experiment. The permeation rate of FITC–dextran to the receiver compartment over a period of 1 h was less than 0.1%.

2.6. Analysis

The concentrations of drugs were determined by HPLC (L-6000, Hitachi Co., Ltd, Tokyo, Japan) using an L-4200H UV-Vis detector or F-1050 fluorescence spectrophotometer (Hitachi Co., Ltd). The HPLC conditions used to determine the concentration of each drug are shown in Table 2. The measurement of FITC–dextran was carried out in a 650-60 fluorescence spectrophotometer (Hitachi Co., Ltd) with an excitation wavelength of 495 nm and emission wavelength of 514 nm.

3. Results

3.1. Efflux of tryptamine to the donor compartment of side-by-side diffusion chamber when flow rate is changed

Fig. 3a shows the efflux of tryptamine to the donor compartment of side-by-side diffusion chamber under the following conditions: dose of drug added, 10 mg; volume of liquid in each vessel, 10 ml; flow rates (Q), 0.35, 0.50, 0.95, and 1.10 ml/min. Each parameter (k_a, k_e, and X_0/V_d) was calculated according to a nonlinear regression least-squares procedure with the Mac Curve Fit 1.0.8. program by fitting to Eq. (1). The relationship between each parameter and flow rate is shown in Fig. 3b–d. X_0/V_d (X_0/V_d = 322.4 μg/ml, Fig. 3b) was not changed by change in flow rate, and k_a ($k_a = 0.0826Q\,\mathrm{min}^{-1}$) and k_e ($k_e = 0.2931Q\,\mathrm{min}^{-1}$, Fig. 3c) showed good linear correlations with flow rate. X_0/V_d and k_a were used in the calculation of AUC using Eq. (2), so,

$$\mathrm{AUC}(\mu\mathrm{g\,min/ml}) = \frac{3903}{Q(\mathrm{ml/min})} \quad (3)$$

AUC was found to be inversely proportional to flow rate. The theoretical AUC can be obtained by performing on Eq. (3). Fig. 3d shows the AUC of tryptamine on the donor phase for each flow rate. The experimental values agree well with the theoretical values. A flow rate of 0.5 ml/min was used in subsequent experiments.

3.2. Permeation of relatively water-soluble drugs across a Caco-2 monolayer

The amounts of permeation across the Caco-2 monolayer of eight relatively water-soluble drugs that are used clinically were determined using the system shown in Fig. 1. The time course of permeation of caffeine into the receiver compartment of side-by-side diffusion chamber is shown in Fig. 4a, and the cumulative Caco-2 permeations (percents of doses) of the eight drugs are shown in Table 3. The oral absorptions

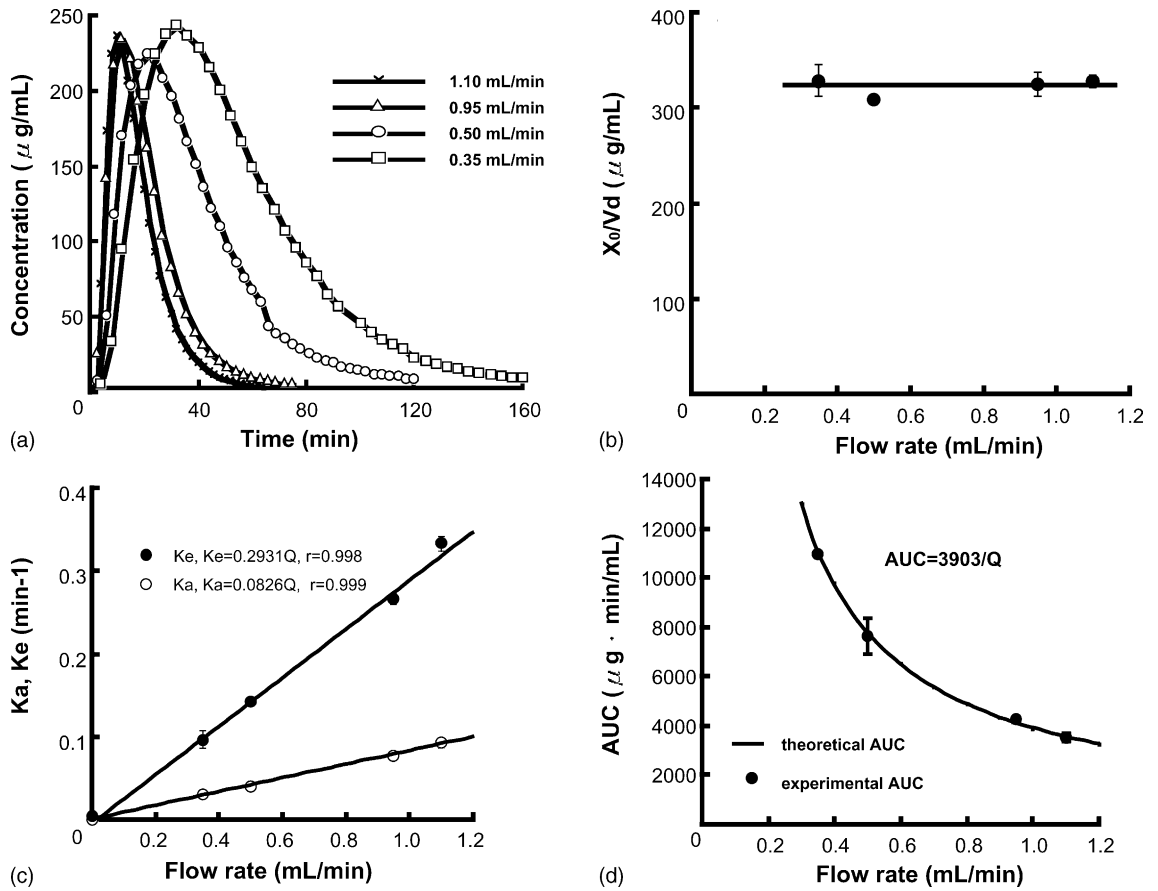

Fig. 3. Efflux of tryptamine to the donor compartment of side-by-side diffusion chamber (a) and the relationship between each parameter and flow rate (b–d). Each value represents the mean with S.E.M. of three experiments.

Table 3
Cumulative permeation of drugs across Caco-2 monolayers or intestine of rat

Drug	Caco-2 (%)	Rat intestine (%)
Acebutolol	0.004 ± 0.001	0.013 ± 0.005
Atenolol	0.002 ± 0.000	0.009 ± 0.001
Caffeine	0.396 ± 0.031	0.205 ± 0.014
Levofloxacin	0.032 ± 0.002	0.030 ± 0.004
Metoprolol	0.030 ± 0.003	0.040 ± 0.005
Ozagrel	0.022 ± 0.002	0.029 ± 0.003
Ritodrine	0.003 ± 0.000	0.010 ± 0.002
Theophylline	0.248 ± 0.025	0.105 ± 0.014

Each value represents the mean ± S.E.M. of the results of three to five experiments.

in humans of ritodrine (Essed et al., 1988), metoprolol (Regardh et al., 1974), levofloxacin (Nakashima et al., 1992), and ozagrel (Fukushima et al., 1990) were taken from the literature, that of caffeine and theophylline were taken from The Pharmacological Basis of Therapeutics by Benet et al. (1996), and that of acebutolol and atenolol are from Therapeutic Drugs by Colin Dollery (1999). The relationship between cumulative Caco-2 permeation and oral absorption of these drugs in humans is shown in Fig. 5a. The cumulative Caco-2 permeation of drugs that are almost completely absorbed in humans, such as caffeine and theophylline, was over 0.03%. At a cumulative permeation across a Caco-2 monolayer of less than 0.03%, there was a good correlation between oral absorption in humans and cumulative permeation ($R = 0.967$).

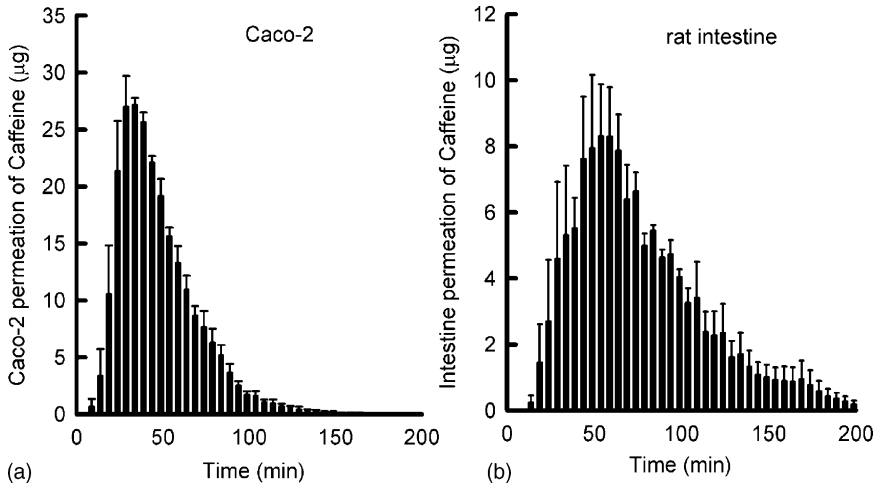

Fig. 4. Time courses of permeation of caffeine across a Caco-2 cell monolayer (a) and a rat intestine (b). Each value represents the mean with S.E.M. of three experiments.

3.3. Permeation of relatively water-soluble drugs across a rat intestine

The amounts of permeation across a rat intestine of eight relatively water-soluble drugs used clinically were determined. The time course of the permeation of caffeine into the receiver compartment of side-by-side diffusion chamber is shown in Fig. 4b, and the cumulative intestine permeations (percents of doses) of the eight drugs are shown in Table 3. The relationship between cumulative intestine permeation and oral absorption of these drugs in humans is shown in Fig. 5b. The cumulative intestine permeations of drugs that are almost completely absorbed in humans, such as caffeine and theophylline, were over 0.04%. At a cumulative permeation across a rat intestine of

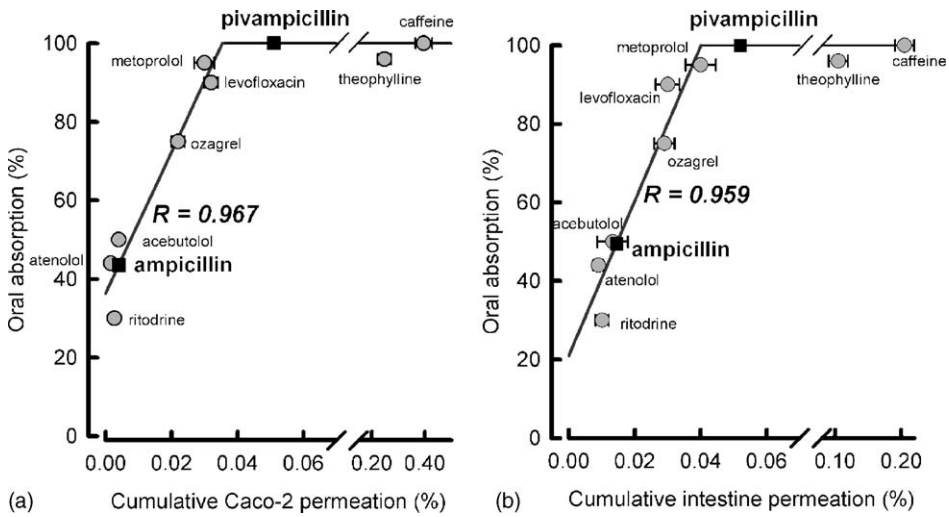

Fig. 5. Relationship between cumulative Caco-2 (a) or intestine (b) permeation and the oral absorption in humans. Predicted oral absorptions of ampicillin and pivampicillin based on this relationship are plotted (■). Each point represents the mean ± S.E.M. of three to five experiments.

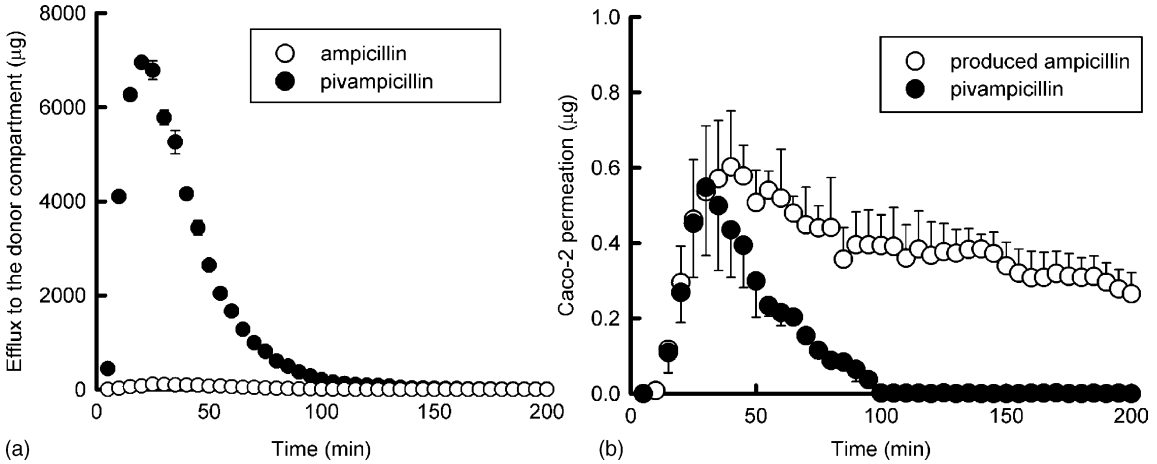

Fig. 6. (a) Efflux of pivampicillin to the donor compartment of side-by-side diffusion chamber. A very small amount of ampicillin was also detected. (b) Time course of permeation of ester prodrug pivampicillin across a Caco-2 cell monolayer. Both pivampicillin and ampicillin, a decomposed form of pivampicillin, were detected. Each point represents the mean ± S.E.M. of three experiments.

less than 0.04%, there is a good correlation between oral absorption in humans and cumulative permeation ($R = 0.959$).

3.4. Efflux of pivampicillin to the donor compartment of side-by-side diffusion chamber

The amounts of pivampicillin and ampicillin eluted into the donor compartment of side-by-side diffusion chamber after pivampicillin had been added to the DDV were measured using the system shown in Fig. 1. The time course of the efflux of pivampicillin is shown in Fig. 6a. Measurement showed that 92.2% of pivampicillin was eluted into the donor compartment. A very small amount of ampicillin was also detected. The total amount of pivampicillin eluted into the donor compartment, i.e. the amount of pivampicillin and the amount of ampicillin produced from pivampicillin in the donor compartment, was 94.7%.

3.5. Permeation of pivampicillin across a Caco-2 monolayer

In the case of a Caco-2 monolayer, when pivampicillin was added to the DDV, both pivampicillin and ampicillin were detected in the receiver compartment of side-by-side diffusion chamber. The time course of the permeation of pivampicillin into the receiver compartment is shown in Fig. 6b. The calculation of the amount of decomposition of pivampicillin was based on the amount of ampicillin that was produced by hydrolysis of pivampicillin. The cumulative permeation of pivampicillin was 0.051%. When ampicillin was used, the cumulative permeation was 0.004% (Table 4). Based on these results and the results shown in Fig. 5, the oral absorption of ampicillin is predicted to be 43.5%, while that of pivampicillin is predicted to be almost 100%.

3.6. Permeation of pivampicillin across a rat intestine

Permeation of pivampicillin across a rat intestine was also studied. When pivampicillin was added, ampicillin produced from pivampicillin was detected in the receiver compartment of side-by-side diffusion chamber, but pivampicillin was not detected. Based on the amount of ampicillin produced (23.58 μg), it was estimated that 31.29 μg of pivampicillin was decomposed into ampicillin. As shown in Table 4, the cumulative permeation of pivampicillin was 0.052%, and the cumulative permeation into the receiver compartment was 0.015% when ampicillin was used. Based on these results and the results shown in Fig. 5, the oral absorption of ampicillin is predicted to be 49.5%, while that of pivampicillin is predicted to be almost 100%.

Table 4
Amounts of ampicillin and pivampicillin that permeated across Caco-2 monolayers or rat intestine

	Drug	Amount added to dissolving vessel (mg)	Amount of pivampicillin detected in receiver side (μg)	Amount of ampicillin detected in receiver side (μg)	Amount decomposed (μg)	Cumulative permeation (%)	Predicted oral absorption in humans (%)
Caco-2	Pivampicillin	60	8.55 ± 0.99	16.77 ± 3.03	22.25 ± 4.01	0.051 ± 0.008	100
	Ampicillin	60	–	2.43 ± 0.69	–	0.004 ± 0.001	43.5
Rat intestine	Pivampicillin	60	N.D.	23.58 ± 2.72	31.29 ± 3.61	0.052 ± 0.006	100
	Ampicillin	60	–	8.75 ± 1.96	–	0.015 ± 0.032	49.5

Each value represents the mean ± S.E.M. of the results of three experiments. N.D.: not detected.

4. Discussion

Caco-2 monolayers are generally accepted to be a suitable in vitro model for drug transport studies as these cells have been shown to express most of the enzymatic, functional and morphological characteristics of the intestinal mucosa (Hidalgo et al., 1989; Audus et al., 1990; Hilgers et al., 1990; Gan et al., 1994). The aim of this study was to determine the reliability of a newly developed system for prediction of oral absorption in humans. Our proposed system includes a DDV (assumed stomach), a PAV (assumed intestine) and a side-by-side diffusion chamber mounted not only by Caco-2 monolayer but also by rat intestine. We studied the permeations of eight relatively water-soluble drugs using this system. It was found that there was a relationship between the cumulative permeation and oral absorption in humans. Moreover, we found that in the case of those eight drugs, there was a good linear correlation between cumulative permeation across a Caco-2 monolayer and that across a rat intestine ($r = 0.986$, data shown in Table 3). Based on the above-mentioned observations and the results obtained, it is thought that both Caco-2 monolayers and rat intestine are useful for evaluation of gastrointestinal absorption properties of relatively water-soluble drugs.

We also tried to assess the oral absorption of ester prodrugs, which is metabolized in the intestine. Lipophilic ester prodrugs are widely used to enhance oral delivery of poor membrane-permeable compounds. Pivampicillin has been selected as a model compound for transport studies. In the present study, we investigated the effect of intestinal metabolism on transepithelial flux of pivampicillin using Caco-2 monolayers and rat intestine. The oral absorption of ampicillin, a typical β-lactam antibiotic, is as low as 30–40%. On the other hand, pivampicillin, the pivaloyloxymethyl ester of ampicillin, possesses enhanced lipophilicity and optimized passive permeation in GI tract. In studies with rat intestines using pivampicillin, almost complete hydrolysis and an increased transepithelial flux of ampicillin have been observed. Based on the correlation between cumulative permeation across a rat intestine and oral absorption in humans (Fig. 5b), the predicted oral absorption of ampicillin produced from pivampicillin after oral administration of pivampicillin was two times higher than that after oral administration of ampicillin (Table 4). In studies on the permeation of pivampicillin across Caco-2 monolayers, both pivampicillin and ampicillin were detected in the receiver compartment. Based on the correlation between cumulative permeation across Caco-2 monolayers and oral absorption in humans (Fig. 5a), the predicted oral absorption of ampicillin that was calculated from the total amount of permeated pivampicillin and ampicillin after administration of pivampicillin was 2.3 times higher than that after administration of ampicillin (Table 4). It has been reported that plasma concentrations of ampicillin from an oral dose of pivampicillin are two to three times higher than those obtained from equimolar doses of ampicillin (Colin Dollery, 1999). The findings in the present study are consistent with those findings. However, the time course of permeation of the ester prodrug pivampcillin into the receiver compartment using Caco-2 monolayers was different from that using rat intestine. This finding is in agreement with results reported by Augustijns et al. (1998) showing that there was an extensive interspecies difference in esterase activity along the GI tract and that enzymatic

activity in homogenates from the Caco-2 system was much lower than that in homogenates from the small intestine of rats. The results of our experiments show that ampicillin kept appearing in receiver compartment after pivampicillin disappeared from both donor and receiver compartments, suggesting that ampicillin might be gradually generated by pivampicillin trapped in the cells.

The Caco-2 system or the small intestine of a rat is considered to be a good model for studying oral absorption in humans. Our studies stated that it is possible to predict the oral absorption of a relatively water-soluble drug or an ester prodrug in humans based on the cumulative permeation across a Caco-2 monolayer or a rat intestine, and furthermore to obtain information on the mechanism of absorption or absorption enhancement of (pro)drugs. Our newly developed system enables more kinds of oral preparations and also pH-dependent soluble drugs to be evaluated.

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