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Development of a new system for prediction of drug absorption that takes into account drug dissolution and pH change in the gastro-intestinal tract

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Abstract

A new system for prediction of drug absorption that takes into account drug dissolution and pH change in the gastro-intestinal tract was developed. In this new system, a drug (solid form) is added into a drug-dissolving vessel (pH 1.0) and the dissolved drug is transferred to a pH adjustment vessel (pH 6.0). Then the drug solution is transferred to the apical surface of Caco-2 cells, and the permeation rate of the drug across a Caco-2 monolayer is determined. This system was able to predict the oral absorption ratios of ten water-soluble drugs in humans. Using this system, it was predicted that drugs that permeated Caco-2 at a rate of more than 0.1% of the dose in 200 min would be almost completely absorbed after oral administration in humans. For a drug whose permeation ratio was less than 0.03%, the absorption ratio was predicted to be less than 30%. This system also enabled prediction of the absorption rate and variability in the absorption of albendazole, a drug with poor water solubility. It also enabled assessment of the improvement in absorption using a solid dispersion of albendazole-polymers that improved the water solubility. The results suggest that this system is useful for oral absorption screening of new drugs and pharmaceutical products.

Keywords: Intestinal absorption; Prediction; Caco-2; Human; Screening

1. Introduction

A simple and easy method to predict the absorption rate of a drug after oral administration in humans is a powerful tool for the development of medicinal drugs. Recently, many groups have

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reported methods for predicting absorption of an intestinal drug using artificial membranes, cultured cells, isolated tissues and organ perfusion (Pagliara et al., 1999). Currently, transport study using Caco-2 cells, a human colon carcinoma cell line, is a widely used method. In experiments using this cell line, the drug has usually been completely dissolved in a buffer and added to the apical side of Caco-2, and the transportation of the drug to basal side was determined. However, most drugs for oral administration are in solid form. Thus, a lipophylic drug, whose solubility and/or dissolving rate are extremely low, will show a high absorption rate in a transport study using a completely dissolved drug solution. More recently, Ginski and co-workers reported a continuous dissolution/Caco-2 system to overcome this problem (Ginski and Polli, 1999; Ginski et al., 1999). Their system consisted of two steps. In the first step, a solid-form drug is dissolved in a dissolution vessel, and the solution is transferred to Caco-2 mounted on a chamber by a peristaltic pump. In the second step, the drug is transported from the apical side to the basal side of Caco-2. Their system therefore enables prediction of the absorption rate of a drug that has extremely low solubility. However, in a patient, an orally administered drug is dissolved in the stomach (pH 1) and the drug solution is transferred to the intestine (pH 6). This drastic change in pH affects the solubility of anionic or cationic drugs. Particularly in the case of cationic drugs, the solubility in an acidic condition (stomach) is greater than that in a basic or neutral condition (intestine). Water-insoluble cationic drugs have relatively high solubility in the stomach, but they are sometimes recrystallized in the intestinal lumen. Therefore, it would be difficult to use previously reported drug absorption prediction systems for predicting the absorption rate of a water-insoluble cationic drug. We therefore developed a new system for predicting the absorption rate of drugs that takes into account solid drug dissolution and the change in pH in the gastro-intestinal tract, and we tested the new system using both water-soluble and water-insoluble drugs.

2. Materials and methods

2.1. Chemicals

[^{3}H]-Inulin (21.1 GBq/mmol) was purchased from Amersham (Buckinghamshire, UK). Cefdinir, ozagrel hydrochloride and levofloxacin were kindly donated by Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan), Ono Pharmaceutical Co. Ltd. (Osaka, Japan) and Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan), respectively. Albebdazole, enoxacin, metoprolol tartrate, ranitidine hydrochloride and ritodrine were purchased from Sigma (St. Louis, MO). Caffeine anhydrous and cimetidine 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. Drug absorption rate predicting system

As shown in Fig. 1, our system for predicting drug absorption rate takes into account drug dissolution and change in pH in the gastro-intestinal tract. In this system, a drug (3 mg, solid form) is added to a drug-dissolving vessel (DDV; assumed stomach, pH 1.0, 3 ml) and the dissolved drug is transferred to a pH adjustment vessel (PAV; assumed intestine, pH 6.0, 3 ml). Each of these

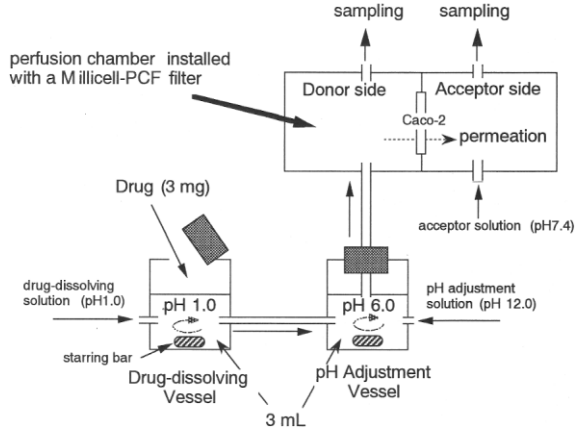

Fig. 1. Scheme of the drug absorption predicting system.

Table 1
Compositions of flowing solutions (mM)

	Drug-dissolving solution	pH adjustment solution[a]	Acceptor solution[b]
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	50.0		25.0
$CaCl_2$	2.52		1.26
$MgSO_4$	0.81		0.41
MES		20.0	
HEPES			10.0
HCl	68.0		
NaOH		68.0	

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

vessels is a glass vial. The compositions of the drug-dissolving solution (pH 1.0), pH adjustment solution (pH 12.0) and acceptor solution (pH 7.4) are shown in Table 1. The flow rate of each solution (0.13 ml/min) is controlled by a peristaltic pump. The drug solution is transferred to the donor side of a diffusion chamber (Milli-12, World Precision Instruments Inc., Sarasota, FL). Mounted between the donor and acceptor compartments is a Caco-2 monolayer grown on a polycarbonate Millicell-PCF filter (0.4 μm in pore size, 0.6 cm^2 in growth area, Millipore Co., Bedford, MA). The drug is permeated to the acceptor side of the chamber and collected by a fraction collector every 5 min. A silicon tube (i.d., 0.5 mm) is used to connect each vessel and the chamber.

2.3. 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 contained Dulbecco's modified Eagle's medium (Gibco, Life Technologies, Inc., Grand Island, NY) 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 58th passages were used in this study.

For the transport studies, Caco-2 cells were seeded on Millicell-PCF at a cell density of 8×10^4 cells/filter. The cell monolayers were fed a fresh growth medium every 2 days and were then used on the 15–21th day for the transport experiments. To evaluate the integrity of the monolayer, transepithelia electrical resistance (TEER) was measured in the Millicell-PCF with the supplemented incubation medium using Millicell-ERS (Millipore Co., Bedford, MA). TEERs of the filter were subtracted from the total TEER measurements of Caco-2 cell epithelia. The monolayers were used when their TEERs were $> 600\ \Omega\ cm^2$.

2.4. Preparation of albendazole-polymers solid dispersion

A solid dispersion was prepared by use of a solvent method (Kohri et al., 1999). Albendazole (0.1 g), hydroxypropylmethylcellulose (0.5 g) and hydroxypropylmethylcellulose phthalate (0.5 g) were completely dissolved in 75 ml of ethanol-dichloromethane (1:1), and then lactose (0.5 g) was added. The suspension was evaporated immediately at 45°C, and the residue was dried under vacuum and passed though a 60-mesh sieve. A

Table 2
Conditions used to determine the concentration of each drug[a]

Drug	Column	Wavelength	Mobile phase
Caffeine	A	273	50 mM KH_2PO_4 in 10% CH_3CN
Cefdinir	A	286	50 mM KH_2PO_4 in 10% CH_3CN
Cimetidine	A	201	50 mM KH_2PO_4 in 20% CH_3CN
Enoxacin	A	265	50 mM KH_2PO_4 in 40% CH_3CN
Ozagrel	A	270	50 mM KH_2PO_4 in 15% CH_3CN
Ranitidine	A	217	50 mM KH_2PO_4 in 35% 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
Albendazole	C	310 Ex/Em	50 mM KH_2PO_4 in 45% CH_3CN
Metoprolol	C	225/320	20 mM KH_2PO_4, 2 mM 1-octanesulfonic acid in 45% CH_3CN
Ritodrine	C	280/305	20 mM KH_2PO_4, 0.3 mM 1-octanesulfonic acid in 30% CH_3CN

[a] 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. (Tokyo, Japan). C: ERC-ODS-1161; length, 100 mm; i.d., 6 mm; ERC Inc. (Tokyo, Japan).

physical mixture was prepared by mixing albendazole, polymers and lactose with a pestle and mortar, and then passing the mixture though a 60-mesh sieve.

2.5. Analytical methods

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). HPLC condition of each drug was shown in Table 2. [^{3}H]-Inulin was measured by liquid scintillation counting.

The coefficient of variation (CV) for evaluating the variability of drug permeation across the Caco-2 monolayer was calculated by the following equation: CV (%) = (standard deviation/mean) × 100.

3. Results

3.1. Elution of drugs into the donor vessel and permeation across the Caco-2 monolayer

At first, we determined the elution behavior into the donor side after administration of [^{3}H]-inulin (solution form, 9 μl; 14.5 nmol) into DDV. As shown in Fig. 2, the elution of [^{3}H]-inulin into donor side reached maximum at 25 min, and it was undetectable after 180 min. In the next study, the permeation behaviors across the Caco-2 monolayer of drugs after co-administration of [^{3}H]-inulin (solution form) and ozagrel (solid form, 3 mg) into DDV were determined. The permeation of each drug reached maximum at about 50 min, and the behaviors were almost the same (Fig. 3). The cumulative permeation ratio of

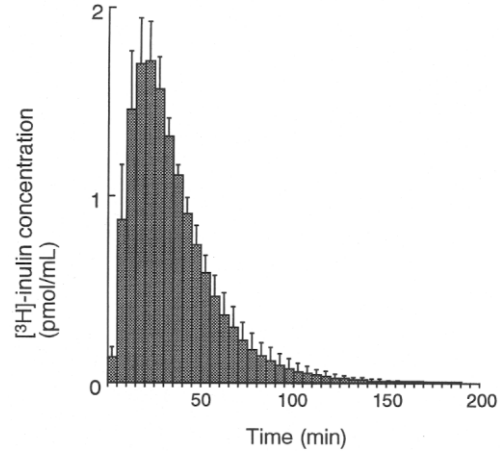

Fig. 2. Time course of [^{3}H]-inulin elution into the donor side. Each column represents the mean ± SEM of the results of three experiments.

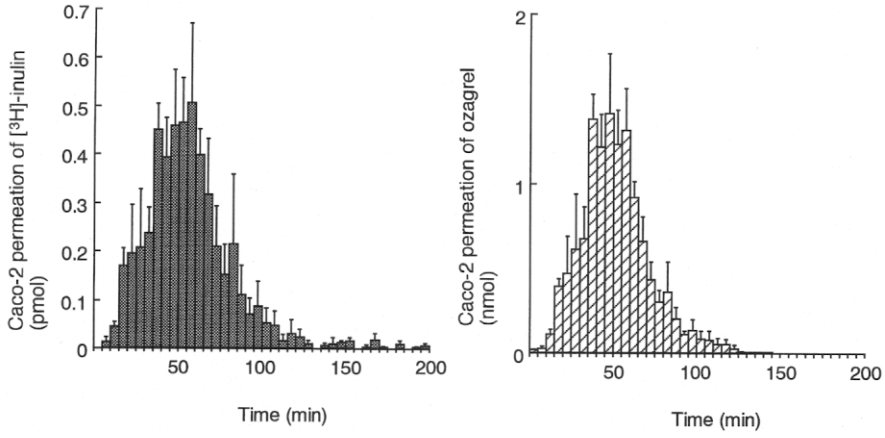

Fig. 3. Time courses of [^{3}H]-inulin (left) and ozagrel (right) permeation across the Caco-2 cell monolayer. Each column represents the mean ± SEM of the results of three experiments.

ozagrel across the Caco-2 monolayer was 0.086 ± 0.025% of the dose.

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

The amounts of permeation across the Caco-2 monolayer of ten relatively water-soluble drugs used clinically were determined. The cumulative Caco-2 permeation (percent of dose) and the CV value of each drug are shown in Table 3. The CV values of all drugs were less than 40%. The relationship between cumulative Caco-2 permeation and absorption ratio of these drugs after oral administration in humans is shown in Fig. 4. The absorption ratios of cefdinir (Maeda et al., 1989), ritodrine (Essed et al., 1988), metoprolol (Regardh et al., 1974), levofloxacin (Nakashima et al., 1992), enoxacin (Somogyi and Bochner, 1988) and ozagrel (Fukushima et al., 1990) were taken from the literature. The ratios of ranitidine, cimetidine, theophylline and caffeine are from Goodman and Gilman's (1996), the pharmacological basis of therapeutics. The cumulative Caco-2 permeation of drugs that are almost completely absorbed in humans, such as caffeine and theophylline, was over 0.1%. At a permeation ratio of less than 0.1%, there was a good correlation between the absorption rate in humans and the permeation ratio of Caco-2. In the case of drugs with poor absorption, such as cefdinir and ritodrine, the cumulative Caco-2 permeation was less than 0.03%.

3.3. Permeation of albendazole, a drug that has poor water solubility, across the Caco-2 monolayer

Albendazole is an orally administered drug used for the treatment of human echinococcosis caused by the larval forms of *Echinococcus multilocularis*. This drug is cationic and has low solubility. The solubility of albendazole is about 900

Table 3
Cumulative Caco-2 permeation and coefficient variance of drugs used[a]

Drugs	Cumulative Caco-2 permeation (percent of dose)	CV (%)
Cefdinir	0.028 ± 0.009	32.1
Ritodrine	0.038 ± 0.002	5.3
Metoprolol	0.071 ± 0.017	23.9
Ranitidine	0.078 ± 0.031	39.7
Levofloxacin	0.083 ± 0.031	37.3
Enoxacin	0.085 ± 0.017	20.0
Ozagrel	0.086 ± 0.025	29.1
Cimetidine	0.225 ± 0.027	12.0
Theophylline	0.717 ± 0.056	7.8
Caffeine	0.839 ± 0.115	13.7

[a] Each datum represents the mean ± SEM of the results of three to five experiments.

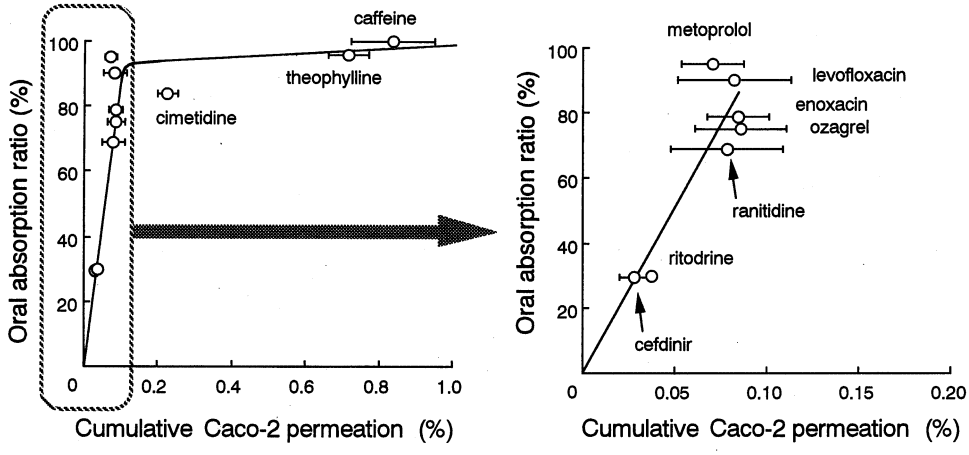

Fig. 4. Relationship between cumulative Caco-2 permeation rate and oral absorption rate in humans. Each point represents the mean ± SEM of the results of three to five experiments.

μg/ml at pH 1.2 but less than 1 μg/ml at pH above 5 (Kohri et al., 1998). It is thought that albendazole is moderately dissolved in the stomach, but recrystallized in the intestine due to the rise in the pH value. This is one of the reasons for the great inter-subject variability in the bioavailability of albendazole in clinical studies (Marriner et al., 1986; Jung et al., 1992; Sato et al., 1994). We therefore determined the Caco-2 permeation of albendazole using our system. In Fig. 5, most of the data of permeation of albendazole across Caco-2 are very low and vary widely. Since the Caco-2 permeation rates of [^{3}H]-inulin in all studies were almost the same (data not shown), the high permeation rate of albendazole was not thought to be caused by cell injurty or filter damage. In the next study, we used a commercial albendazole tablet, Escazol® (SmithKline Beecham, Tokyo, Japan), and an albendazole-polymer solid dispersion that is prescribed to improve the water-solubility of albendazole (Kohri et al., 1999). As shown in Table 4, the Caco-2 permeation rate of albendazole when Escazol® powder was used was very low and varied, like that of albendazole powder. On the other hand, the permeation rate of albendazole when the solid dispersion was used about 4-fold larger than that of the physical mixture. Furthermore, the CV value of permeation across Caco-2 of the solid dispersion was very low.

4. Discussion

Ginski and co-workers reported the system for predicting the drug absorption that took into account solid drug dissolution (Ginski and Polli, 1999; Ginski et al., 1999). Their system can predict the absorption of water soluble and poorly soluble drugs. However, they did not take into account change in pH at the gastro-intestinal tract. Thus their system could not predict the accurate absorption of the unstable or insoluble drug in acidic solution. Base on these problems,

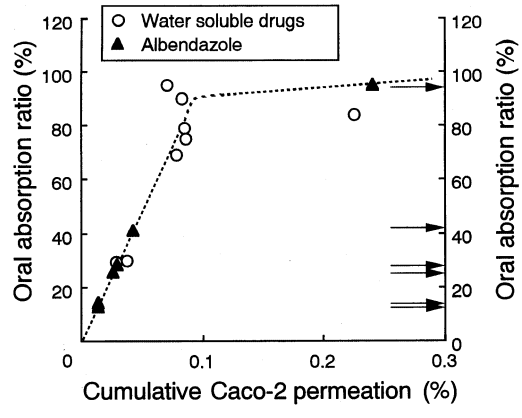

Fig. 5. Prediction of oral absorption ratio of albendazole from cumulative Caco-2 permeation. The data of water-soluble drugs in Fig. 4 were used. Arrows show the prediction of oral absorption of albendazole.

Table 4
Donor side elution, Caco-2 permeation and its coefficient variance of albendazole, Escazol®, albendazole-polymer solid dispersion and its physical mixture[a]

	Donor side elution (percent of dose)	Caco-2 permeation (percent of dose)	CV (%)
Albendazole powder	2.32 ± 0.55	0.061 ± 0.088	143.9
Escazol® powder[b]	3.03 ± 1.56	0.014 ± 0.012	85.0
Physical mixture[c]	19.6 ± 4.4	0.056 ± 0.035	62.2
Solid dispersion[c]	≈ 100	0.210 ± 0.021	9.7

[a] Each value is represent the mean $\pm$ SD of 3–6 determinations.
[b] Escazol® tablet was pulverized, and 10.2 mg of powder (albendazole; 3 mg) was added to DDV.
[c] The compositions of albendazole-polymers are stated in Section 2. In this study, 10 mg of an albendazole-polymers mixture (albendazole; 0.625 mg) was added to DDV.

we developed a new system for predicting the drug absorption that took into account solid drug dissolution and change in pH at the gastro-intestinal tract. This system enables prediction of the absorption rate of water-soluble drugs, because the drugs are immediately dissolved in a dissolving vessel. Many studies have shown that the Caco-2 permeation of drugs is correlated to the absorption rate in humans. However, this new system was designed to enable prediction of the absorption rate of not only water-soluble drugs but also drugs that have poor water solubility. Thus, we examined the absorption of albendazole, an extremely water-insoluble drug, in this system. The permeation rate of albendazole across the Caco-2 monolayer was very low, and there was a large variation in data between studies. This variability could be explained as follows. Albendazole is a cationic drug and is therefore soluble in DDV (pH 1.0); however in PAV, the solubility of albendazole is remarkably decreased and albendazole would recrystallize in the vessel. In fact, it was confirmed by the naked eye that the buffer in PAV became clouded. It was thought that this phenomenon also occurs in the human gastro-intestinal tract, causing the poor and variable albendazole bioavailability in patients. In Fig. 5, there was an extremely high datum of cumulative Caco-2 permeation of albendazole. This phenomenon was not thought to be caused by cell injurty or filter damage. This could be explained that the re-crystallized albendazole adhered the surface of Caco-2 or membrane filter and it dissolved slowly, but high concentration of albendazole was kept at there. Therefore, the permeation of albendazole might be risen in this case. A commercially available albendazole tablet, Escazol®, contains surfactants to improve its solubility in the stomach. In the present study (Table 4), the elution of Escazol® into the donor side was slightly larger than that of albendazole powder, but its permeation rate across Caco-2 was almost the same as that of albendazole powder. On the other hand, we previously reported a solid dispersion of albendazole-polymers improved the solubility in weakly acidic and neutral solutions (Kohri et al., 1999). This solid dispersion increased the albendazole absorption after oral administration in rabbits with low gastric acidity. In the present study, the elution and permeation of albendazole using this solid dispersion were remarkably larger than those of physical mixtures of albendazole-polymers. Moreover, the CV value of permeation across Caco-2 was much lower. Clinical studies have shown a great inter-subject variability in the bioavailability of albendazole after oral administration. Our system would also enable evaluation of this variability in absorption rate.

In this study, our system could predict the absorption of the solid dispersion. Therefore, it will also enable to predict the absorption of the other pharmaceutical product, such as microsphere and control-released granule. However, the volume of DDV of this system is only 3 ml, thus a product that is above 30 mg can not be used for this system. To settle this problem, it is necessary to make the vessel larger. Moreover, this system will be able to evaluate the drug–drug interaction

in gastro-intestinal tract, such as new quinolone antibiotic and aluminum-contained antiacid.

In conclusion, we constructed a new system for predicting drug absorption that takes into account dissolution of solid drugs and change in pH in the gastro-intestinal tract. This system enables evaluation of the absorption rate of water-soluble drugs after oral administration in humans. Furthermore, the system predicted that albendazole, a drug with poor water solubility, has low absorption and variability. The results suggest that this new system will be useful for prediction of the absorption rates of new compounds or new products.

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