In Vitro System to Evaluate Oral Absorption of Poorly Water-Soluble Drugs: Simultaneous Analysis on Dissolution and Permeation of Drugs

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Purpose. The aim of the present work was to develop a new *in vitro* system to evaluate oral absorption of poorly water-soluble drugs by utilizing Caco-2 monolayers.

Methods. Caco-2 monolayer was mounted between side-by-side chambers, which enabled the simultaneous assay of dissolution and permeation of drugs (dissolution/permeation system; D/P system). Apical and basal sides of the chamber were filled with buffer solutions. Drugs were applied to the apical side as powder, suspension, or solution, and then, the permeated amounts into the basal side were monitored for 2 h. At the same time, dissolved amounts of drugs at the apical side were detected. The amount of drug applied to the D/P system was based on its *in vivo* clinical dose.

Results. Sodium taurocholate (5 mM, apical side) and bovine serum albumin (4.5% w/v, basal side) increased the permeated amount of poorly water-soluble drugs. Both additives were considered to be effective at mimicking *in vivo* conditions of intestinal drug absorption. From the correlation between the permeated amount of 13 drugs (% dose/2 h) in the D/P system and their percentage dose absorbed in humans *in vivo*, this system was found to be useful in evaluating oral absorption of poorly water-soluble drugs.

Conclusions. With attempts made to mimic the physiologic conditions of the human GI tract, *in vivo* oral absorption of drugs was quantitatively assessed in the D/P system *in vitro*. This system is quite useful to predict the oral absorption of poorly water-soluble drugs after administration as solid dosage forms.

KEY WORDS: Caco-2 monolayers; intestinal absorption; dissolution; permeation; poorly water-soluble drug; *in vitro–in vivo* correlation.

INTRODUCTION

Recently, in pharmaceutical companies, the lead or candidate compounds for new medicines that are selected by high-throughput screening from a huge compound library often show very poor suitability as a medicine in respect to ADME properties. Insolubility or poor solubility in water is one of the most serious problems in the subsequent processes of drug development. In the case of oral drugs, because the drug must be dissolved in the gastrointestinal (GI) tract before absorption, poor solubility leads to low bioavailability after oral administration.

Among the many factors that influence oral drug absorption, solubility in water and permeability of the gastrointestinal membrane are keys to determining the fraction dose absorbed. Amidon *et al.* have defined three parameters for drug absorption—absorption number, dose number, and dissolution number, which are based on the permeability and solubility of drugs and the successfully demonstrated three-dimensional relationship among permeability, solubility (dissolution), and the fraction dose absorbed (1,2). Moreover, they proposed a biopharmaceutics classification system of drugs (BCS) in which drugs are classified into four classes according to their permeability and solubility. These theoretical analyses enable the prediction of *in vivo* oral absorption of drugs administered as a solid dosage form, if enough information is given concerning the permeability and solubility.

In order to evaluate the permeability of drugs, various *in vivo* to *in vitro* methodologies have been developed. The *in vitro* permeation study using Caco-2 cell (3–5) or MDCK cell (6) monolayers is one of the most promising techniques and now widely used in the pharmaceutical companies. The permeability of drugs to Caco-2 or MDCK monolayers has been reported to correlate well with their oral absorption. With the data from the *in vitro* solubility study, therefore, it is possible to predict the oral absorption of drugs by theoretical calculations, as proposed by Amidon *et al.* However, in the case of poorly soluble drugs, several difficulties still remain to predict absorption because (a) their absorption is markedly affected by the physiologic conditions in the GI tract such as bile acid secretion and some other endogenous components (7), (b) their absorption is subject to food effects (8,9), and, finally, (c) the effect of various formulations to improve their oral absorption such as micronization, solid dispersion, or lipid-based formulation (10,11) should be evaluated. In addition, very limited solubility of compounds sometimes causes difficulty in supplying enough concentration of drugs for permeability measurements. *In vivo* absorption studies (oral administration studies) with animals are useful to know the total absorption of drugs. However, the data obtained (bioavailability) include all factors of absorption and thus tend to be variable and not informative for further analysis or improvement. Also, the interspecies differences in oral absorption might be great for poorly soluble drugs, reflecting the differences in physiologic conditions of the GI tract between animals and humans.

In this study, we have tried to develop a new *in vitro* system to evaluate the oral absorption of poorly water-soluble drugs. The system [dissolution/permeation (D/P) system] consists of two half-chambers and a Caco-2 monolayer mounted between them. Drugs were applied to the D/P system in solid dosage form, then both dissolution and permeation processes were evaluated simultaneously to predict quantitatively the *in vivo* oral absorption in humans.

MATERIALS AND METHODS

Materials

Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD) at passage 17. Dulbecco's modified Eagle medium (D-MEM), nonessential amino acids (NEAA), fetal bovine serum (FBS), L-glutamate, trypsin (0.25%), EDTA (1 mM), and antibiotic-antimycotic mixture (10,000 U/ml penicillin G, 10,000 μg/ml streptomycin sulfate,

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and 25 μg/ml amphotericin B in 0.85% saline) were purchased from Gibco Laboratories (Lenexa, KS). [^{14}C]Mannitol (mannitol) (specific activity of 55 mCi/mmol) was purchased from New England Nuclear (Boston, MA). Griseofulvin was obtained from Shionogi Pharmaceutical Co., Ltd. Pranlukast was obtained from Ono Pharmaceutical Co., Ltd. Cellulose acetate filter was purchased from Advantec (Toyo Roshi Kaisha, Ltd., Japan). All other reagents used were of the highest purity.

Preparation of Caco-2 Monolayer

Caco-2 cells were grown in D-MEM supplemented with 10% FBS, 1% L-glutamate, 1% NEAA, and 5% antibiotic-antimycotic solution as culture medium at 37°C in culture flasks (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) in humidified air at 5% CO_2 atmosphere. Cells were harvested with typsin-EDTA and seeded on polycarbonate filters (0.3-μm pores, 4.20 cm^2 growth area) inside cell culture inserts (Nippon Becton Dickinson Co., Ltd., Tokyo Japan) at a density of 3×10^5 cells/filter. The culture medium (1.5 ml in the insert and 2.6 ml in the well) was replaced every 48 h for the first 6 days and every 24 h thereafter. After 18–21 days in culture, the Caco-2 monolayer was used for the following experiments.

Chambers for the D/P System

The D/P system used in this experiment is illustrated in Fig. 1. Chambers were made of acrylic plastic. To avoid damage to the Caco-2 monolayer mounted between chambers, O-rings made of fluorinate rubber were attached at the edge of the inner opening of both apical and basal chambers. The effective surface area of the Caco-2 monolayer was 1.77 cm^2. Both sides of the Caco-2 monolayers were consistently stirred at 100, 200, or 400 rpm by magnetic stirrers. The volume of apical and basal sides was set at 8 ml and 5.5 ml, respectively.

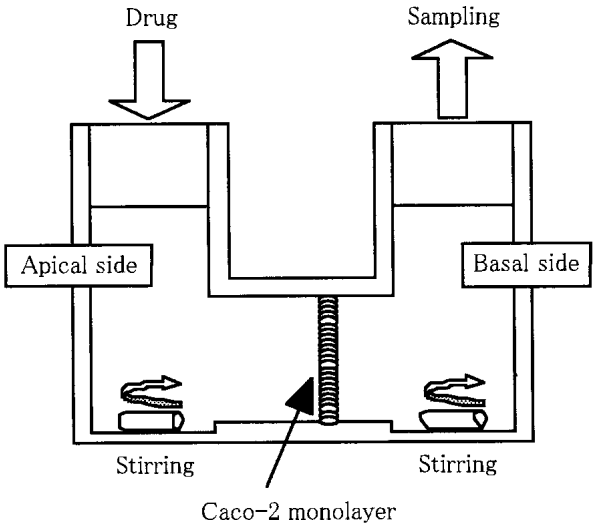

Fig. 1. Schematic illustration of the dissolution/permeation system (D/P system). Caco-2 monolayer was mounted between the apical and basal chambers. Both sides of the monolayer were filled with transport medium (apical side; pH = 6.5, volume = 8 ml, basal side; pH = 7.4, volume = 5.5 ml) and stirred by magnetic stirrers constantly. Drugs were applied to the apical side as solid, suspension, or solution.

As a buffer solution for both sides (transport medium, TM), Hanks balanced salts solution (HBSS) supplemented with 25 mM glucose was used in all studies after adjusting the pH to 6.5 as apical solution or 7.4 as basal solution with HEPES. All experiments were performed at 37°C.

Dissolution Profile of Griseofulvin in the D/P System

Dissolution profiles of griseofulvin were observed by using only the apical chamber without mounting Caco-2 monolayers. Instead of Caco-2 monolayers, a flat sheet of aluminum foil was mounted between the chambers to prevent the leak of apical solution (8 ml of TM adjusted to pH = 6.5, with or without 5 mM TCA) to the basal compartment. One milligram of griseofulvin was applied to the apical chamber as a powder. The stirring rate of the solution was set to 100, 200, or 400 rpm. After applying griseofulvin, aliquots of samples were routinely taken from the apical solution for 2 h. Samples were filtered through cellulose acetate filters to determine the dissolved amount of griseofulvin.

Permeation Profiles of Various Drugs in the D/P System

Apical solution (TM with or without 5 mM TCA; pH = 6.5) and basal solution (TM with or without 4.5% w/v BSA; pH = 7.4) were introduced to the apical and basal sides of the Caco-2 monolayer in the well, respectively. After incubation for 20 min, the Caco-2 monolayer with support filter was taken from the insert and was mounted between the chambers of the D/P system. The apical and basal sides of the monolayer were filled with TM (pH = 6.5 for apical and 7.4 for basal) and stirred at 100, 200, or 400 rpm. Drugs were applied to the apical side as suspension or powder. Only [^{14}C]mannitol was applied as a solution because mannitol dissolved quickly and the dissolution process has no effect on its permeated amount in this study. Then, aliquots of samples were taken from the basal side at appropriate intervals over 2 h. The volume of the basal side was maintained by adding fresh basal solution. After the end of the experiment, apical solution was immediately collected and filtered through cellulose acetate filter to determine the final concentration of the dissolved drug. The transepithelial electric resistance (TEER) of the monolayer was checked before and after the experiment. In all experiments, there was no significant decrease in the TEER value during the experiment (data not shown).

Analytic Methods

The concentration of [^{14}C]mannitol in the sample solution was determined by using a liquid scintillation counter (LSC 3500, Aloka, Tokyo, Japan). Unlabeled samples were analyzed with a reversed-phase HPLC system (LC-10A Shimadzu Co., Kyoto, Japan) equipped with a variable wavelength ultraviolet detector (SPD-10A, Shimadzu Co., Kyoto Japan) or a fluorescence spectromonitor (RP-10A, Shimadzu Co., Kyoto Japan). The column (J'sphere ODS-H80 75 × 4.6 mm I.D., YMC, Japan) was used with a mobile phase consisting of 50 mM phosphate buffer (pH = 2.5) and acetonitrile. Albendazole, atenolol, carbamazepine, chlorpheniramine, danazol, ketoprofen, metoprolol, piroxicam, pranlukast, propranolol, and warfarin were quantified with the variable ultraviolet detector at 310 nm, 226 nm, 285 nm, 225

nm, 286 nm, 260 nm, 225 nm, 326 nm, 260 nm, 228 nm, and 311 nm, respectively. Griseofulvin was quantified by fluorescence detection with excitation and emission wavelengths of 303 and 428 nm, respectively.

RESULTS

Experimental Conditions in the D/P System

Effect of Stirring Rate on Dissolution and Permeation of Griseofulvin

Fig. 2 shows the dissolution and permeation profiles of griseofulvin (applied amount 1 mg) in the D/P system obtained at stirring rates of 100, 200, and 400 rpm. For dissolution in the apical solution, no significant differences were observed in the dissolved amount among the three different stirring rates (Fig. 2a). However, the amount of griseofulvin permeated into the basal solution was apparently higher (approximately twofold) at a high stirring rate (200 and 400 rpm) than at 100 rpm (Fig. 2b). Because the permeated amount of griseofulvin at 200 and 400 rpm was almost the same, it was obvious that the permeation, but not the dissolution, was diffusion-limited at the slow (100 rpm) stirring rate. In the following experiments, therefore, the stirring rate was fixed to 200 rpm on both sides.

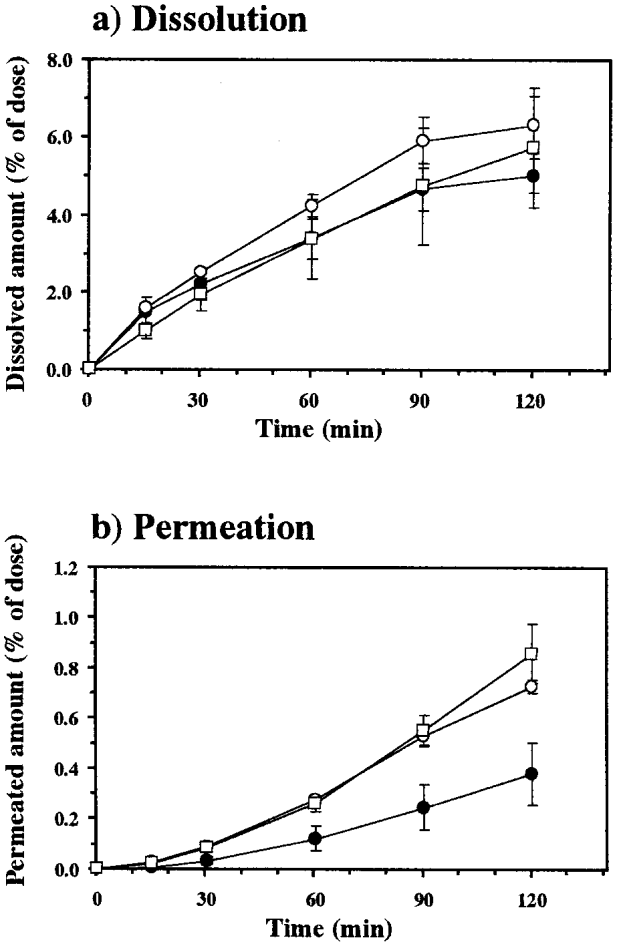

Fig. 2. Effect of stirring rate on dissolution (a) and permeation (b) of griseofulvin in the D/P system. Both apical and basal sides were stirred at 100 (●), 200 (○), or 400 (□) rpm by magnetic stirrers. One milligram of griseofulvin was applied to the apical side as a powder, and then the time course of its dissolution and permeation was observed for 2 h. The data are expressed as the mean ± SE of at least three independent experiments.

Effect of Bovine Serum Albumin on Permeation of Griseofulvin

Fig. 3 shows the permeation profiles of griseofulvin with or without BSA (4.5% w/v) in the basal solution. In this experiment, the saturated suspension of griseofulvin (13.5 μg/ml) was applied to the apical side to avoid the effect of lag-time of dissolution. In the presence of BSA in the basal solution, the permeated amount of griseofulvin increased linearly with time, whereas it reached the ceiling in the absence of BSA. This suggests that BSA worked as a reservoir of drugs through the binding with drugs in the basal solution, to maintain a sink-condition for drug permeation across Caco-2 monolayers.

Effect of Sodium Taurocholate on Dissolution and Permeation of Griseofulvin

Fig. 4 shows the effect of sodium taurocholate (TCA) added to the apical solution on griseofulvin dissolution and permeation. One milligram of griseofulvin was applied to the apical side as a powder in the presence or absence of 5 mM TCA. Basal solution always contained 4.5% w/v of BSA. With 5 mM TCA, griseofulvin dissolved rapidly, and the dissolved amount reached a plateau level around 10% of dose. In contrast, dissolution was slow and reached 6.33% of dose after 2 h in the absence of TCA (Fig. 4a). The permeated amount was also enhanced by TCA about 1.5-fold; however, the effect of TCA was rather mild, and the time course of permeation was similar in both conditions (Fig. 4b). These results indicated that most of the griseofulvin that dissolved rapidly in the presence of TCA was incorporated in the micelles; then, only the free fraction in the apical solution permeated Caco-2 monolayers.

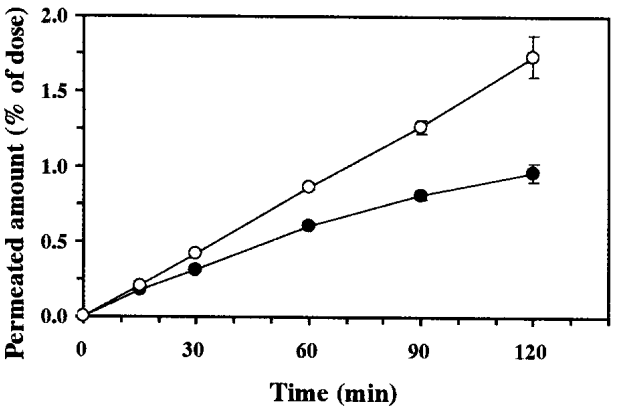

Fig. 3. Effect of BSA on permeation of griseofulvin in the D/P system. Suspension of griseofulvin (13.5 μg/ml) was applied to the apical side; then the time course of its permeation was observed for 2 h with (○) or without (●) 4.5% BSA in the basal solution. The data are expressed as the mean ± SE of at least three independent experiments.

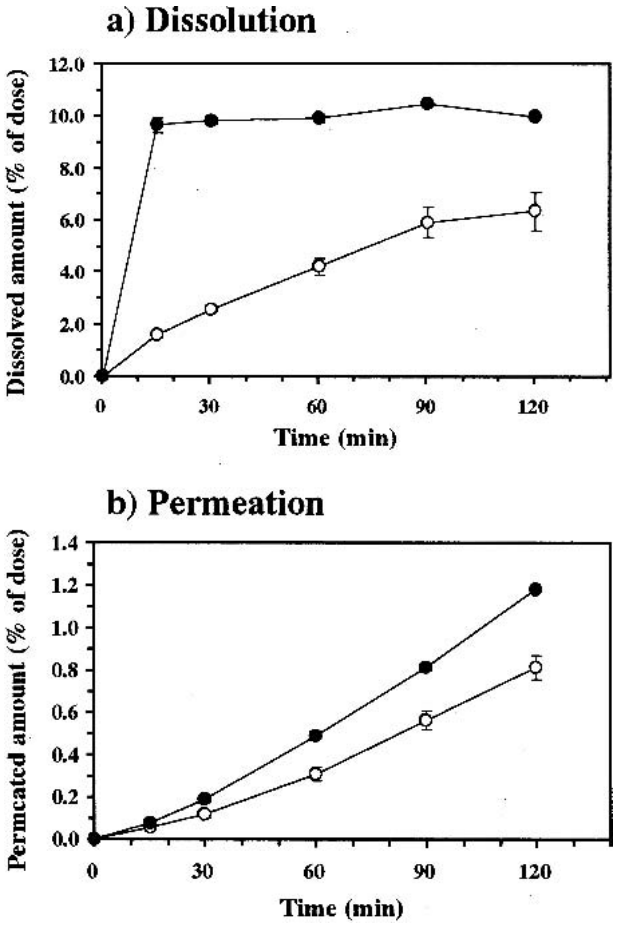

Fig. 4. Effect of bile acid on dissolution (a) and permeation (b) of griseofulvin in the D/P system. One milligram of griseofulvin was applied to the apical side as a powder, and then the time course of its dissolution and permeation was observed for 2 h with (●) or without (○) 5 mM TCA in the apical solution. The data are expressed as the mean ± SE of at least three independent experiments.

Effect of Applied Amount on Dissolution and Permeation of Propranolol and Griseofulvin

Oral dose is a key factor to determine the fraction of the dose that is absorbed, especially for drugs having poor water solubility. As shown in Table I, a 2.5-fold increase in the applied amount of griseofulvin induced approximately a 50% decrease in its permeated amount (percentage of dose) because of the decrease in percentage of dissolved amount. In the case of propranolol, which has a higher water solubility and is completely dissolved in the apical solution, a 10-fold increase in the applied amount induced only an 18% decrease in the permeated amount. These findings clearly indicate that the applied amount affected the permeated amount in the D/P system, depending on the solubility of drugs. Therefore, in order to evaluate *in vivo* absorption by using *in vitro* experiments with the D/P system, the applied amount should be decided based on the *in vivo* clinical dose of each drug. Volume of gastrointestinal (GI) fluid was reported as approximately 500 ml and 900–1000 ml at fasted and fed state, respectively (12). In the D/P system used here, the apical volume (8 ml) corresponds to about 1/100 of *in vivo* volume; thus, in the following study, 1% of clinical dose was applied to the D/P system.

Correlation between *In Vivo* Absorption and *In Vitro* Permeation in the D/P System

Various drugs were applied to the D/P system, and obtained data are summarized in Table II. The following conditions were used for this experiment: apical side, pH 6.5, stirring rate 200 rpm, containing 5 mM TCA; basal side, pH 7.4, stirring rate 200 rpm, containing 4.5% BSA. Each drug was applied to the apical side as a powder, and the applied amount was set to 1% of the clinical dose. In Table II, permeability (P_{app}) of drugs was calculated from the slope of the time course of permeated amount from 1.5 to 2 h, assuming that the dissolved concentration of drugs in apical solution was almost constant during final 30 min as,

$$P_{app}\ (cm/s) = \frac{\text{permeated amount at 2 h} - \text{permeated amount at 1.5 h}}{\text{apical (dissolved) concentration at 2 h} \cdot A \cdot 1800}$$

where A is the effective area of Caco-2 monolayer (1.77 cm^2). Because some drugs were taken up into micelles of TCA in apical solution, the permeability calculated here should be considered as an apparent permeability (P_{app}).

Fig. 5 shows the relationship between dissolved amount at 2 h and P_{app} of each drug. Thirteen drugs used were classified into three different groups: completely dissolved and high-P_{app} drugs (Class 1), incompletely dissolved but high-P_{app} drugs (Class 2), and completely dissolved but low-P_{app} drugs (Class 3). The permeated amounts of these drugs were plotted against their *in vivo* human absorption (fraction dose absorbed) in Fig. 6. Drugs classified in Class 1 in Fig. 5 were

Table I. Effect of Applied Amount of Drugs to D/P System on Their Dissolution and Permeation

	Clinical dose* (mg)	Applied dose‡ (mg)	Dissolved amount (% dose/2 h)	Permeated amount (% dose/2 h)
Propranolol	10	1.0	Completely dissolved	7.00 ± 0.32
		0.1	Completely dissolved	8.52 ± 0.17
Griseofulvin	250	1.0	9.96 ± 0.03	1.18 ± 0.01
		2.5	4.75 ± 0.01	0.65 ± 0.01

* Oral dose.
† Applied amount to D/P system.
Values were expressed as the mean ± SD of at least three experiments.

Table II. Parameters of 13 Drugs for Dissolution and Permeation in D/P System and Their Absorption in Humans

	Clinical dose* (mg)	Applied dose* (mg)	Dissolved amount (% dose/2 h)	Permeated amount (% dose/2 h)	P_{app} ($\times 10^{-6}$ cm/s)	Human abs.‡ (%)
Albendazole	200	2.0	0.626	0.065	79.71	20
Atenolol	50	0.5	100	0.085	1.75	50
Carbamazepine	100	1.0	100	12.26	75.66	83
Chlorpheniramine	6	0.06	100	6.84	71.58	80
Danazol	100	1.0	3.74	0.12	27.09	30
Griseofulvin	250	2.5	4.75	0.647	130.94	80
Ketoprofen	50	0.5	100	8.62	68.30	85
Mannitol	—	1.0	100	0.078	0.42	16
Metoprolol	40	0.4	100	4.51	27.16	94.5
Piroxicam	10	0.1	100	10.17	80.10	95
Pranlukast	225	2.25	0.737	0.061	25.21	15
Propranolol	10	0.1	100	8.52	33.14	95
Warfarin	5	0.05	100	7.48	65.88	93.5

* Oral dose.
† Applied amount to D/P system (1% of clinical dose).
‡ Represents mean values for % absorption obtained from individual drug references or the *Goodman & Gilman's The Pharmacological Basis of Therapeutics* (10th ed.).
Values were expressed as the mean of at least three experiments.

found to be absorbed almost completely *in vivo* and, in the D/P system, more than 1% of the applied amount was permeated over 2 h. Other drugs in Class 2 or 3 showed incomplete absorption *in vivo,* and the *in vitro* permeated amount in the D/P system was less than 1% of dose. Although the theoretical analysis of the correlation between *in vivo* absorption and *in vitro* permeated amount in the D/P system has not been completed, this correlation strongly suggested the possibility of predicting the *in vivo* drug absorption from the *in vitro* experiments in the D/P system.

DISCUSSION

The number of new compounds with very poor water solubility has increased dramatically after the paradigm shift in drug discovery occurred in the past two decades. To avoid a risk at the later stage of drug development, systems of screening the physicochemical properties of new compounds, especially on their water solubility, have been widely introduced at the discovery stage. Nevertheless, many of the compounds selected as leads or candidates for new medicine show very poor water solubility. In these stages, more precise characterizations of their ADME properties are demanded for decision making about their further development. Only a limited number of those compounds are evaluated in animal models because of the severe capacity limits of *in vivo* studies. Moreover, an estimation based on the animal studies is not guaranteed in humans. In this study, therefore, we have tried to develop a new *in vitro* system to evaluate oral absorption of poorly water-soluble drugs that can be used as an alternative to *in vivo* animal studies in the future.

A study with similar types of *in vitro* systems that combined dissolution and permeation processes of drug absorption has been published. Ginski *et al.* have reported the continuous dissolution/Caco-2 system to investigate drug absorption (13,14). They observed the dissolution–absorption relationships and determined the rate-limiting process of absorption in fast- and slow-dissolving formulations. Kobayashi *et al.* have reported a system in which the effect of pH change

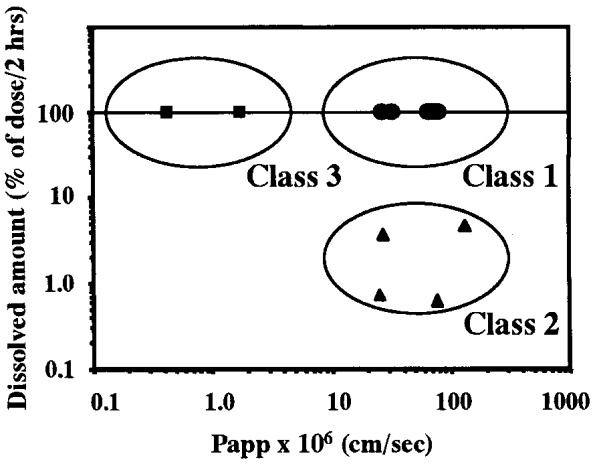

Fig. 5. Relationship between dissolved amount (% dose/2 h) and apparent permeability (P_{app}, cm/s) in the D/P system. Twelve drugs were classified into three classes as Class 1 (●), completely dissolved with high P_{app} (7 drugs), Class 2 (▲), incompletely dissolved with high P_{app} (4 drugs), and Class 3 (■), completely dissolved with low P_{app} (2 drugs).

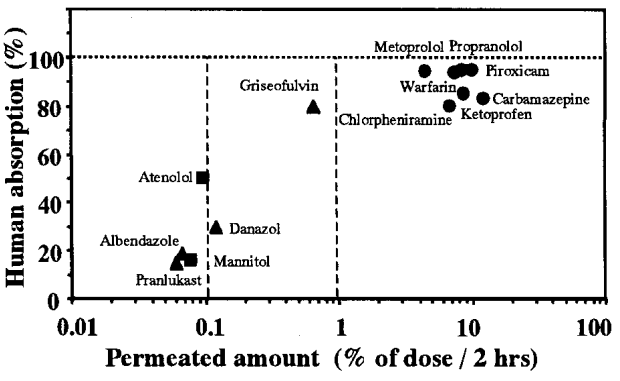

Fig. 6. Correlation between *in vivo* human absorption and *in vitro* permeated amount in the D/P system. The same symbols defined in Fig. 5 are used for each drug.

in the GI tract on drug dissolution and permeation was evaluated (15). Those studies have given useful information to consider the dissolution–permeation relationship in drug absorption and the effects of formulation and additives. However, in terms of the prediction of *in vivo* absorption, especially that of poorly water-soluble drugs, their system is insufficient because they did not take into account the clinical dose of drugs and the physiologic conditions of the human GI tract. The ratio of dose and water volume in the GI tract is one of the key factors to determine the absorption of poorly soluble drugs. Also, the absorption was markedly influenced by the physiologic conditions of the GI tract.

In our system, experimental conditions were considered based on *in vivo* conditions in the human GI tract. The amounts of drugs applied in Fig. 6 were set to 1% of their clinical dose because the volume of the apical chamber, 8 ml, could be regarded as about 1/100 of the physiologic volume of human GI tract, which was reported as approximately 500–1000 ml (12). Bile acids are secreted into the duodenum from the gallbladder, and their average concentration in human intestinal tract (of healthy subjects) was reported to be 5–15 mM (16,17) and highly affected by food intake. In this study, a 5 mM concentration of TCA was added to the apical solution, which corresponds to the concentration during fasting conditions in human. TCA at this concentration showed no effect on the integrity of Caco-2 monolayers.

Addition of TCA facilitated both dissolution and permeation of griseofulvin, although TCA was less effective on the permeation than on the dissolution (Fig. 4). Because Kimura *et al*. reported that the critical micellar concentration of TCA was 2.9 mM (18), a large fraction of dissolved griseofulvin was considered to be taken up into micelles, which decreased the fraction available for membrane permeation. This might also be the situation *in vivo,* and thus, the result with TCA in the D/P system corresponds to the fact that the *in vivo* oral absorption of griseofulvin is facilitated by the secretion of bile acids (7).

During the *in vivo* oral absorption process, drugs can rapidly enter into the blood circulation after penetration across the intestinal epithelial layer. In the blood, most drugs with high lipophilicity exist in a bound form with serum proteins such as albumin. Protein binding can enhance the solubility of drugs in the serum, which might facilitate their absorption into the blood. We have already reported that the addition of BSA to the basal side facilitates the transmembrane transport of various drugs *in vitro*, which might give a reasonable estimation of drug permeability in the study with Caco-2 monolayers (19). In the D/P system, results in Fig. 3 clearly demonstrated the importance of serum protein in the basal solution to promote the absorption of poorly water-soluble drugs into the blood circulation.

In this study, dissolution and permeation of drugs were observed over 2 h, and, finally in Fig. 6, the permeated amount of each drug at 2 h was correlated with *in vivo* absorption. The importance of GI transit time to consider drug absorption was well described in the theory presented by Amidon *et al.* (1,2), where the average transit time in the small intestine was defined as 3 h (20). However, the relative time scale in the D/P system might differ from that in human intestine *in vivo*. We have observed the relationship between human absorption of several drugs and their permeated amount in the D/P system at different time points. Then, results in Figs. 5 and 6 have suggested the possibility of using the data at 2 h in the D/P system as surrogates of *in vivo* drug absorption.

In addition, it was possible to classify the drugs as shown in Fig.5 based on the solubility and permeability obtained in the D/P system. Although the boundary of the criteria for each class was not the same as that described in the BCS guideline presented by the FDA, this type of analysis should be helpful to recognize the main cause of incomplete absorption and then to consider the way to improve it.

By taking into account the *in vivo* physiologic conditions of the human GI tract as described above, it became possible to evaluate *in vivo* absorption in humans from the permeated amount in the D/P system. In Fig. 6, atenolol showed the low permeated amount (less than 0.1%) in spite of 50% absorption in human. Atenolol is a drug classified in Class III in BCS; thus, its absorption is limited by the permeability. Although *in vivo* absorption was assumed to be 50% of dose, atenolol was reported to show quite low permeability to Caco-2 monolayers, which was almost the same or rather lower than that of mannitol (4). Therefore, the deviation in Fig. 6 could be explained by the fact that the permeability of atenolol to Caco-2 monolayers often failed to describe its absorption in humans. Differences in the membrane properties between human intestine and Caco-2 monolayers, mainly the difference in tightness of paracellular pathway, might be a factor to explain this inconsistency. To overcome this problem, the use of other membranes having a leakier structure, such as MDCK (6) or short-term cultured Caco-2 monolayers (21), should be tested.

Finally, the D/P system developed here is expected to be useful for the understanding factors that cause the absorption of poorly water-soluble drugs to vary. For instance, by applying the intestinal simulated fluids for the fasted and fed states proposed by Dressman *et al.* (22,23), food effect on total absorption, not only on the dissolution, could be monitored. The effect of formulation to improve the absorption of poorly water-soluble drugs such as micronization, solid dispersion, and lipid-based systems can be evaluated quantitatively in the D/P system. Furthermore, if the dissolution in the stomach under the acidic condition influences the subsequent absorption in the intestine, as is the case for drugs that are weak bases, drugs can be preincubated in the low-pH solution before being applied to a D/P system. These studies are now under investigation and are the subjects for future reports.

CONCLUSION

In this report, we have developed a new *in vitro* system, the D/P system, which enables the quantitative assessment of drug absorption, especially for poorly water-soluble drugs. Experimental conditions of the system were set taking into account the physiologic conditions of the human GI tract that could be a key to success in evaluating *in vivo* oral absorption. The D/P system is considered to be quite useful not only to predict drug absorption but also to determine the main cause of incomplete absorption and various factors that affect drug absorption.

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