In Vitro Dissolution/Permeation System to Predict the Oral Absorption of Poorly Water-Soluble Drugs: Effect of Food and Dose Strength on It

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The aim of the present work was to confirm the usefulness of the dissolution/permeation system (D/P system) in the estimation of human oral absorption of poorly water-soluble drugs. The D/P system, which can simultaneously evaluate drug absorption processes, dissolution and permeation, can predict the oral absorption of poorly water-soluble drugs in fasted and fed humans, with a correlation between in vivo oral absorption (% of absorbed) and in vitro permeated amount (% of dose/2 h) in the D/P system. The oral absorption (fraction of absorbed dose, %) of poorly water-soluble drugs in the fasted and fed states was predicted using the D/P system. The effect of food on the oral absorption of various drugs estimated by the D/P system significantly correlated with clinical data (correlation coefficient: 0.924). Moreover, the proportion of oral absorption of cilostazol was predicted to decrease with an increase in its dose strength, which significantly correlated with in vivo human absorption. Consequently, the D/P system was demonstrated to be a useful in vitro system for prediction of the oral absorption of poorly water-soluble drugs.

Key words poorly water-soluble drug; food effect; absorption; dissolution; solubility

The oral absorption of drugs and drug candidates often varies, when they are administered after food intake.1—4) The effect of food on oral drug absorption may alter the effectiveness of drug therapy. Thus, dosing instructions should be carefully set on the basis of the characteristics of drug absorption in order to ensure a high level of effectiveness in drug therapy. The oral drug absorption under the fed state can be more or less than that under the fasted state, which are often called positive and negative food effects, respectively.5—8) Fleisher et al. stated that drugs classified into class I, class II and class III using the biopharmaceutics classification system (BCS) are likely to be not affected by food, be positively affected by food and be negatively affected by food, respectively, while the effects are unclear for class IV drugs.9)

As the main cause of the positive food effect, it is considered that the ingestion of food stimulates the secretion of bile juice into the gastrointestinal tract, which accelerates the solubility and dissolution of poorly water-soluble drugs and then enhances oral absorption.10—12) The oral absorption of drugs with high lipophilicity was affected by the food state, which is shown in Table 1. For instance, the oral absorption of albendazole was enhanced 4-fold by the intake of food. However, the total absorbed amount of nateglinide was almost the same between the fasted and fed states because the intake of food did not enhance area under curve (AUC) but induced a decreased absorption rate (decreased Cmax and prolonged Tmax).13) Moreover, the oral absorption of poorly water-soluble drugs is often proportionally reduced with increasing dose strength owing to solubility- and dissolution-rate-limited absorption.14,15) Thus, these effects on drug absorption should be considered when the oral absorption of poorly water-soluble drugs is predicted.

Gu et al. proposed a statistical model for prediction of the effect of food on drug absorption.16) The maximum absorbable dose (MAD) of 92 drugs was calculated using solubility, fluid volume (250 ml), transit time (180 min) and absorption rate constant (min⁻¹). Then, the effect of food on drug absorption was predicted by comparing MAD with clinical dose and using logistic regression with physicochemical properties. For the 92 drugs, 80% were correctly categorized into positive, negative or no effect of food groups in the prediction. In the case of a positive effect of food, 83% were successfully classified. Although the adapted method is useful for the qualitative prediction of the effect of food on drug absorption, it may not be suitable for its quantitative prediction.

In previous studies, we developed an in vitro system (Dissolution/Permeation system: D/P system, Fig. 1) to evaluate the dissolution and permeation of drugs in solid dosage form.17) The D/P system enables simultaneous determination of drug dissolution and permeation after a drug is applied to the apical side of the system. The applied amount of drugs was set to 1% of the clinical dose, because the volume of clinical dose and using logistic regression with physicochemical properties. For the 92 drugs, 80% were correctly categorized into positive, negative or no effect of food groups in the prediction. In the case of a positive effect of food, 83% were successfully classified. Although the adapted method is useful for the qualitative prediction of the effect of food on drug absorption, it may not be suitable for its quantitative prediction.

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Table 1. Oral Absorption of Drugs under the Fasted and Fed States in Humans

<table>
<thead>
<tr>
<th>Drug</th>
<th>cLog P0</th>
<th>Clinical dose (mg)</th>
<th>AUCb (ng · h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>3.1</td>
<td>400</td>
<td>1327/5280</td>
</tr>
<tr>
<td>Cilostazol</td>
<td>3.4</td>
<td>100</td>
<td>8087/10150</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>3.3</td>
<td>900</td>
<td>7881/11430</td>
</tr>
<tr>
<td>Danazol</td>
<td>2.4</td>
<td>100</td>
<td>204/639</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>6.1</td>
<td>250</td>
<td>2281/3118</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>2.9</td>
<td>500</td>
<td>7200/11400</td>
</tr>
<tr>
<td>Nateglinide</td>
<td>4.2</td>
<td>60</td>
<td>6930/6540</td>
</tr>
<tr>
<td>Quazepam</td>
<td>4.9</td>
<td>20</td>
<td>291/468</td>
</tr>
</tbody>
</table>

a) Predicted by Pallas 3.0. b) Data from refs. 1, 2, 7, 13, 15, 27—29.
fluid, FaSSIF and FeSSIF, established by Galia et al.\textsuperscript{(19)} were used in the D/P system. When various drugs were applied to the apical side of the D/P system, the permeated amounts of drugs were monitored for 2 h. We carried out a study of the dissolution and permeation of 13 drugs with the D/P system in order to obtain correlations between oral absorption in humans and the permeated amounts of drugs. Then, correlations were individually observed under fasted and fed conditions. With these correlations, the predicted positive food effect on the oral absorption of albendazole corresponded to \textit{in vivo} observations.\textsuperscript{(18)} However, our \textit{in vitro} system might not be sufficient for quantitative prediction of positive food effect.

In this study, we tried to predict the oral absorption of poorly water-soluble drugs in humans from \textit{in vitro} experiments with the D/P system. In addition, we also performed in the estimation of effect of dose strength on the oral absorption of poorly water-soluble drugs from the assay with the D/P system. Then, we compared the results for the D/P system with \textit{in vivo} observations in humans to confirm the usefulness of our \textit{in vitro} system to quantitative estimate the oral absorption of poorly water-soluble drugs.

MATERIALS AND METHODS

Materials The Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD, U.S.A.) at passage 17. Dulbecco’s modified Eagle’s medium (D-MEM) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Non-essential amino acids (NEAA), fetal bovine serum (FBS), l-glutamate, trypsin (0.25%-)-ethylene-diaminetetraacetic acid (EDTA) (1 mM) and antibiotic–antimycotic mixture (10000 U/ml penicillin G, 10000 µg/ml streptomycin sulfate and 25 µg/ml amphotericin B in 0.85% saline) were obtained from Gibco Laboratories (Lenexa, KS, U.S.A.). Egg-phosphatidylcholine (lecithin) was purchased from NOF Corp. (Tokyo, Japan). Sodium taurocholate (NaTC), bovine serum albumin (BSA) and cyclosporine A were obtained from WAKO Pure Chemical Industries, Ltd. (Osaka, Japan). Cilostazol (Pletaal® tablet), gefitinib (Iressa® tablet), nateglinide (Starlix® tablet) and quazepam (Doral® tablet) were purchased from Otsuka Pharmaceutical Co., Inc. (Tokyo, Japan), AstraZeneca Japan (Osaka, Japan), Astellas Pharma Inc. (Tokyo, Japan) and Mitsubishi Pharma Corp. (Osaka, Japan), respectively. A cellulose acetate filter (0.2 µm) was obtained from Toyo Roshi Kaisha Ltd. (Tokyo, Japan). All other reagents used were of the highest purity.

Preparation of the 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 (Nippom Becton Dickinson Co., Ltd., Tokyo, Japan) in humidified air with a 5% CO\textsubscript{2} atmosphere. Cells were harvested with trypsin-EDTA and seeded onto polycarbonate filters (3.0 µm pores, 4.20 cm\textsuperscript{2} growth area) inside a cell culture insert (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) at a density of 3×10\textsuperscript{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 d and every 24 h thereafter. After 18—21 d in culture, the Caco-2 monolayer was utilized for the following experiments.

Chambers for the Dissolution/Permeation System (D/P System) In the D/P system (Fig. 1), the Caco-2 monolayers are mounted in side-by-side chambers. The effective surface area of the Caco-2 monolayer is 1.77 cm\textsuperscript{2}. Both sides of the Caco-2 monolayer are consistently stirred at 200 rpm with magnetic stirrers. The volumes of apical and basal sides are set to 8 ml and 5.5 ml, respectively. As a buffer solution in this study, Hank’s balanced salts solution (HBSS), containing 5.36 mM KCl, 136.89 mM NaCl, 0.34 mM Na\textsubscript{2}HPO\textsubscript{4}, 0.44 mM KH\textsubscript{2}PO\textsubscript{4}, 4.17 mM NaHCO\textsubscript{3}, 1.26 mM CaCl\textsubscript{2}, 0.49 mM MgCl\textsubscript{2}, 0.41 mM MgSO\textsubscript{4} and 25 mm glucose, was used (transport medium, TM). Simulated intestinal media as the apical side of the D/P system were prepared that were based on TM with the addition of NaTC (3 mM) and lecithin (0.75 mM) for FaSSIF\textsubscript{mod} and NaTC (15 mM) and lecithin (3.75 mM) for FeSSIF\textsubscript{mod6.5}. The pH of FaSSIF\textsubscript{mod} and FeSSIF\textsubscript{mod6.5} was adjusted to 6.5 with N-(2-hydroxyethyl)piperazine-N’-2-ethanesulfonic acid (HEPES). As a basal medium, TM containing BSA (4.5 w/v%) with a pH adjusted to 7.4 with HEPES was used in the D/P system.

Dissolution and Permeation of Various Drugs in D/P System A solution (TM, pH adjusted to 6.5) and the basal medium were introduced to the apical and basal sides of the Caco-2 monolayer in the well, respectively. After preincubation for 20 min, the Caco-2 monolayer with support filter was mounted between the chambers of the D/P system. Then, the apical side of the Caco-2 monolayer was filled with the apical medium, FaSSIF\textsubscript{mod} or FeSSIF\textsubscript{mod6.5}. The basal side was filled with the basal medium in all experiments. Drugs were applied to the apical side as a bulk powder or crushed tablets. The applied amount of drugs was set to 1% of the clinical dose. Then, aliquots of samples were taken from the basal solution at intervals over 2 h. The volume of the basal solution was maintained by adding fresh medium. After completion of the experiment, the apical solution was immediately collected and filtered through a cellulose acetate filter to determine the final concentration of the dissolved drug. All apical samples (0.2 ml) were mixed with 0.2 ml of the solution consisting of 50 mM phosphate buffer (pH 2.5) and acetonitrile (50/50) to prevent the dissolved drug from precipitating in the apical solution. The transepithelial electric resistance (TEER) of the Caco-2 monolayer was checked before and after the experiment. All experiments were performed at 37°C.

Analytical Methods All basal samples (0.2 ml) were
mixed with 1.5 ml of methanol. The mixture was shaken and the supernatant (1.3 ml) was collected after centrifugation at 15000 rpm for 20 min. After removal of the solvent under vacuum at 40 °C, the residue was dissolved in 0.1 ml of the solution consisting of 50 mm phosphate buffer (pH 2.5) and acetonitrile (50/50). This solution was used for the assay.

**Cilostazol and Danazol** Cilostazol and danazol in the solution was analyzed with a reversed-phase HPLC system (LC-10A, Shimadzu Co., Kyoto, Japan). A column (J'sphere C18, 10 μm) maintained at 40 °C using a binary pump, a vacuum degasser, an autosampler, a column oven and a mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) interface (LCMS-2010A, Shimadzu Co., Kyoto, Japan). The mobile phase was composed of 0.1% formic acid in water (A) and acetonitrile (B) and the flow rate was 1.0 ml/min. Cilostazol and danazol were quantified with a variable wavelength ultraviolet detector (SPD-10A, Shimadzu Co., Kyoto, Japan) at 254 and 286 nm, respectively.

**Cyclosporine A** Cyclosporine A in the solution was analyzed with the reversed-phase HPLC system (LC-VP, Shimadzu Co., Kyoto, Japan) with a binary pump, a vacuum degasser, an autosampler, a column oven and a mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) interface (LCMS-2010A, Shimadzu Co., Kyoto, Japan). The mobile phase was composed of 0.1% formic acid in water (A) and acetonitrile (B) and the flow rate was 0.5 ml/min. Cyclosporine A was trapped and eluted from an analytical column (Mercury MS Luna 5 μm, 0.5 ml/min. Cyclosporine A was trapped and eluted from an in water (A) and acetonitrile (B) and the flow rate was 1.0 ml/min. Cyclosporine A was trapped and eluted from an in water (A) and acetonitrile (B) and the flow rate was 1.0 ml/min. Cyclosporine A was trapped and eluted from an in water (A) and acetonitrile (B) and the flow rate was 1.0 ml/min. Cyclosporine A was trapped and eluted from an in water (A) and acetonitrile (B) and the flow rate was 1.0 ml/min. Cyclosporine A was trapped and eluted from an in water (A) and acetonitrile (B) and the flow rate was 1.0 ml/min.

**Geftinib, Nateglinide and Quazepam** The concentrations of gefitinib, nateglinide and quazepam in the solution were analyzed with the reversed-phase HPLC system (LC-VP, Shimadzu Co., Kyoto, Japan) with a binary pump, a vacuum degasser, an autosampler, a column oven and a mass spectrometer equipped with an electrospray ionization (ESI) interface (LCMS-2010A, Shimadzu Co., Kyoto, Japan). Selected ion monitoring was used for detection of protonated molecules of gefitinib (m/z 447.25), nateglinide (m/z 318.30) and quazepam (m/z 388.00). Other analytical methods were the same as those used when cyclosporine A was analyzed.

**Data Analysis** All analytical data from the D/P system were expressed as percentages of applied amount (% of dose). The oral absorption (% of absorbed) of drugs in fasted and fed humans was predicted by substituting the permeated amount during 2 h (% of dose/2 h) into the following equation:

$$Abs_{\text{max}} = \frac{PA_{\text{50}} \cdot PA^\gamma}{PA_{\text{50}} + PA^\gamma}$$  \hspace{1cm} (1)

where $Abs_{\text{max}}$ is the maximum absorption (defined as 100%), $PA_{\text{50}}$ is the in vitro permeated amount in the D/P system (% of dose/2 h), $PA_{\text{50}}$ is the permeated amount, which corresponds to 50% of the absorption in vivo, and $\gamma$ is Hill’s coefficient. In the previous study, $PA_{\text{50}}$ and $\gamma$ were obtained by fitting the permeated amount (PA) of drugs in the D/P system and their oral absorption in humans and then there was a good correlation between $Fa$ and % of dose/2 h with these substitutions.\(^\text{18}\)

**RESULTS**

**Dissolution and Permeation Study** The dissolution and permeation of cilostazol, cyclosporine A, gefitinib, nateglinide and quazepam, for which the applied amounts were based on the clinical doses shown in Table 1, were measured in the D/P system with FaSSIF\(_{\text{mod}}\) and FeSSIF\(_{\text{mod.5}}\) as apical media. Figures 2 and 3 show results obtained from this study with the D/P system, together with previous results. When FeSSIF\(_{\text{mod.5}}\) was used, for all drugs, except nateglinide, the dissolved amount (% of dose/2 h) was significantly greater than that with FaSSIF\(_{\text{mod}}\). In contrast, nateglinide was com-
Completely dissolved in both apical media during 2 h. The permeated amount of drugs in the D/P system was variable (Fig. 3). The permeated amounts of albendazole, cyclosporine A, danazol and griseofulvin with FeSSIFmod6.5 were significantly greater than those with FaSSIFmod. In contrast, the permeated amounts of gefitinib, nateglinide and quazepam with FeSSIFmod6.5 were less than those with FaSSIFmod. In the case of cilostazol, no significant difference was observed in the permeated amount between FaSSIFmod and FeSSIFmod6.5.

**Prediction of Oral Absorption**

Using the *in vivo* and *in vitro* correlation (IVIVC) of oral drug absorption, we have tried to predict the oral absorption of drugs from the *in vitro* data with the D/P system. The oral absorption of all drugs in humans under the fasted and fed states was estimated with Eq. 1 using the permeated amount in the D/P system during 2 h (% of dose/2 h), and predicted absorption under the fasted and fed states is shown in Table 2. When 50, 100 and 200 mg of cilostazol were administered to fasted humans, the oral absorption (fraction of absorbed dose) was predicted to be 53%, 41% and 31%, respectively. When 100 and 200 mg of danazol were administered to fasted humans, the oral absorption was predicted to be 30% and 21%, respectively.

**Correlation between *in Vivo* Observation and *in Vitro* Prediction (IVIVC)**

As the effect of food on drugs in humans (observed FE), the ratio of the oral absorption between the fasted and fed states was used \((\frac{AUC_{fed}}{AUC_{fasted}})\). The effect of food on each drug in the D/P system (predicted FE) was evaluated on the basis of the dissolved and permeated amounts (FaSSIFmod/FeSSIFmod6.5) and predicted human absorption under the fasted and fed states (fed/fasted).

Figure 5 shows the relationship between observed and predicted effects of food on oral drug absorption. The correlation coefficients \((r^2)\) were 0.820 for dissolution results, 0.852 for permeation results and 0.924 for estimated absorption results using unweighted least-squares regression analysis. The slopes of correlation were 0.427 (dissolution results), 1.205 (permeation results) and 1.239 (estimated absorption results). The root-mean-square errors (RMSE, Eq. 2), which are predictability indicator, were 1.682 (dissolution results), 0.785 (permeation results) and 0.396 (predicted absorption results).

In order to compare *in vitro* prediction with *in vivo* observations of the effect of dose strength on oral absorption, estimated amounts of cilostazol and danazol absorption were

### Table 2. Predicted Absorption of Drugs under the Fasted and Fed States in Humans from the D/P System

<table>
<thead>
<tr>
<th>Drug</th>
<th>Fasted state</th>
<th>Fed state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>14</td>
<td>49</td>
</tr>
<tr>
<td>Cilostazol</td>
<td>41</td>
<td>69</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Danazol</td>
<td>30</td>
<td>71</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>74</td>
<td>92</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>43</td>
<td>75</td>
</tr>
<tr>
<td>Nateglinide</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>Quazepam</td>
<td>75</td>
<td>91</td>
</tr>
</tbody>
</table>

### Table 3. *In Vivo* and *In Vitro* Prediction of Oral Absorption of Cilostazol in Fasted Humans

<table>
<thead>
<tr>
<th>Clinical dose (mg)</th>
<th><em>In vivo absorption</em> (humans)</th>
<th><em>In vitro prediction</em> (D/P system)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC (µg h/l)</td>
<td>Predicted absorption (%)</td>
</tr>
<tr>
<td>50</td>
<td>4334±924</td>
<td>53</td>
</tr>
<tr>
<td>200</td>
<td>8087±2300</td>
<td>41</td>
</tr>
<tr>
<td>100</td>
<td>12943±4380</td>
<td>31</td>
</tr>
</tbody>
</table>

*a* Data from ref. 15.
calculated with the following equation:

\[
\text{estimated amount of absorption (mg)} = \frac{\text{oral absorption (\%)} \times \text{dose (mg)}}{100} \tag{3}
\]

where oral absorption is the predicted absorption by the D/P system and dose is the clinical dose. Then, the estimated amount of cilostazol absorption significantly correlated with the AUC reported in humans \((r^2 = 0.999)\). In case of danazol, when 100 and 200 mg were administered to fasted humans, the estimated amount of absorption was calculated to be 30 and 42 mg, respectively.

**DISCUSSION**

Several groups have reported on the use of dissolution in biorelevant media as a predictor of drug performance in humans under fasted and fed states.\(^{20-22}\) The effects of food on drug absorption are generally better predicted when using biorelevant media containing bile salts and lecithin, FaSSIF and FeSSIF, compared with traditional United States Pharmacopeia (USP) compendial media, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). We have reported that danazol dissolution in the D/P system corresponds to dissolution study with FaSSIF and FeSSIF, which indicates that drug dissolution in the D/P system could provide the same results as dissolution study with the USP apparatus.\(^{2,18}\)

The apical media affected drug dissolution in the D/P system. Increasing the concentrations of NaTC and lecithin enhanced the dissolved amount of most drugs used in this study. However, the dissolved amount of nateglinide in apical media was not observed to exhibit a significant difference between FaSSIF and FeSSIF because it exhibited complete dissolution in both media during 2h. Nateglinide was categorized into the low soluble group on the basis of BCS because the dose number \((D_0)\) of nateglinide was calculated to be 3.6 using the water solubility (0.066 mg/ml) and the clinical dose (60 mg). In the apical side of the D/P system, nateglinide was completely dissolved in apical media within 15 min after applying to the apical side (data not shown), indicating that the dissolution of nateglinide was not a rate limiting step. This difference between \(D_0\) and the dissolved amount must be explained their definitions. Therefore, some of poorly water-soluble drugs, for which \(D_0\) was greater than 1, could be completely dissolved in the D/P system. The ratio of the dissolved amount \((\text{FeSSIF}_\text{mod6.5}/\text{FaSSIF}_\text{mod})\) correlated with in vivo observations of the effect of food \((\text{AUC}_{\text{fed}}/\text{AUC}_{\text{fasted}})\) (Fig. 5a). However, this correlation \((r^2 = 0.820)\) was qualitative but not quantitative for the prediction of the effect of food because the RMSE \((1.682)\) of the correlation was too large and the slope of this correlation \((0.427)\) could induce overestimation. This correlation with lower slope might be caused with that poorly water-soluble (high lipophilicity) drugs were likely to be taken up into micelles formed by NaTC and lecithin which resulted in higher dissolution in the FeSSIF\_mod6.5. Thus, the dissolved amount in the D/P system could be used for estimation of whether or not the intake of food affects the oral absorption of a drug.

The relationship \((r^2 = 0.852, \text{slope} = 1.205, \text{RMSE} = 0.785)\) of the effect of food between in vivo observation and in vitro permeated amount was determined (Fig. 5b). The permeated amounts of albendazole, cyclosporine A, danazol, gefitinib, griseofulvin, nateglinide and quazepam with FeSSIF\_mod6.5 were significantly greater than those with FaSSIF\_mod. This corresponds to the effect of food observed in humans. On the other hand, the permeated amounts of other drugs with FeSSIF\_mod6.5 were equal to or less than that with FaSSIF\_mod. Apical media in the D/P system could affect not only drug dissolution, but also drug permeation. These differences in drug dissolution and permeation between FaSSIF\_mod and FeSSIF\_mod6.5 could be due to the fact that micelles formed by NaTC and lecithin enhanced drug dissolution but reduced the free concentration of drugs owing...
to taking up into micelles. The effects of micelles on drug dissolution and permeation are complicated because they depend on individual interaction between micelles and the drug. However, the permeated amount is a hybrid parameter of drug dissolution in apical media and permeation in Caco-2 monolayers, which could include the effect of micelles on dissolution and permeation of drugs individually.

In a previous study, we obtained correlation curves between human absorption and the permeated amount from the D/P system with FaSSIFmod, the dissolved solubility- and dissolution rate-limited. When cilostazol was completely in fasted humans and that its absorption could be reported that cilostazol in tablet could not be absorbed increase in the dose strength of cilostazol from 50 to 200 mg amounts of cilostazol decreased at rates slightly greater than amount was lower than 10% for all applied amounts (0.5, dissolution and permeation of drugs individually.

Singh reported correlations of \( \text{AUC} \) ratio (fed/fasted) with aqueous solubility, dose/solubility ratio and \( \log P \) that were derived and statistically evaluated by Pearson’s correlation test. A positive correlation \( (r=0.514, 0.551) \) was obtained between \( \text{AUC} \) ratio and \( \log P \) and between \( \text{AUC} \) ratio and dose/solubility ratio, whereas a negative correlation was determined between \( \text{AUC} \) ratio and the logarithms of aqueous solubility. The prediction of the effect of food with the D/P system significantly correlated with clinical observations when the oral absorption of drugs was calculated with Eq. 1 using the permeated amount \( (r^2=0.924, \text{Fig. 5c}) \), whereas the dissolved and permeated amounts became less predictive of the effect of food than the predicted absorption. These results clearly indicate that calculation of the oral absorption with \( \text{in vivo} \) results in the D/P system is advantageous to predict the effect of food on the oral absorption of poorly water-soluble drugs in humans. Additionally, the correlation of the effect of food between that observed in humans and that predicted by the D/P system \( (\text{RMSE} = 0.382) \) could be sufficient to predict the effect of food on the oral absorption of poorly water-soluble drugs in drug development.

Bramer et al. investigated the oral absorption of cilostazol administered to humans under the fasted state as various dose strengths and as ethanolic solution. In their investigations, the \( \text{AUC} \) increased at a rate less than proportional to dose strength (dose range: 50—200 mg) and the absorption after the oral administration of 50 mg of cilostazol in tablet was less than that in ethanolic solution. Jinno et al. reported that reduction of particle size of cilostazol improved oral absorption and could mask the effect of food in dogs. These reports indicate that cilostazol in tablet could not be absorbed completely in fasted humans and that its absorption could be solubility- and dissolution rate-limited. When cilostazol was applied to the D/P system with FaSSIFmod, the dissolved amount was lower than 10% for all applied amounts (0.5, 1.0, 2.0 mg). Moreover, the dissolved and permeated amounts of cilostazol decreased at rates slightly greater than proportional to the applied amount. Using the D/P system, an increase in the dose strength of cilostazol from 50 to 200 mg was predicted to induce a decrease in oral absorption in fasted humans. The estimated amounts of cilostazol absorption upon administration of 50, 100 and 200 mg were calculated to be 27, 42 and 62 mg, respectively, from the predicted absorption and the dose strength. These amounts significantly correlate with the clinical data \( (r^2=0.999) \). Additionally, we carried out a similar prediction with danazol. It was reported that the oral absorption of danazol \( (\text{AUC}) \) increased less than the dose-proportional manner. When 200 mg of danazol was administered to fasted humans, the \( \text{AUC} \) increased 1.6-fold compared to that administered 100 mg. In the D/P system, danazol absorption was predicted to decrease with the increase in the dose strength, which corresponds to \( \text{in vivo} \) observations. The oral absorption of cilostazol and danazol could be solubility- and dissolution-limited absorption because the oral absorption of both drugs was enhanced by formulation technique to improve drug solubility and dissolution. These estimations suggest that the D/P system is an advantageous tool for consideration of the dose dependency of absorption before clinical studies in drug development.

Various formulation techniques enhance the oral absorption of poorly water-soluble drugs and can mask the positive food effect. The D/P system could evaluate the effect of formulation on the human oral absorption of poorly water-soluble drugs. The effect of formulation on oral drug absorption is now under investigation in the D/P system and will be a subject for future reports.

CONCLUSION

Prediction of the human oral absorption of poorly water-soluble drugs using the D/P system significantly correlated with \( \text{in vivo} \) observations. Consequently, the D/P system was found to be a powerful tool to predict not only the effect of food on the oral absorption of poorly water-soluble drugs but also the effect of dose strength.

REFERENCES