Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutics and Biopharmaceutics 58 (2004) 659–665
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

# A possibility to predict the absorbability of poorly water-soluble drugs in humans based on rat intestinal permeability assessed by an in vitro chamber method

Etsushi Watanabe[a,*], Masayuki Takahashi[b], Masahiro Hayashi[a]

[a]*Faculty of Pharmaceutical Sciences, Science University of Tokyo, Tokyo, Japan*
[b]*Pharmaceutical Formulation Research Laboratory, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan*

Received 6 December 2002; accepted in revised form 9 March 2004

Available online 11 June 2004

**Abstract**

This paper describes a means to predict the absorbability of poorly water-soluble drugs in humans based on rat intestinal permeability assessed by the in vitro Ussing-type chamber method. We investigated the correlation between the apparent permeability coefficients ($P_{app}$) of 10 water-soluble drugs obtained by the in vitro chamber method, in which the excised rat small intestinal tissue was used as the membrane, and the fractions absorbed ($F_a$) in humans. Using this correlation, we predicted $F_a$ values of 5 poorly water-soluble drugs based on their $P_{app}$ obtained through our modified chamber method using an additive. For water-soluble drugs, a good correlation between $P_{app}$ and $F_a$, expressed by the equation: $F_a = 1 - \exp(-P_{app} \times 1.51 \times 10^5)$($r^2 = 0.920$), was found. The poorly water-soluble drugs used in the present study could be solubilized with 5% (final concentration) dimethylsulfoxide, and their $P_{app}$ could be obtained through our modified chamber method. For poorly water-soluble drugs whose dose:solubility ratio ranged from 2500 to 3500 ml, predicted $F_a$ values were favorably comparable with their $F_a$ values reported in humans in the literature. These results showed that the in vitro Ussing-type chamber method was a useful method for predicting the $F_a$ of poorly water-soluble drugs.
© 2004 Elsevier B.V. All rights reserved.

*Keywords:* Poorly water-soluble drugs; Permeability; In vitro chamber method; Intestinal tissue; Fraction absorbed

## 1. Introduction

Permeability and solubility are key underlying parameters for controlling drug absorption [1]. Therefore, in the development of the drugs given orally, those which have high solubility and high permeability have been chosen. For estimating permeability, in vitro chamber experiments using Caco-2 cell monolayers or isolated intestinal tissues are widely used. Arturrson et al. report a good correlation between the apparent permeability coefficients ($P_{app}$) of water-soluble drugs obtained in the in vitro chamber experiment using Caco-2 cell monolayers, and fraction absorbed ($F_a$) in humans [2]. Therefore, the Caco-2 system has been used not only for the research of absorption mechanisms in academic fields but also as part of the high throughput screening system (HTS) in the pharmaceutical industry [3].

New drug compounds such as those synthesized by combinatorial chemistry tend to be large molecules, and almost all of them are poorly water-soluble [3]. Therefore, the development of poorly water-soluble drugs for oral administration is increasing, and many attempts to improve solubility of poorly water-soluble drugs using the techniques/materials such as solid dispersion, melt extrusion and cyclodextrin have been reported [4–6]. However, even if the poorly water-soluble drug improves solubility using the above-mentioned techniques, the dissolution rate is generally slow and high absorbability is not expected. More recently, a technology has been developed to seal liquids into hard gelatin capsules (e.g. Licaps™ produced by the Capsugel Division of Pfizer Inc.) [7]. Since the oral dosage

* Corresponding author. Shizuoka Pharmaceutical Research Center, Daiichi Pharmaceutical Co., Ltd., 588, Kanaya-Kawara, Kanaya-cho, Haibara-gun, Shizuoka-ken 428-0021, Japan. Tel.: +81-547-45-3191; fax: +81-547-46-3426.
*E-mail address:* watanqsf@daiichipharm.co.jp (E. Watanabe).

0939-6411/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2004.03.029

form in which the drug solution is encapsulated does not require the process of drug dissolution in the gastrointestinal tract, the high permeability drug can be considered to have a high absorbability regardless of its solubility. Therefore, estimation of permeability of poorly water-soluble drugs will be more important in the near future.

However, estimation of permeability of poorly water-soluble drugs is almost never reported because they easily precipitate in the transport medium during the permeation experiment.

As an approach to overcome this problem, additives are used as solubilizing agents. Though additives prevent precipitation and maintain high drug concentrations in the transport medium during the experiment, they cannot be used for the Caco-2 system because of its tendency to be easily damaged by them [8,9]. In this respect, isolated rat intestinal tissues mounted on the chamber are not affected by the additives [10]. Therefore, this modified chamber method using an additive has the potential to be applied to poorly water-soluble drugs as well as water-soluble drugs. However, it is unknown whether $P_{app}$ of water-soluble drugs obtained through the in vitro chamber experiment using isolated rat intestinal tissues and $F_a$ in humans have a quantitative correlation, as with $P_{app}$ obtained through the Caco-2 system.

In the present study, we investigated the correlation between $P_{app}$ values of 10 water-soluble drugs obtained through the usual chamber method [10] and their $F_a$ values obtained in humans [2,11–15]. Secondly, $P_{app}$ values of 5 poorly water-soluble drugs were obtained through our modified method using an additive. Finally, the relationship between $P_{app}$ values of these poorly water-soluble drugs and their $F_a$ values previously obtained in humans [11,13,16–18] were investigated, and the relationship between $P_{app}$ and $F_a$ values for these poorly water-soluble drugs were compared with that for water-soluble drugs.

## 2. Experiment

### 2.1. Chemicals

Sulfasalazine, furosemide, atenolol, acetaminophen, propranolol, theophyline, diclofenac, metoprolol, antipyrine, naproxen, indomethacin (IDM), triamterene (TAT), nifedipine (NFD), phenytoin (PHT) and griseofulvin (GSF) were purchased from Sigma Chemical Co. Ltd. (St Louis, MO). Other chemicals used were of analytical grade.

### 2.2. Poorly water-soluble drugs

In Japanese Pharmacopoeia XIV, the degree of dissolution of the drug whose solubility in water was lower than 100 μg/ml was defined as 'practically insoluble' or 'insoluble'. Therefore, we defined a drug whose solubility in water was lower than 100 μg/ml as a poorly water-soluble drug. In the present study, we used IDM, TAT, NFD, PHT and GSF as poorly water-soluble drugs.

### 2.3. Animals

Male Wistar rats at age of 7 weeks or older (body weight about 200–250 g) were used. The rats were fasted for about 24 h with free access to water before the experiment. Necessary approvals for the experimental protocol of animals were obtained from the Ethical Committee of Science University of Tokyo (Tokyo, Japan).

### 2.4. Methods

#### 2.4.1. In vitro Ussing-type chamber method

Details have been previously reported [10,19,20]. In the present study, a jejunum segment was mounted to the Ussing-type chamber. Samples (100 μl) were withdrawn from both sides every 20 min up to 100 min. For the chamber experiment with a poorly water-soluble drug, the drug solution solubilized by 5% (final concentration) dimethylsulfoxide (DMSO) was added to the mucosal side. The initial drug concentration on the mucosal side was 0.2 mM in all cases.

#### 2.4.2. Measurement of solubility and distribution coefficients of poorly water-soluble drugs

The experimental method to obtain the apparent solubility of a poorly water-soluble drug has been previously reported [10]. The solvents used were distilled water, Japanese Pharmacopoeia XIV (JP) disintegration media 1st-fluid (JP1; pH 1.2), JP 2nd-fluid (JP2; pH6.8) and DMSO. Drug distribution coefficients [$D$] were calculated by the flask method. Two millilitres of a poorly water-soluble drug (0.5 mg/ml) in octyl alcohol was accurately pipetted into a glass tube. Then an equal volume of phosphate buffer saline (pH 6.8) was pipetted into the glass tube, and the tube was closed with a stopper. The glass tube was vigorously shaken for 30 min. The glass tube was allowed to sit in a tube stand until the two phases separated. Each layer was collected for assay. The octyl alcohol phase was diluted by pipetting 0.1 ml into 4.9 ml of the HPLC mobile phase in a clean glass tube. The drug distribution coefficient was calculated based on the difference in concentration between the two layers.

### 2.5. Assay

The concentration of the drug was determined by reversed-phase HPLC using an isocratic system (Alliance 2690, Waters Corporation, MA, USA) and a Waters 2487 Dual Absorbance Detector (Waters). Details of HPLC conditions for the determination of water-soluble drugs have been previously reported [19,20]. For the poorly

Table 1
HPLC conditions for the determination of poorly water-soluble drugs

| Drug | Column | Eluent | Wavelength of detection (nm) | Flow rate (ml/min) |
|---|---|---|---|---|
| Indomethacin | A | 1% acetic acid solution/acetonitrile = 40/60 | 240 | 1.0 |
| Triamterene | B | 20 mM diammonium hydrogen phosphate/methanol = 55/45 | 260 | 0.8 |
| Nifedipine | B | 50 mM phosphate buffer (pH 2.5)/acetonitrile = 40/60 | 240 | 1.0 |
| Phenytoin | B | 50 mM phosphate buffer (pH 2.5)/acetonitrile = 55/45 | 210 | 1.0 |
| Griseofulvin | A | 50 mM phosphate buffer (pH 2.5)/acetonitrile = 50/50 | 280 | 0.8 |

A: Symmetry $C_{18}$; i.d., 4.6 mm; length, 150 mm; octadecylsilarized silica gel column (3.5 μm in particle diameter), Waters Corporation, MA, USA. B: L-column ODS; i.d., 4.6 mm; length, 150 mm; octadecylsilarized silica gel column (5 μm in particle diameter), Chemicals Evaluation and Research Institute, Japan. Column temperature was 40 °C.

water-soluble drugs, the experimental conditions of the various HPLC methods are outlined in Table 1.

### 2.6. Data analysis

#### 2.6.1. Calculation of the apparent permeability coefficient

The apparent permeability coefficient per unit membrane surface area ($P_{app}$ (cm/s)) was calculated according to the following equation:

$$P_{app} = dM/dt \times 1/AC_0 \quad (1)$$

where $dM/dt$ was the steady-state appearance rate of drugs to the serosal side (μmol/s), $C_0$ was the initial drug concentration on the mucosal side (mM), and $A$ was the surface area of the membrane ($cm^2$) [10,19,20].

#### 2.6.2. Estimation of the fraction absorbed in humans

$F_a$ values of 10 water-soluble drugs and 5 poorly water-soluble drugs were taken from the literature [2,11–18].

#### 2.6.3. Calculation of parameters to indicate the relationship between the apparent permeability coefficient and fraction absorbed in humans

The relationship between the in vitro permeability of water-soluble drugs in rats and in vivo absorption in humans was investigated using complete radial mixing model (CRM) [12,21,22]. The numerical expression which indicated a relationship between $P_{app}$ (cm/s) of water-soluble drugs and the fraction absorbed in humans ($F_a$) was described by:

$$F_a = 1 - \exp(-P_{app} \times f) \quad (2)$$

The relationship between $P_{app}$ and $F_a$, which was expressed as the correction factor $f$ was determined by non-linear regression (Levenberg–Marquardt least squares algorithm; Delta Graph 4.0(r) by SPSS Inc., USA).

#### 2.6.4. Calculation of dose:solubility ratio of poorly water-soluble drugs

The dose:solubility ratio ($R_{D/S}$) was calculated as proposed by Dressman et al. [23]. $R_{D/S}$ was described by the following equation:

$$R_{D/S} = M_0/C_s \quad (3)$$

where $M_0$ (mg) was the dose, and $C_s$ (mg/ml) was the luminal solubility. In the present study, the solubility in JP1 or JP2 was substituted for $C_s$. The $M_0$ of poorly water-soluble drugs was taken from the literature [11,13,16–18].

## 3. Results

### 3.1. Estimation of relationship between the in vitro permeability and in vivo absorption of water-soluble drugs

Table 2 shows $P_{app}$ values of 10 water-soluble drugs and their $F_a$ values. $P_{app}$ values ranged from $3 \times 10^{-6}$ to $30 \times 10^{-6}$ cm/s, and $F_a$ values from 0.1 to 1.0. The relationship between $P_{app}$ values and $F_a$ values is shown in Fig. 1. The best fit for the data ($r^2 = 0.920$) was observed when $f$ was $1.51 \times 10^5$. Consequently, $F_a$ could be predicted from $1 - \exp(-P_{app} \times 1.51 \times 10^5)$.

### 3.2. Solubility and distribution coefficients of poorly water-soluble drugs

Table 3 shows solubility and distribution coefficients of 5 poorly water-soluble drugs. The molecular weights of the poorly water-soluble drugs used in the present study were

Table 2
The apparent permeability coefficients ($P_{app}$) of 10 water-soluble drugs obtained through the usual in vitro Ussing-type chamber method, and their fractions absorbed ($F_a$) in humans

| Water-soluble drugs | $P_{app}$, mean ± SD ($10^{-6}$ cm/s) | $F_a$ [a] |
|---|---|---|
| Sulfasalazine | 2.76 ± 0.19 | 0.13 [11] |
| Furosemide | 6.06 ± 0.74 | 0.60 [12] |
| Atenolol | 6.95 ± 3.07 | 0.50 [12] |
| Acetaminophen | 8.67 ± 0.01 | 0.80 [13] |
| Propranolol | 11.80 ± 3.51 | 0.90 [2] |
| Theophyline | 12.82 ± 0.14 | 1.00 [14] |
| Diclofenac | 14.26 ± 2.11 | 0.99 [15] |
| Metoprolol | 23.99 ± 1.98 | 0.95 [12] |
| Antipyrine | 24.19 ± 1.99 | 1.00 [12] |
| Naproxen | 30.13 ± 3.15 | 1.00 [12] |

[a] $F_a$ values were quoted from references shown in square brackets.

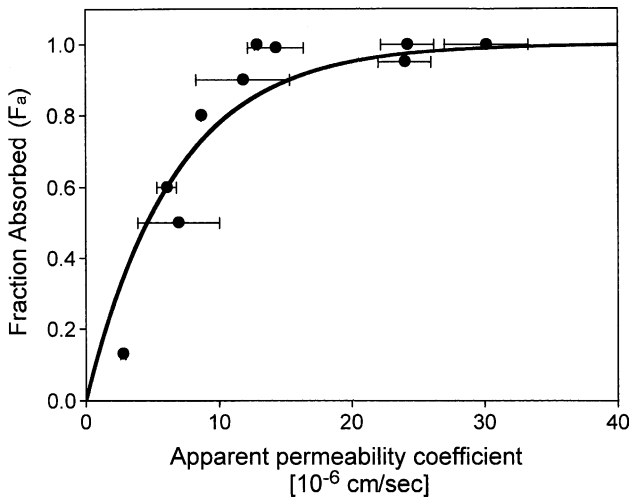


Fig. 1. Plot of the fraction absorbed in humans versus the apparent permeability coefficients ($P_{app}$) of 10 water-soluble drugs. The fraction absorbed was adapted from the data in Refs. [2,11–15]. The theoretical line (solid line) was calculated using the data of the 10 water-soluble drugs in Table 2 by non-linear regression as described in the text. $P_{app}$ values represent the mean of the data from more than three experiments.

between 250 and 360. Their solubility in distilled water, JP1 and JP2 was below 0.2 mM, except for solubility of IDM in JP2. They showed good solubility in DMSO. The log $D$ values were more than 1.0 in all the 5 poorly water-soluble drugs (from the lowest to the highest, IDM, TAT, GSF, PHT, and NFD).

### 3.3. Permeability of poorly water-soluble drugs

$P_{app}$ values for 5 poorly water-soluble drugs were obtained through the modified method using an additive. During the experiment, each poorly water-soluble drug did not precipitate out in aqueous media. Additionally, the drug concentration on the serosal side of the poorly water-soluble drugs used in the present study after 100-min transportation was less than 10% of the solubility in JP2 (data not shown). Since pH value of the transport medium was similar to that of JP2, the drugs transported to the serosal side were expected to be dissolved completely. Table 4 shows $P_{app}$ values and $F_a$ values. TAT had the lowest $P_{app}$ value, followed by IDM, NFD, PHT, and GSF. Though a drug with high log $D$ value was expected to have a high permeability according to the pH partition theory, there was no parallelism with the order of log $D$ values (Table 3). Winiwarter et al. have also reported that $P_{app}$ of water-soluble drugs does not necessarily correlate to physicochemical properties of a drug such as log $D$ and the polar surface area [24]. This indicates that it is necessary to obtain $P_{app}$ of a drug through an appropriate experiment to evaluate its membrane permeability.

### 3.4. The relationship between the $P_{app}$ of 5 poorly water-soluble drugs and their $F_a$ in humans

The relationship between $P_{app}$ of 5 poorly water-soluble drugs and their $F_a$ is shown in Fig. 2. The correlation curve

Table 3
Solubility and distribution coefficients of poorly water-soluble drugs

| Drug | Solvent | Triamterene (TAT) | Indomethacin (IDM) | Nifedipine (NFD) | Phenytoin (PHT) | Griseofulvin (GSF) |
|---|---|---|---|---|---|---|
| Molecular weight | | 253.27 | 357.79 | 346.34 | 252.27 | 352.77 |
| Solubility[a] μg/ml (mM) | Dist. water | 27.7 (0.11) | 21.8 (0.061) | 3.1 (0.090) | 20.4 (0.081) | 8.7 (0.025) |
| | JP1 | 29.5 (0.12) | <0.1 (<0.00028) | 4.3 (0.012) | 18.8 (0.075) | 8.4 (0.024) |
| | JP2 | 31.2 (0.12) | 218.2 (0.61) | 4.0 (0.012) | 19.1 (0.076) | 7.6 (0.022) |
| | DMSO | >2000 (>7.90) | >2000 (>5.59) | >2000 (>5.77) | >2000 (>7.93) | >2000 (>5.67) |
| log $D_{6.8}$ | Octanol/PBS (pH 6.8) | 1.55 | 1.01 | 3.19 | 2.33 | 2.17 |

Values represent the means of three experiments.
[a] The solubility is determined based on the final concentration obtained in our system.

Table 4
Apparent permeability coefficients ($P_{app}$) of poorly water-soluble drugs, and predicted fractions absorbed ($F_a$) in humans

| Poorly water-soluble drugs[a] | $P_{app}$, mean ± SD[b] ($10^{-6}$ cm/s) | Predicted $F_a$[c] | Previously reported $F_a$ in humans[d] |
|---|---|---|---|
| Triamterene | 5.11 ± 1.91 | 0.467 | 0.40–0.70 [16] |
| Indomethacin | 9.04 ± 1.35 | 0.800 | 1.00 [11] |
| Nifedipine | 15.12 ± 3.19 | 0.956 | 0.90–1.00 [17] |
| Phenytoin | 22.01 ± 2.26 | 0.992 | 1.00 [13] |
| Griseofulvin | 22.12 ± 2.32 | 0.992 | 0.27–0.72 [18] |

[a] Solubilized by 5% (final concentration) dimethylsulfoxide.
[b] Obtained by the modified chamber method.
[c] Predicted based on the relationship in Fig. 1.
[d] Quoted from references shown in square brackets.

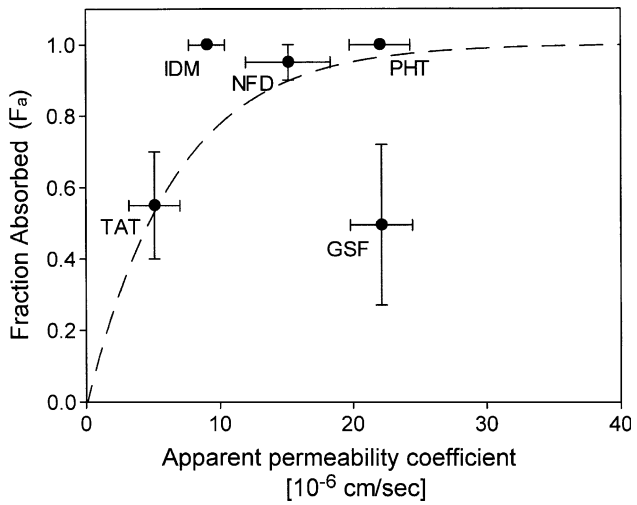


Fig. 2. Plot of the fraction absorbed in humans versus the apparent permeability coefficients ($P_{app}$) of 5 poorly water-soluble drugs. The fraction absorbed was adapted from the data in Refs. [11,13,16–18]. The drugs were solubilized in 5% (final concentration) DMSO. The dashed line was the theoretical line for water-soluble drugs as described in Fig. 1. $P_{app}$ values represent the mean of the data from more than three experiments.

of water-soluble drugs was overlaid with Fig. 2. It is reported by Pruitt et al. [16] that the absorption rate is calculated to be 40–70% when 100 mg of [$^{14}$C]-labeled TAT is administered to humans. Additionally, Gundert et al. [25] report that the areas under the curve (AUCs) normalized by dose (AUCs/dose) after oral administration of 150 and 300 mg TAT are nearly equal. Since this report suggests that TAT is completely dissolved in the gastrointestinal tract and TAT concentration does not exceed its solubility at doses below 300 mg, $F_a$ value of TAT in humans is considered to be their absorption rate. Human $F_a$ values of TAT, NFD and GSF reported in literature vary to some extent. Therefore, when comparing with the predicted $F_a$ values, the median of reported human $F_a$ values was considered to be 'human $F_a$ values' in this study.

$F_a$ values of TAT, NFD and PHT predicted using the correlation curve of water-soluble drugs were favorably comparable with $F_a$ values previously reported in humans. Predicted $F_a$ values of IDM and GSF were lower and higher, respectively, than $F_a$ values previously reported in humans.

### *3.5. Calculation of dose:solubility ratio of poorly water-soluble drugs*

Table 5 shows $R_{D/S}$ of 5 poorly water-soluble drugs. In the drugs except IDM, $R_{D/S}$ values estimated by JP1 did not differ from those estimated by JP2. $R_{D/S}$ values of TAT, NFD and PHT ranged from 2500 to 3500 ml. $R_{D/S}$ value of GSF was approximately 60,000 ml. Though $R_{D/S}$ value of IDM estimated by JP1 was more than 250,000 ml, that estimated by JP2 was about 100 ml.

## 4. Discussion

As shown in Fig. 1, the compounds that had the experimentally derived permeability of greater than approximately $25 \times 10^{-6}$ cm/s were descriptive of 'well-absorbed compounds'. In the Caco-2 system, Stewert et al. define the compounds having a permeability of greater than $33 \times 10^{-6}$ cm/s as well-absorbed compounds [13]. Since the slope based on the relationship between $P_{app}$ and $F_a$ (range 0–1.0) in the present study was much the same as the slope in the study using the Caco-2 system, in vitro Ussing-type chamber method could be justifiably used for prediction of the fraction absorbed of water-soluble drugs in humans, as well as Caco-2 system.

Since poorly water-soluble drugs might easily precipitate out in aqueous media such as the transport medium for the chamber experiment, calculating their $P_{app}$ values was complicated. Generally, in the analysis of results of the chamber experiment, Eq. (1) based on Fick's first law is used assuming the existence of 'sink' condition. Fick's first law is described by the equation of $dM/dt = P_{app} \times A \times (C_{mucosal} - C_{serosal})$, where $dM/dt$ is the appearance rate on the serosal side of the chamber, $P_{app}$ is the apparent permeability coefficient, $A$ is the exposed surface area of the tissue, $C_{mucosal}$ is the drug concentration on the mucosal side, and $C_{serosal}$ is the drug concentration on the serosal side. When $C_{mucosal}$ is kept constant and equal to the initial concentration and is much higher than $C_{serosal}$ during experiment, 'sink' conditions exist. However, it is difficult for poorly water-soluble drugs to keep $C_{mucosal}$ to be a high concentration. Therefore, Eq. (1) was not applied to the calculation of $P_{app}$ values of poorly water-soluble drugs.

Table 5
Calculated dose:solubility ratio ($R_{D/S}$) in poorly water-soluble drugs

| Poorly water-soluble drugs | Dose (mg) | $C_S^{JP1}$ (μg/ml)[a] | $C_S^{JP2}$ (μg/ml)[a] | $R_{D/S}$ (ml) (JP1) | $R_{D/S}$ (ml) (JP2) |
|---|---|---|---|---|---|
| Triamterene | 100 | 29.5 | 31.2 | 3389.8 | 3205.1 |
| Indomethacin | 25 | <0.1 | 218.2 | >25,0000 | 114.6 |
| Nifedipine | 10 | 4.3 | 4.0 | 2325.6 | 2500 |
| Phenytoin | 100 | 18.8 | 19.1 | 5319.1 | 5235.6 |
| Griseofulvin | 500 | 8.4 | 7.6 | 59,523.8 | 65,789.5 |

[a] The soulubility in JP1 or JP2 (see Table 3) were used as the luminal solubility.

DMSO and polyethylene glycol 600 (PEG600) were used as a vehicle for the administration of poorly water-soluble drugs. We had previously reported that 5% (final concentration) DMSO and 10% (final concentration) PEG 600 did not affect the membrane integrity or the permeability of compounds which permeated through the intercellular and intracellular pathways [10]. Therefore, poorly water-soluble drugs as well as water-soluble drugs could be estimated using these additives. In the present study, 5% (final concentration) DMSO was used as an additive. During the experiment, each poorly water-soluble drug did not precipitate out in the transport medium. Additionally, the drug concentration on the serosal side for the poorly water-soluble drugs used in the present study after 100-min transportation was less than 10% of the solubility in JP2 (data not shown). Since the pH value of the transport medium was similar to that of JP2, the drugs transported to the serosal side were expected to be dissolved completely. Therefore, it was considered that measurement of the transported drugs was precise.

Among 5 poorly water-soluble drugs, GSF and IDM did not conform to the relationship between $P_{app}$ and $F_a$ of water-soluble drugs. Predicted $F_a$ of GSF was higher than $F_a$ previously reported in humans. We had expected that $F_a$ of GSF was low because of high $R_{D/S}$. $R_{D/S}$ indicates whether the capacity of the GI fluids is sufficient to dissolve the entire dose administered [26]. Based on the volumes of the upper small intestine in the fed state (as much as 1.5 l [27]), the secretion rate of gastric juice (180 ml/h) and intestinal juice secretion (125 ml/h) in humans [28], the drug in the GI tract 1 h after oral administration was assumed to be diluted with 2 l of GI fluids. $R_{D/S}$ values of TAT, NFD and PHT were within the limits of 4 l. Since TAT, NFD and PHT would be nearly completely solubilized in the gastrointestinal tract, $F_a$ value predicted based on the correlation with water-soluble drugs was favorably comparable with $F_a$ values previously reported in humans.

Though GSF had the highest $P_{app}$ value of the 5 poorly water-soluble drugs, its estimated $F_a$ value in humans was 0.5. Since $R_{D/S}$ value was 30 times as much as the volume of GI fluids, this phenomenon was thought to be caused by incomplete solubilization of GSF in the gastrointestinal tract. Gramatté et al. performed an in vivo perfusion experiment using the human intestine, and suggested that an $F_a$ value of GSF is 1.0 if it is completely dissolved in the intestine [29]. Dressman et al. also report that $F_a$ value of completely dissolved GSF is 1.0 in their simulation study [26]. Additionally, it is frequently reported that the solubility of poorly water-soluble drugs is pharmaceutically improved in recent years [4–6]. Furthermore, the technique of sealing liquids into hard gelatin capsules, which enables the dissolution process to be omitted, has been developed [7]. Therefore, because of its high membrane permeability, GSF might be developed as a 100%-absorbable oral dosage formulations in the near future.

Though $R_{D/S}$ value of IDM estimated by JP1 was more than 250,000 ml, that estimated by JP2 was about 100 ml. This result suggested that IDM had low solubility in the stomach, but it was nearly completely solubilized in the intestinal lumen. $F_a$ value of IDM predicted based on its $P_{app}$ value was 0.80 in the present study, but its $F_a$ value previously reported in humans was 1.0. It is well known that IDM undergoes enterohepatic circulation [30]. Therefore, high $F_a$ value in humans may be ascribable not only to passive diffusion but also to some other factors.

In conclusion, we have established a new system for predicting drug permeability of poorly water-soluble drugs. This system was easy to operate and likely to give reproducible data, and therefore could be widely applied to the study of drug permeability. For poorly water-soluble drugs whose dose:solubility ratio ranged from 2500 to 3500 ml, the relationship between $P_{app}$ and $F_a$ values were found to be equivalent to that for water-soluble drugs. These results suggest that the in vitro Ussing-type chamber method is useful for predicting $F_a$ value of poorly water-soluble drugs.

## Acknowledgements

The authors would like to thank Mr Noriyuki Kinoshita and Miss Maho Kojima for their technical assistance.

## References

[1] G.L. Amidon, H. Lennernas, V.P. Shah, J.R. Crison, A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability, Pharm. Res. 12 (1995) 413–420.
[2] P. Artursson, J. Karlsson, Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells, Biochem. Biophys. Res. Commun. 175 (1991) 880–885.
[3] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 23 (1997) 3–25.
[4] S. Hulsmann, T. Backensfeld, S. Keitel, R. Bodmeier, Melt extrusion—an alternative method for enhancing the dissolution rate of 17beta-estradiol hemihydrate, Eur. J. Pharm. Biopharm. 49 (2000) 237–242.
[5] M.S. Nagarsenker, R.N. Meshram, G. Ramprakash, Solid dispersion of hydroxypropyl beta-cyclodextrin and ketorolac: enhancement of in-vitro dissolution rates, improvement in anti-inflammatory activity and reduction in ulcerogenicity in rats, J. Pharm. Pharmacol. 52 (2000) 949–956.
[6] T. Tanino, T. Ogiso, M. Iwaki, Effect of sugar-modified beta-cyclodextrins on dissolution and absorption characteristics of phenytoin, Biol. Pharm. Bull. 22 (1999) 298–304.
[7] E.T. Cole, Modified-release Drug Delivery Technology, Drugs and the pharmaceutical Science, Marcel Dekker, New York, 2002, pp. 177–190.
[8] M. Sakai, T. Imai, H. Ohtake, M. Otagiri, Cytotoxicity of absorption enhancers in Caco-2 cell monolayers, J. Pharm. Pharmacol. 50 (1998) 1101–1108.

[9] S. Yamashita, T. Furubayashi, M. Kataoka, T. Sakane, H. Sezaki, H. Tokuda, Optimized conditions for prediction of intestinal drug permeability using Caco-2 cells, Eur. J. Pharm. Sci. 10 (2000) 195–204.
[10] E. Watanabe, R. Sudo, M. Takahashi, M. Hayashi, Evaluation of absorbability of poorly water-soluble drugs: validity of the use of additives, Biol. Pharm. Bull. 23 (2000) 838–843.
[11] M. Yazdanian, S.L. Glynn, J.L. Wright, A. Hawi, Correlating partitioning and caco-2 cell permeability of structurally diverse small molecular weight compounds, Pharm. Res. 15 (1998) 1490–1494.
[12] U. Fagerholm, M. Johansson, H. Lennernäs, Comparison between permeability coefficients in rat and human jejunum, Pharm. Res. 13 (1996) 1336–1342.
[13] B.H. Stewart, O.H. Chan, R.H. Lu, E.L. Reyner, H.L. Schmid, H.W. Hamilton, B.A. Steinbaugh, M.D. Taylor, Comparison of intestinal permeabilities determined in multiple in vitro and in situ models: relationship to absorption in humans, Pharm. Res. 12 (1995) 693–699.
[14] L. Hendeles, M. Weinberger, L. Bighley, Absolute bioavailability of oral theophylline, Am. J. Hosp. Pharm. 34 (1977) 525–527.
[15] K. Balon, B.U. Riebesehl, B.W. Müller, Drug liposome partitioning as a tool for the prediction of human passive intestinal absorption, Pharm. Res. 16 (1999) 882–888.
[16] A.W. Pruitt, J.S. Winkel, P.G. Dayton, Variations in the fate of triamterene, Clin. Pharmacol. Ther. 21 (1977) 610–619.
[17] J.S. Grundy, L.A. Eliot, R.T. Foster, Extrahepatic first-pass metabolism of nifedipine in the rat, Biopharm. Drug Dispos. 18 (1997) 509–522.
[18] M. Develoux, Griseofulvine, Ann. Dermatol. Venereol. 128 (2001) 1317–1325.
[19] H. Lennernäs, S. Nylander, A.L. Ungell, Jejunal permeability: a comparison between the Ussing chamber technique and the single-pass perfusion in humans, Pharm. Res. 14 (1997) 667–671.
[20] A.L. Ungell, S. Nylander, S. Bergstrand, Å. Sjőberg, H. Lennernäs, Membrane transport of drugs in different regions of the intestinal tract of the rat, J. Pharm. Sci. 87 (1998) 360–366.
[21] P.J. Sinko, G.D. Leesman, G.L. Amidon, Predicting fraction dose absorbed in humans using a macroscopic mass balance approach, Pharm. Res. 8 (1991) 979–988.
[22] D.M. Oh, R.L. Curl, G.L. Amidon, Estimating the fraction dose absorbed from suspensions of poorly soluble compounds in humans: a mathematical model, Pharm. Res. 10 (1993) 264–270.
[23] J.B. Dressman, C. Reppas, In vitro–in vivo correlations for lipophilic, poorly water-soluble drugs, Eur. J. Pharm. Sci. 11 (2000) 73–80.
[24] S. Winiwarter, N.M. Bonham, F. Ax, A. Hallberg, H. Lennernas, A. Karlen, Correlation of human jejunal permeability (in vivo) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach, J. Med. Chem. 41 (1998) 4939–4949.
[25] R.U. Gundert, K.D. Von, E. Weber, H.E. Geissler, B. Grebian, E. Mutschler, Plasma and urinary levels of triamterene and certain metabolites after oral administration to man, Eur. J. Clin. Pharmacol. 16 (1979) 39–44.
[26] J.B. Dressman, D. Fleisher, Mixing-tank model for predicting dissolution rate control or oral absorption, J. Pharm. Sci. 75 (1986) 109–116.
[27] J.S. Fordtran, T.W. Locklear, Ionic constituents and osmolality of gastric and small-intestinal fluids after eating, Am. J. Dig. Dis. 11 (1966) 503–521.
[28] T. Hiroshige, M. Kato, Elementary Physiology (in Japanese), Nanzando Company Ltd., Section 6.
[29] T. Gramatté, Griseofulvin absorption from different sites in the human small intestine, Biopharm. Drug Dispos. 15 (1994) 747–759.
[30] B. Terhaag, U. Hermann, Biliary elimination of indomethacin in man, Eur. J. Clin. Pharmacol. 29 (1986) 691–695.