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Common solubilizers to estimate the Caco-2 transport of poorly water-soluble drugs

Yutaka Takahashi *, Hiromu Kondo, Tatsuo Yasuda, Takashi Watanabe, Shin-Ichiro Kobayashi, Shigeharu Yokohama

Novel Pharmaceutical Laboratories, Institute for Drug Development Research, Yamanouchi Pharmaceutical Company Limited, 180 Ozumi, Yaizu-shi, Shizuoka 425-0072, Japan

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Abstract

Solubilizers are often used to enhance the bioavailability of drugs with poor aqueous solubility. This study focuses on the use of the Caco-2 system containing solubilizers to predict the absorption of poorly water-soluble drugs in humans. First, the effects of propylene glycol (PG), hydroxypropyl-β-cyclodextrin (HP-β-CD), polyethylene glycol 400 (PEG 400), and Tween 80 on the viability (transepithelial electrical resistance, TEER) of 3-day cultured Caco-2 monolayers were evaluated. These solubilizers, even at the low concentration, reduce the viability of Caco-2 monolayers; these results indicate the impossibility for 3-day cultured Caco-2 monolayers to be used for this test. Next, the effects of PG, Tween 80, PEG 400, HP-β-CD, Pluronic F-68 (Pluronic), HCO-40, sodium lauryl sulfate (SLS), Gelucire 44/14, Transcutol P, and extract gall powder on the viability of 21-day cultured Caco-2 monolayers and the apparent permeability (P_{app}) of propranolol (PPL), Nadolol (NDL), and FITC-dextran 4000 (FD-4) were investigated. Five different solubilizing methods (20% PG, 5% Tween 80, 5% PEG 400, 5% HP-β-CD, and 5% Tween 80+5% PEG 400) did not affect the viability of 21-day cultured Caco-2 monolayers. Furthermore, the P_{app} values of the three compounds containing these solubilizers did not differ from the values for control formulations (without solubilizers). These results clearly suggest that the use of PG, Tween 80, PEG 400, or HP-β-CD as solubilizing excipients and the testing of these formulations on 21-day cultured Caco-2 monolayers can predict intestinal absorption of poorly water-soluble drugs in humans.

Keywords: Caco-2; Membrane permeability; Intestinal absorption; Poorly water-soluble compounds; Solubilizers

1. Introduction

Drug absorption of orally administered drugs, a major determinant of bioavailability, is mainly determined by a drug's membrane permeability and its solubility in the intestinal lumen. During the development of a drug, several methods are commonly used to estimate in vivo intestinal drug

* Corresponding author. Tel.: +81-54-627-5155; fax: +81-54-621-0106

E-mail address: takaha_y@yamanouchi.co.jp (Y. Takahashi).

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permeability; these methods include in vivo experiments such as oral administration to animals; in situ experiments such as intestinal perfusion or construction of intestinal loops; and in vitro experiments such as the use of cultured cells or artificial membranes. The appropriate experimental method is usually chosen to match where a drug is in the process of development. Consequently, although there is no best method, better techniques have been sought to estimate in vivo absorption earlier in the development process. Such a test would save time and money, and possibly quicken the development of life-saving therapies.

Recently, an in vitro method that uses human colon carcinoma Caco-2 cells to estimate in vivo drug absorption has emerged as a leading method to test potential drugs in the early development stages. Caco-2 cells retain many features of small intestinal cells (Hidalgo and Borchardt, 1990a,b; Hilgers et al., 1990; Dantzig and Bergin, 1990; Chen et al., 1994). Therefore, the use of Caco-2 cell monolayers has been widely adopted for the rapid screening of intestinal drug absorption. Several groups have reported a good correlation between drug permeability measured in 21-day cultured Caco-2 monolayers and the oral absorption of the same drug in humans (Artursson and Karlsson, 1991; Yee, 1997; Yamashita et al., 1997). Furthermore, similar correlations have been obtained from 3-day cultured Caco-2 monolayers (Chong et al., 1997). However, these results were obtained, for the most part, from compounds with good water solubility. In contrast, many new drugs and compounds just entering development have high lipophilicity and poor water solubility (Lipinski et al., 1997). Accordingly, one of the biggest problems that prevent wider use of the Caco-2 system is the poor water solubility of many compounds.

Nonetheless, several groups have recently tried to use the in vitro Caco-2 system to evaluate the permeability of lipophilic compounds. It has been shown that it is possible to use several additives, such as dimethylsulfoxide, in the Ussing chamber method (Watanabe et al., 2000). It has also been investigated whether several solubilizing excipients effect on the transmembrane transport of poorly water-soluble NCEs (new chemical entities) into Caco-2 monolayers (Saha and Kou, 2000). Additionally, efforts have been made to characterize the effects of bile acids, commonly used to solubilize drugs, on the permeability and transepithelial electrical resistance (TEER) of Caco-2 monolayers; their results showed that taurocholic or cholic acid at a concentration of 10 mM can be used to evaluate the absorption of poorly water-soluble drugs (Yamashita et al., 2000). Furthermore, it has shown that bovine serum albumin (BSA) applied to the basolateral side of Caco-2 monolayers helps to prevent the non-specific binding of highly lipophilic compounds (Krishna et al., 2001).

Since, with few exceptions, drugs are transported over the intestinal epithelium by passive diffusion (Artursson, 1990), we have tried to optimize the Caco-2 system evaluating the intestinal permeability of poorly water-soluble drugs, which permeate via passive diffusion mechanism. For the objective, in this study, we examined not only the effects of several solubilizers on TEER of Caco-2 monolayers and on paracellular drug transports, but also the effect on transcellular drug transports which have different in vivo absorption. Furthermore, we evaluated this system from the aspect of culture conditions. The previous literature contained a few studies to evaluate the effects of commonly used solubilizers on the performance and reliability of this system. Therefore, we focused on testing many more solubilizers than have been previously reported.

2. Materials and methods

2.1. Materials

Caco-2 cells were obtained from Dainippon Pharmaceutical Co. (Osaka, Japan). Dulbecco's modified Eagle medium (DMEM), non-essential amino acids (NEAA), fetal bovine serum (FBS), l-glutamate, 0.25% (w/v) trypsin–1 mM EDTA, and an antibiotic–antimycotic mixture (10,000 U/ml penicillin G, 10,000 μg/ml streptomycin sulfate, and 25 μg/ml amphotericin B in 0.85% w/v saline) were purchased from Life Technologies, Inc. (Rockville, MD). The BIOCOAT® HTS Caco-2

Assay System and HTS Multiwell™ inserts were purchased from Nippon Becton Dickinson Co. (Tokyo, Japan). Nadolol (NDL), FITC-dextran 4000 (FD-4), lucifer yellow (LY), and BSA were purchased from Sigma Chemical Co. (St. Louis, MO). Propranolol (PPL) and dexamethasone (DMS) were purchased from Wako Pure Chemicals (Osaka, Japan). YM-X was provided by the Chemical Research Division, Yamanouchi Pharmaceutical Co. (Ibaraki, Japan). Propylene glycol (PG), Tween 80, and polyethylene glycol 400 (PEG 400) were of JP or NF/USP grade and were purchased from Kanto Chemicals (Tokyo, Japan). Pluronic F-68 (Pluronic) was obtained from Asahi Denka Kogyo Co. (Tokyo, Japan) and HCO-40 was obtained from Nikko Chemicals (Tokyo, Japan). Sodium lauryl sulfate (SLS) was purchased from Katayama Chemical, Inc. (Osaka, Japan). Hydroxypropyl-β-cyclodextrin (HP-β-CD) was purchased from Nippon Syokuhin Kako Co. (Tokyo, Japan). Extract gall powder was purchased from Sigma Aldrich Japan (Tokyo, Japan). Gelucire 44/14 and Transcutol P were provided by Gattefosse Co. (Westwood, NJ). Other reagents were of analytical grade.

2.2. Cell culture

Caco-2 cells were grown as described previously (Hidalgo et al., 1989). Briefly, Caco-2 cells were grown in DMEM with 4.5 g/l D-glucose supplemented with 10% (v/v) FBS, 1% (v/v) L-glutamate, 1% (v/v) NEAA, and 1% (v/v) of the antibiotic–antimycotic mixture, and were grown in a humidified incubator at 37 °C in an atmosphere of 95% air–5% CO_2. Culture medium was changed every 48 h.

2.3. Preparation of 3-day cultured Caco-2 monolayers

Caco-2 cells at passage numbers 58–78 were harvested with the trypsin–EDTA solution and seeded onto fibrillar collagen-coated HTS Multiwell™ inserts (1 μm pore size, 0.31 cm^2 area) at a density of 2×10^5 cells/insert in Basal Seeding Medium containing MITO+™ Serum Extender. The cells were then incubated for 24 h. Medium was then changed to Differentiation Medium containing MITO+™ Serum Extender and the cells were incubated for 48 h more. After that time, medium was removed to prepare for subsequent drug transport experiments.

2.4. Preparation of 21-day cultured Caco-2 monolayers

Caco-2 cells at passage numbers 58–78 were harvested with the trypsin–EDTA solution and seeded onto HTS Multiwell™ inserts (1 μm pore size, 0.31 cm^2 area) at a density of 1×10^5 cells/insert. Culture medium (0.5 ml in the insert and 1.5 ml in the well) was replaced 3 days after initial plating and every 48 h thereafter. After 21–25 days in culture, the Caco-2 monolayers were used for drug transport experiments.

2.5. Evaluation of Caco-2 monolayer viability

The EVOM™ epithelial voltohmeter (World Precision Instruments, Sarasota, FL) was used to check the transepithelial electric resistance (TEER) of Caco-2 monolayers before and after 1 h of incubation at 37 °C with a solubilizer applied to the apical side of monolayers at 37 °C. If the TEER value for monolayers after treatment with a given concentration of solubilizer showed no significant ($P > 0.05$) decrease from the TEER value in the absence of solubilizers (control), that concentration of solubilizer was considered to have no effects on the viability of Caco-2 monolayers.

2.6. Drug transport experiments

Hank's balanced salts solution (HBSS) supplemented with 20 mM glucose was used in all experiments, after adjusting the pH to 6.0 with 20 mM MES for the apical side and the pH to 7.4 with 20 mM HEPES for the basolateral side. After making solubilizer solution at concentrations that did not affect the TEER of monolayers in the apical incubation solution, three model compounds with different in vivo permeabilities (PPL: high, NDL: intermediate, FD-4: low) were separately dissolved in the solubilizer solution. The

permeability of these drug solutions through Caco-2 monolayers was measured and compared with the permeability of the control solution. Transport was initiated by adding sufficient drug-solubilized solution to yield a final drug concentration of 300 μM to the apical chamber of inserts bathed with 1.5 ml basolateral solution. Samples (100 μl) were withdrawn from the basolateral side at 15 min and 1 h after administration. The volume of the basolateral solution was maintained by adding fresh medium. All experiments were performed at 37 °C. The apical-to-basolateral permeability (apparent permeability coefficient, P_{app}) of each drug was calculated according to the following equation (Mainprize and Grady, 1998):

$$P_{app}\ (\mathrm{cm/s}) = \frac{dQ}{dt}\left(\frac{1}{C_0 A}\right),$$

where dQ/dt is the rate of appearance of drugs on the basolateral side (mg/s), C_0 is the initial drug concentration on the apical side (mg/ml), and A is the surface area of the monolayers (cm^2).

A check of the LY permeability and the monolayer TEER was performed at each experiment to confirm that the integrity of Caco-2 monolayers was maintained during these flux studies.

2.7. Analytical methods

All samples were analyzed using a reverse-phase HPLC system (LC-10AVP; Shimadzu, Kyoto, Japan) equipped with either a variable-wavelength ultraviolet detector or a fluorescence detector.

2.8. Statistical analysis

Comparison of the TEER values of Caco-2 monolayers between in presence and absence of the solubilizers was determined using two-tailed Student's t-test at the 5% significance level. The statistical software package SAS (v8.2; SAS Institute, Inc., Cary, NC) was used for analysis of data.

3. Results and discussion

3.1. Suitability of 3-day cultured Caco-2 monolayers

Fig. 1 shows the effects of PG, HP-β-CD, PEG 400, and Tween 80 on the TEER of 3-day cultured Caco-2 monolayers. One of these excipients was added to the apical side of separate test monolayers. The excipient concentrations that caused a decrease in the TEER value of no significant different from the value in the absence of solubilizers ($204 \pm 42\ \Omega\ cm^2$) during 1 h incubation were 5% (w/v) for PG, 1% (w/v) for HP-β-CD, 1% (w/v) for PEG 400, and 0.5% (w/v) for Tween 80. These results indicate that all solubilizers affected the TEER of 3-day cultured Caco-2 monolayers, even when applied at relatively low concentrations.

In this study, the concentrations of 300 μM were chosen as tested drug solutions (Mainprize and Grady, 1998), because drugs at these high concentrations are most likely transported across Caco-2 monolayers by passive diffusion. Additionally, in our preliminary experiments, we have obtained the linear rate of permeability of model compounds from 15 min to 1 h after administration. Therefore, samples were collected at these time points.

Two solubilizers, 5% PG and 1% HP-β-CD, were chosen to test their effects on the permeability of each of the three model compounds that have different in vivo permeabilities; these results were compared with the permeability of each drug in the absence of solubilizers. Table 1 summarizes the P_{app} values for each drug. The results show that the P_{app} values of all drugs in the presence of a solubilizer were higher than those in the absence of a solubilizer. Furthermore, the P_{app} value of LY, which is absorbed exclusively through a paracellular route (Hidalgo et al., 1989), was much greater in the presence of the solubilizers than in the absence of the solubilizers, indicating that these solubilizers cause leaks in the integrity of 3-day cultured monolayers that cannot be detected by TEER.

Additionally, Table 1 also shows that for drugs dissolved in the vehicle alone, in the absence of a solubilizer, the P_{app} values range between 0.40 ×

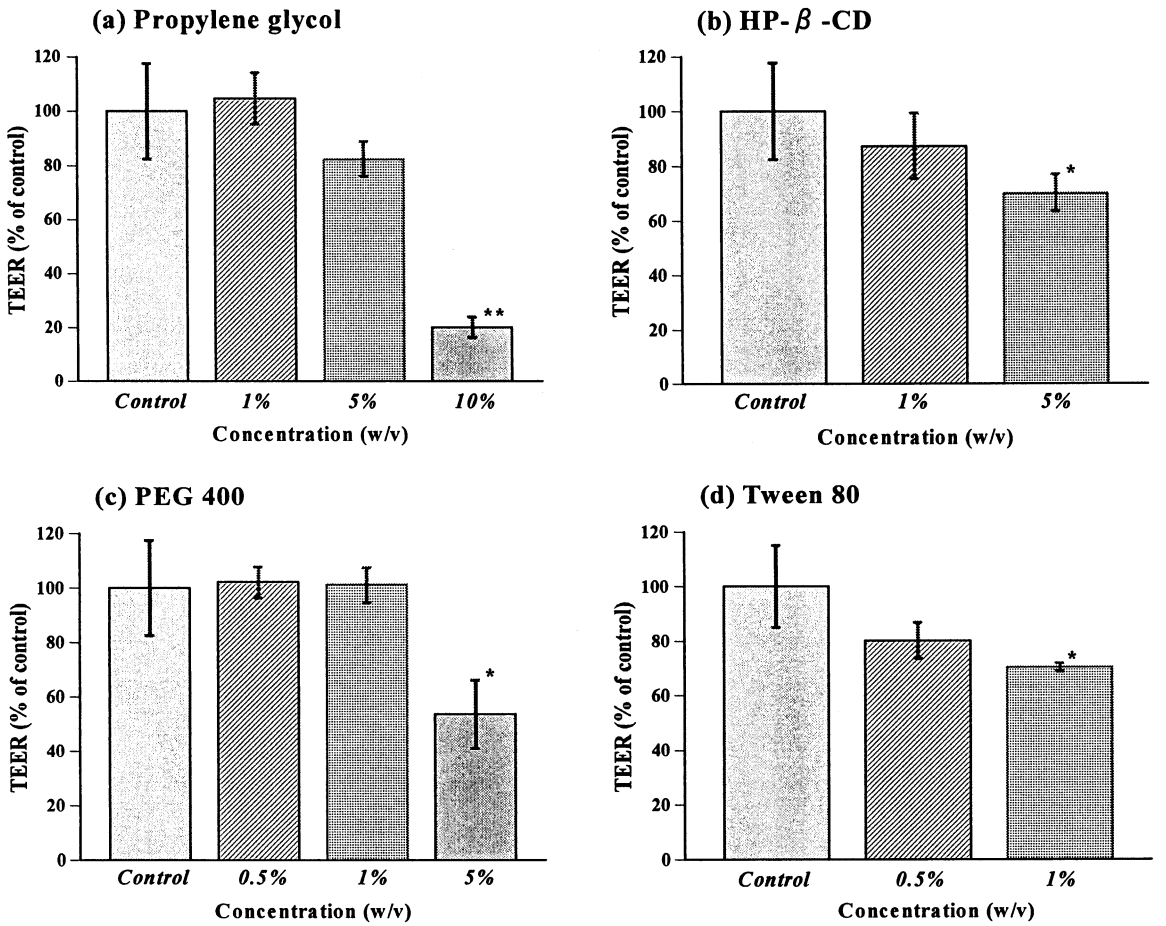

Fig. 1. Effect of (a) PG, (b) HP-β-CD, (c) PEG 400, and (d) Tween 80 on the TEER of 3-day cultured Caco-2 monolayers. One of the concentrations of (a), (b), (c), or (d) was added to the apical side of the monolayers. The TEER of the monolayers was measured 1 h after treatment began and is represented as a percentage of the TEER value of the monolayers not treated with any solubilizer. The data are expressed as the mean ± SD of at least three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$.

10^{-6} cm/s for FD-4, which represents a typical drug with low permeability in humans, and 11.0×10^{-6} cm/s for PPL, which represents a typical drug with high permeability. This range represents a difference of about 28-fold. In contrast, the P_{app} range in the presence of 5% PG is only about sevenfold ($(3.1–22.0) \times 10^{-6}$ cm/s), and the range for 1% HP-β-CD is about threefold ($(5.8–14.7) \times 10^{-6}$ cm/s), being quite different from the corresponding values that received the control treatment. These results indicate that 3-day cultured Caco-2 monolayers are leaky (TEER, 3-day = 204 Ω cm^2; 21-day = 454 Ω cm^2 shown below) and sensitive to solubilizers. Consequently, the use of solubilizers with 3-day cultured Caco-2 monolayers does not yield reliable data.

3.2. Suitability of 21-day cultured Caco-2 monolayers

Fig. 2 shows the effects of PG, Tween 80, PEG 400, HP-β-CD, Pluronic, HCO-40, Transcutol P, Gelucire 44/14, SLS, and extract gall powder on the TEER of 21-day cultured Caco-2 monolayers. One of these excipients was added to the apical side of separate test monolayers, and the excipient concentrations that caused a decrease in the TEER value of no significant different from the value in

Table 1
Effect of 5% PG and 1% HP-β-CD on the permeability of model compounds across 3-day cultured Caco-2 monolayers

Compound	F^a (%)	P_{app} ($\times 10^{-6}$ cm/s)		
		Control	5% PG	1% HP-β-CD
PPL	90	11.0±7.5	22.0±3.6	14.7±2.5
NDL	35	2.5±1.3	5.5±1.0	9.3±2.1
FD-4	0	0.40±0.10	3.1±0.4	5.8±2.6
LY	–[b]	1.3±0.4	17.7±1.5	19.7±1.5

The P_{app} values are given as the mean±SD of at least three independent experiments.

[a] The percentage of the administered dose absorbed in humans, cited from the Refs. Chong et al. (1996) and Yamashita et al. (2000).

[b] There are no data.

the absence of solubilizers (454±32 Ω cm^2) during 1 h incubation were 20% (w/v) for PG, 5% (w/v) for Tween 80, PEG 400 and HP-β-CD, and 10% (w/v) for Pluronic, HCO-40, and Transcutol P. Gelucire 44/14, SLS, and extract gall powder, even at the lowest concentrations tested (1% w/v, 1% w/v, and 0.5% w/v, respectively), decreased the TEER drastically, indicating these solubilizers damage Caco-2 monolayers severally. Consequently, these solubilizers were excluded from further study.

As a result of the initial survey, seven solubilizers remained. These excipients can be used at concentrations which do not affect the TEER of the monolayers. The permeability of each of the three model compounds in the presence of one of these solubilizers was measured and compared with the permeability of each drug in the absence

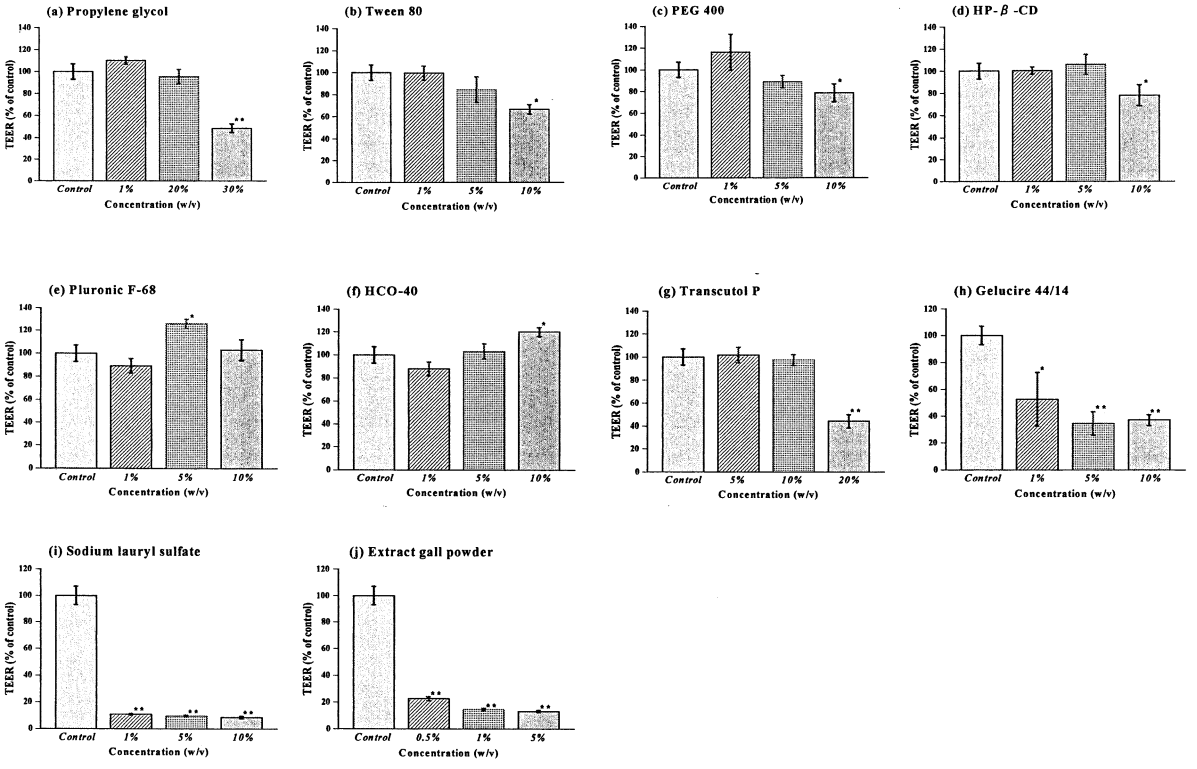

Fig. 2. Effect of (a) PG, (b) Tween 80, (c) PEG 400, (d) HP-β-CD, (e) Pluronic, (f) HCO-40, (g) Transcutol P, (h) Gelucire 44/14, (i) SLS, and (j) extract gall powder on the TEER of 21-day cultured Caco-2 monolayers. One of the concentrations of each solubilizer was added to the apical side of the monolayers. The TEER of the monolayers was measured 1 h after treatment began and is represented as a percentage of the TEER value of the monolayers not treated with any solubilizer. The data are expressed as the mean±SD of at least three independent experiments. $^*P < 0.05$; $^{**}P < 0.01$.

Table 2
Effect of several solubilizers on the permeability of model compounds across 21-day cultured Caco-2 monolayers

Compound	F^a (%)	P_{app} ($\times 10^{-6}$ cm/s)								
		Control	20% PG	5% Tween 80 and +	5% PEG 400	5% HP-β-CD	5% Tween 80+5% PEG 400	10% Pluronic	10% HCO-40	10% Transcutol P
PPL	90	1.9±0.3	1.6±0.2	1.9±0.3	2.3±0.6	1.7±0.4	1.7±0.3	0.62±0.39	0	4.0±0.5
NDL	35	0.49±0.08	0.33±0.05	0.50±0.06	0.48±0.05	0.55±0.1	0.48±0.09	0.25±0.02	0.18±0.02	0.96±0.14
FD-4	0	0.07±0.01	0.05±0.01	0.08±0.01	0.05±0.02	0.03±0.01	0.03±0.01	0.07±0.06	0.04±0.02	0.13±0.01
LY	–[b]	0.22±0.04	0.30±0.05	0.32±0.06	0.31±0.07	0.15±0.03	0.15±0.09	0.13±0.03	0.08±0.002	0.06±0.01

The P_{app} values are given as the mean±SD of at least three independent experiments.
[a] The percentage of the administered dose absorbed in humans, cited from the Refs. Chong et al. (1996) and Yamashita et al. (2000).
[b] There are no data.

of solubilizers. The results are summarized in Table 2. The difference in P_{app} values for each of the three model compounds in the presence of 20% PG, 5% Tween 80, 5% PEG 400, and 5% HP-β-CD compared with the P_{app} value in the absence of any solubilizer is slight. The difference in the P_{app} values between FD-4 and PPL for these solubilizers is about 32-fold ($0.05–1.6 \times 10^{-6}$ cm/s) for 20% PG, about 24-fold ($0.08–1.9 \times 10^{-6}$ cm/s) for 5% Tween 80, about 46-fold ($0.05–2.3 \times 10^{-6}$ cm/s) for 5% PEG 400, and about 57-fold (($0.03–1.7) \times 10^{-6}$ cm/s) for 5% HP-β-CD. The P_{app} range for each solubilizer is equal to or greater than the range for the control treatment, about 27-fold (($0.07–1.9) \times 10^{-6}$ cm/s). Consequently, these four solubilizers can be used with 21-day cultured Caco-2 monolayers in vitro to estimate the absorption of poorly water-soluble compounds in vivo. Additionally, since combinations of solubilizers are often used in formulations, the combination of 5% Tween 80 and 5% PEG 400 on drug permeability was assessed. The P_{app} values of the three model compounds and the difference in the P_{app} values between FD-4 and PPL, 57-fold (($0.03–1.7) \times 10^{-6}$ cm/s), is not so different from the control values. Consequently, the results indicate that this excipient combination can also be used with this test system.

The results were not as good for other solubilizers tested. In the presence of 10% Pluronic or 10% HCO-40, the P_{app} values of the three model compounds were smaller than the control values, and the ranges of the P_{app} values between FD-4 and PPL are more narrow than the control P_{app} values. The results suggest that 10% Pluronic and 10% HCO-40 inhibit drug permeability in some way, perhaps by interacting with lipophilic drugs. Therefore, these solubilizers are not appropriate for this study.

The P_{app} values for model compounds in the absence or in the presence of Transcutol P are also shown in Table 2. In the presence of 10% Transcutol P, the P_{app} values for all the model compounds were much larger than the corresponding values that received the control treatment. These results indicate this excipient heightens drug permeability across Caco-2 monolayers; therefore, it is not appropriate for these kind of in vitro permeability studies.

3.3. Application of the method to poorly water-soluble compounds

Finally, in order to determine whether this system can be used to test new poorly water-soluble compounds, the permeabilities of two lipophilic compounds were tested. DMS is poorly soluble (< 100 μg/ml in the apical solution) but well absorbed in humans. In contrast, YM-X (molecular weight = 516.6; $pK_a = 3.6$; log $D_{7.4} = 4.2$) is poorly soluble (< 1 μg/ml in the apical solution) and poorly absorbed in some animals (Table 3). Highly lipophilic compounds are well known both to be retained by Caco-2 monolayers and to bind non-specifically to plastic materials. However, it is shown that serum protein (BSA 4.5% w/v) added to the basolateral side of monolayers could prevent these interactions (Yamashita et al., 2000). In this study, therefore, the basolateral solution contained 4.5% (w/v) BSA. The TEER and the P_{app} values of DMS and YM-X in the presence of 5% Tween 80 or 5% HP-β-CD were compared with the TEER and P_{app} values of previously tested model compounds (Table 3). As previously observed, the mean TEER values of monolayers treated with either 5% Tween 80 or 5% HP-β-CD were similar to that of the control. However, the P_{app} values for both DMS and YM-X in the presence of HP-β-CD are markedly smaller than the values for the compounds in the presence of Tween 80. This result indicates that HP-β-CD retains lipophilic compounds much more strongly than does Tween 80. Nonetheless, when compared with P_{app} data from the three model compounds, the high P_{app} value for DMS reflects its high in vivo absorption (cf. PPL data) as accurately as the low P_{app} value for YM-X reflects its low in vivo absorption (cf. FD-4 data). Therefore, 21-day cultured Caco-2 monolayers can accurately estimate the in vivo absorption of potential drugs with poor water solubility formulated with the solubilizers listed in Table 4.

Table 3
Effect of 5% Tween 80 and 5% HP-β-CD on TEER and the permeability of DMS and YM-X across 21-day cultured Caco-2 monolayers

Compound	F^a (%)	Control	+5% Tween 80	+5% HP-β-CD
TEER (Ω cm^2)				
		454±40	450±23	460±43
P_{app} ($\times 10^{-6}$ cm/s)				
DMS	100	ND[c]	2.4±0.4	1.4±0.4
YM-X	5.7[b]	ND[c]	0.12±0.01	0.09±0.03
PPL	90	1.9±0.3	1.9±0.3	1.7±0.4
NDL	35	0.49±0.08	0.39±0.07	0.55±0.1
FD-4	0	0.07±0.01	0.08±0.01	0.03±0.01
LY	–[d]	0.22±0.04	0.23±0.02	0.15±0.03

TEER and P_{app} values are given as the mean±SD of at least four independent experiments.
[a] The percentage of the administered dose absorbed in humans, cited from the Refs. Chong et al. (1996) and Yamashita et al. (2000).
[b] The value was taken from an in situ absorption study. Using the closed loop technique, 0.15 mg/0.5 ml of ^{14}C–YM-X was injected into jejunal region of rats in situ and the absorption rate was calculated from residual radioactivity in the segment at 1 h after injection.
[c] Not determined.
[d] There are no data.

Table 4
List of appropriate solubilizers for in vitro permeability studies using 21-day cultured Caco-2 monolayers

Excipient	Concentration (%, w/v)
PG	20
Tween 80	5
PEG 400	5
HP-β-CD	5
Mixtures (Tween 80+PEG 400)	5+5

4. Conclusions

This report presents evidence that excipients commonly used in drug formulations to solubilize poorly water-soluble compounds can be used with 21-day cultured Caco-2 monolayers to predict the human intestinal absorption of drugs early in their development. Potential drugs can be assigned to one of the three absorption categories: high, intermediate, and low, which correspond to their permeability. This is especially useful, since during early stages the physicochemical properties of potential drugs are usually poorly understood. The results also show that appropriate solubilizers can be chosen from among PG, Tween 80, PEG 400, and HP-β-CD or co-mixtures according to the physicochemical properties of the compounds to be tested. However, because lipophilic compounds are retained differently by each solubilizer, the permeability measured using only one solubilizer might be an underestimate of the actual permeability. Therefore, to obtain a better estimate of in vivo absorption, at least two solubilizers should be used to evaluate a compound. Additionally, since the results of this study also indicate that differences exist between the TEER and the LY permeability in the same monolayers, at least two separate checks for the integrity of Caco-2 monolayers should be performed to generate reliable absorption data.

In conclusion, appropriate solubilizers with 21-day cultured Caco-2 monolayers can be used to estimate the in vivo absorption of potential lipophilic drugs in early development stage. This method can greatly accelerate the development of life-saving drugs by allowing the proper allocation of resources to the most promising NCEs. However, this method could evaluate the passive absorption of drugs, but could not estimate the carrier (e.g., peptide transporter and P-glycoprotein)-mediated transport of drugs. In future, these active transport of drugs should also be accurately estimated in vitro studies.

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