ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 50 (2000) 403–411

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

Effect of solubilizing excipients on permeation of poorly water-soluble compounds across Caco-2 cell monolayers

Pratik Saha*, Jim H. Kou

Formulation Research, Schering-Plough Research Institute, Kenilworth, NJ, USA

Received 27 April 2000 accepted in revised form 30 June 2000

Abstract

The purpose of this study was to evaluate the effects of solubilizing excipients on Caco-2 transport parameters of poorly water-soluble NCEs (new chemical entities), and determine their permeability class under the BCS guidance (Biopharmaceutics Classification System). The effect of solubilizing excipients on soluble donor concentration of Sch 56592, Sch-X and Sch-Y was estimated. The transport of reference compounds and NCEs was studied across Caco-2 monolayers in absence or presence of solubilizing agents. The Caco-2 permeability of reference compounds showed good correlation with their extent of human oral absorption data. Sch 56592, Sch-X and Sch-Y exhibited high baseline Caco-2 permeability ($>10^{-5}$ cm/s). Povidone (1%) improved soluble donor concentration and flux of Sch 56592 by 40%. Other solubilizing excipients predominantly improved Sch 56592 soluble donor concentration, with either no change or a decrease in flux. With Sch-X, 1% povidone, pluronic F68, gelucir 44/14, and 3:2 propylene glycol/Tween-80 markedly improved soluble donor concentration, while increasing Sch-X flux by 40–65%. The soluble donor concentration of Sch-Y was also enhanced by excipients; however, only 1% pluronic F68 and PEG 300 increased Sch-Y flux by 35–50%. Sch 56592, Sch-X and Sch-Y are low solubility–high permeability compounds under the BCS guidance. For such poorly water-soluble NCEs, solubilizing excipients should be carefully screened based on their effects on solubility profiles and membrane transport.

Keywords: Caco-2; Transport; Soluble donor concentration; Apparent solubility; Biopharmaceutics; Oral absorption

1. Introduction

For orally administered compounds, drug dissolution generally precedes gastrointestinal absorption and systemic availability. The intrinsic solubility and dissolution profile of a drug often determines the rate and extent of drug absorption from an intestinal site. With the advent in recent years of combinatorial chemistry and high throughput biological screening, new entities tend to be more lipophilic and less water-soluble [1]. For poorly water-soluble compounds, solubility and dissolution appear to be the rate-limiting steps in their overall oral absorption process. In the preclinical development phase, formulation optimization to improve the poor solubility/dissolution profile of such candidates is critical for oral absorption. These efforts focus on use of solubilizing excipients and dissolution enhancers to improve the poor solubility/dissolution of such compounds. However, the effect of these excipients on membrane transport across intestinal epithelial barrier is not well characterized. It is to be emphasized that the mass transport of drug across a membrane is dependent not only on drug concentration but also on the thermodynamic activity of the permeant and the permeability of the biological membrane [2].

The present study characterizes the effects of solubilizing excipients on transport parameters of poorly water-soluble NCEs (new chemical entities) across Caco-2 monolayers (human colon adenocarcinoma cell line) [3,4]. The studies evaluate excipient effects on soluble donor (radiolabeled) drug concentration as well as on Caco-2 membrane permeability and drug partitioning (from vehicle), leading to the delineation of their effects on the overall intestinal permeation rate of such poorly water-soluble compounds. Caco-2 monolayers [3,4] were selected as an in vitro model to evaluate transepithelial intestinal permeability due to their successful application (in the literature) for the prediction of or correlation with human absorption [5,6]. The transport of BCS (Biopharmaceutics Classification System) reference compounds [7–9] was also studied across Caco-2 monolayers. From the correlation of our Caco-2 permeability values with human oral absorption data (from literature)

* Corresponding author. Present address: EnTec Drug Delivery, FMC Biopolymer, Box 8, Route 1 & Plainsboro Road, Princeton, NJ 08543, USA. Tel.: +1-609-951-3779; fax: +1-609-951-3680.

E-mail address: pratik_saha@fmc.com (P. Saha).

0939-6411/00/$ - see front matter
PII: S0939-6411(00)00113-2

[8,9] for these reference compounds, it was possible to assign a permeability ranking for NCEs, and classify new entities based on the 'solubility/permeability' paradigm proposed by Amidon et al. [7].

Based on preformulation data, three poorly water-soluble compounds of unrelated pharmacological class: Sch 56592 (a triazole), Sch-X (a himbacine analog), and Sch-Y (an imidazole derivative) were studied. Solubility and dissolution may play rate-limiting roles in the oral absorption process of these compounds due to their poor intrinsic aqueous solubility. For these compounds, the effect of solubilizing excipients (to improve solubility/dissolution) on intestinal drug permeation may play an equally important role in assessing oral absorption. Potentiometric determinations [10–12] using the PCA101/GLpK_a instrument (Sirius Analytical Instruments, UK) gave the following pK_a and intrinsic log partition coefficient (log P) values: Sch 56592: p$K_a = 4.64$, 3.58, log $P = 2.4$; Sch-Y: p$K_a = 6.45$, log $P = 4.0$. Similar determinations were not made for Sch-X; however, pH-solubility studies revealed that the solubility of Sch-X increased with decreasing pH, suggesting that it was a weak base. The BCS reference molecules that were studied included: class I (high solubility–high permeability) compounds – propranolol and diltiazem; class II (low solubility–high permeability) compound – phenytoin; and class III (high solubility–low permeability) compounds – cimetidine and polyethylene glycol (PEG) 4000 [8,9].

2. Materials and methods

2.1. Materials

[^{14}C]Sch 56592 (specific activity 78 μCi/mg), [^{3}H]Sch-X (specific activity 13.27 mCi/mg), and [^{3}H]Sch-Y (specific activity 1.73 mCi/mg) were provided by Schering–Plough Radiochemistry (Kenilworth, NJ). The ^{3}H-label on Sch-X and Sch-Y was in a non-exchangeable position of the molecule so that washout of the tritium will not be a concern. D-[^{3}H]Mannitol (specific activity 19.7 Ci/mmol), D-[^{14}C]mannitol (specific activity 51.5 mCi/mmol), [^{3}H]diltiazem (specific activity 85.5 Ci/mmol), and [^{14}C]5,5-diphenylhydantoin (specific activity 53.1 mCi/mmol) were obtained from Dupont NEN (Boston, MA). DL-[^{3}H]propranolol hydrochloride (specific activity 28.0 Ci/mmol), [^{3}H]cimetidine (specific activity 13.3 Ci/mmol), and [^{14}C]polythylene glycol 4000 (specific activity 15.3 mCi/g) were obtained from Amersham Co. (Downers Grove, IL). Unlabeled Sch 56592, Sch-X and Sch-Y were supplied by Schering-Plough Chemical Research (Kenilworth, NJ). Unlabeled D-mannitol, diltiazem, 5,5-diphenylhydantoin (phenytoin), DL-propranolol hydrochloride, cimetidine, polyethylene glycol 4000 (PEG 4000), and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO). All excipients were of USP or NF/EP grade and were obtained from formulation research excipient stockroom, Schering–Plough Research Institute (Kenilworth, NJ). Gelucir 44/14 was provided by Gattefosse Corp. (Westwood, NJ).

Cell culture media and supplies were obtained from Gibco (Grand Island, NY). Transwell inserts (0.4 μm pore size, 12 mm diameter), and cluster plates were obtained from Corning–Costar Corp. (Cambridge, MA).

2.2. Caco-2 cell culture

The Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD) at passage 17. Caco-2 cells were grown as described previously according to Hidalgo et al. [3] and Hilgers et al. [4]. Briefly, cells were cultured in Dulbecco's modified Eagle's medium (DMEM with 4.5 g/l D-glucose) supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 2 mM L-glutamine, and 1% penicillin–streptomycin, and maintained in a humidified incubator at 37°C in 95% air/5% CO_2. Cells were subcultured every 7 days by treatment with 0.05% trypsin–0.53 mM EDTA, and plated at a density of 60 000–100 000 cells/cm^2 on collagen-coated (rat tail collagen, type I) polycarbonate Transwell filters.

Caco-2 cells (on Transwell filters) were grown for at least 21 days to allow formation of physiologically and morphologically well developed confluent cell monolayers prior to initiating drug transport studies [3,4]. The transepithelial electrical resistance (TEER, Ω cm^2) of cultures was monitored with an EVOM epithelial voltohmeter (World Precision Instruments, Sarasota, FL), and corrected for background TEER contributed by the blank filter and culture medium. Confluent Caco-2 monolayers as observed under light microscopy (300× magnification) and with TEER values (mean ± SEM, $n = 62$) of 311 ± 5 Ω cm^2 were used for drug transport studies.

2.3. Estimation of soluble donor concentration of radiolabeled Sch 56592, Sch-X and Sch-Y in modified Ringer's solution

Unlabeled drug saturated MRS (modified Ringer's solution, pH 7.4) in absence or presence of excipient vehicle was spiked with 1 μCi/ml of [^{14}C]Sch 56592, [^{3}H]Sch-X or [^{3}H]Sch-Y, and equilibrated for 3 or 24 h. Excipients were evaluated at concentrations based on typical preclinical formulations and/or dosing vehicles. These were 1% povidone K29/32 (PVP), 0.1% and 1% pluronic F68 (poloxamer 188 NF), 1% gelucir 44/14, 1% 3:2 propylene glycol/Tween-80, 0.5% Tween-80 with 0.4% methyl cellulose, 0.005% Tween-80, and 1% polyethylene glycol (PEG) 300. Two separate study protocols using filtration and centrifugation, and the effect of 3- vs. 24-h equilibration were compared for the estimation of soluble donor concentration of radiolabeled drug. Sch-Y and Sch 56592 were used (in absence of excipients) as probes. In the filtration protocol, 5 ml of radiolabeled sample (unlabeled saturated

drug + 1 μCi/ml radiolabeled drug) was equilibrated (3 or 24 h), filtered using a 0.22-μm syringe filter unit (Fischer Scientific), and collected separately as first, second, third, fourth, and fifth ml filtrates. Each milliliter of filtrate was counted in a liquid scintillation spectrometer (Wallac, Gaithersburg, MD) after mixing with scintillation cocktail. Typically, the radiolabel counts plateaued between the third and fifth ml filtrates; this plateau count was used for calculation purposes to offset the non-specific adsorptive loss of radiolabeled molecule to the filter membrane. The syringes used in the studies were incubated overnight in MRS saturated with unlabeled drug to minimize adsorptive loss as well. In the centrifugation protocol, 0.5 ml of radiolabeled sample (unlabeled saturated drug + 1 μCi/ml radiolabeled drug) was equilibrated (3 or 24 h), and centrifuged in siliconized Eppendorf tubes (to minimize adsorptive loss) at 5000 rev./min for 10 min. The supernatant was sampled using siliconized pipet tips and counted (along with the tip).

2.4. Drug transport across Caco-2 monolayers

Prior to each experiment, Caco-2 monolayers grown on Transwell inserts were washed with modified Ringer's solution (MRS, pH 7.4) containing 1 mM $CaCl_2$, 5.3 mM KCl, 0.4 mM KH_2PO_4, 0.5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 3.3 mM NaH_2PO_4, 137 mM NaCl, 10 mM HEPES, and 25 mM D-glucose. The TEER was then measured to gauge integrity of the monolayers.

2.4.1. Apical–basal transport of Sch 56592, Sch-X and Sch-Y

Transport was initiated by adding 0.5 μCi of radiolabeled drug ([^{14}C]Sch 56592, [^{3}H]Sch-X or [^{3}H]Sch-Y) and 0.5 ml of unlabeled drug saturated excipient vehicle in MRS to the apical chamber (0.5 ml) of Transwell inserts bathed with 1.5 ml MRS in the basolateral chamber. For baseline (control) transport studies, unlabeled drug saturated MRS was added. The transport of D-mannitol (1 mM unlabeled mannitol with 1 μCi/ml [^{3}H]mannitol or [^{14}C]mannitol) (paracellular marker) [13] was monitored simultaneously. The Transwell inserts in cluster plates were agitated on a Rotomix orbital shaker (Thermolyne, Dubuque, IA) inside a 37°C incubator. To estimate apical–basal flux, 200 μl of receiver (basolateral) fluid was sampled using siliconized pipet tips (Fisher Scientific, Pittsburgh, PA) at 0.5, 1, 1.5, and 2 h post-dosing, and counted (along with the tip) in a liquid scintillation spectrometer (Wallac, Gaithersburg, MD). The volume withdrawn was immediately replaced with an equal volume (200 μl) of pre-equilibrated MRS. The integrity of cell monolayers during flux studies was assessed by mannitol permeability, and TEER was also measured at the end of each transport experiment for comparison with initial values. Following the transport study, each Transwell filter with cells was washed with ice-cold MRS buffer to wash out extracellular label; the filter was then cut off, placed in 0.5 ml of 0.5% Triton X-100 for 30 min and vortexed in order to lyse the cells. The radioactivity incorporated into cells or adsorbed on the filter was determined by counting cell lysate or filter with scintillation cocktail in a liquid scintillation spectrometer (Wallac).

2.4.2. Apical–basal transport of propranolol, diltiazem, cimetidine, phenytoin, PEG 4000, and mannitol

The same transport protocol as described for Schering molecules was used for the reference compounds as well. The transport of propranolol, diltiazem, cimetidine, phenytoin, and PEG 4000 was studied at a donor concentration of 0.1 mM unlabeled drug with 1 μCi/ml [^{3}H]propranolol hydrochloride, -diltiazem, -cimetidine, or [^{14}C]phenytoin, -PEG 4000, respectively. For D-mannitol (a paracellular marker) [13], the donor concentration was 1 mM unlabeled mannitol with 1 μCi/ml [^{3}H]mannitol.

2.5. Data analysis

For the estimation of soluble radiolabeled drug concentration in donor, the dpm counts per ml was translated to μg/ml using the specific activity of radiolabeled drug. In transport studies, the steady-state flux was estimated from the slope of the linear portion of a plot of cumulative amount of drug appearing in receiver fluid vs. time. The apparent permeability coefficient (P_{app}, cm/s) was calculated from the observed flux (dQ/dt) normalized against the surface area of filter membrane (A, 1.13 cm^2) and the initial soluble radiolabeled drug concentration (C_0) in donor fluid according to Eq. (1).

$$P_{app} = \frac{dQ/dt}{A \times C_0} \quad (1)$$

Statistical significance was tested by two-tailed Student's t-test or one-way ANOVA using the Excel software package. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Soluble donor concentration of radiolabeled Sch 56592, Sch-X and Sch-Y

A typical profile of dpm counts per ml vs. filtrate volume from the filtration study is shown in Fig. 1A with [^{3}H]Sch-Y. The soluble donor dpm/ml estimates from both filtration and centrifugation protocols were comparable for the two probes, [^{14}C]Sch 56592 and [^{3}H]Sch-Y (Fig. 1B). There was also no significant difference in [^{14}C]Sch 56592 and [^{3}H]Sch-Y dpm counts per ml following 3 or 24 h equilibration (Fig. 1C). The mean dpm counts per ml was translated to μg/ml using the specific activity of radiolabeled drug to estimate the soluble donor concentration of radiolabeled Sch 56592, Sch-X and Sch-Y in absence or presence of excipients (Table 1). As shown in Table 1, the soluble donor concentration for all three radiolabeled compounds was improved by the excipients studied.

Multiplying the soluble fraction of radiolabeled com-

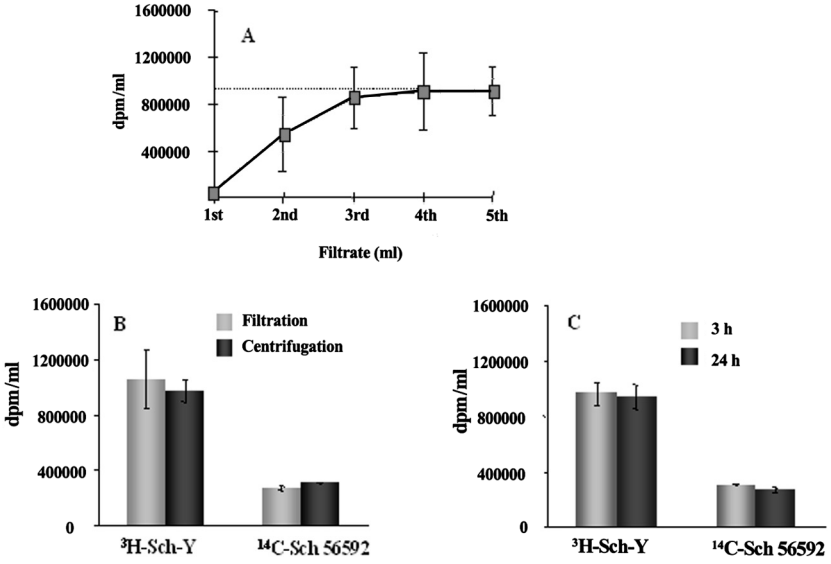

Fig. 1. Soluble donor concentration estimation of radiolabeled drug. (A) Profile of dpm counts per ml vs. filtrate volume from filtration study with [^{3}H]Sch-Y. (B) Soluble donor concentration (dpm/ml) estimates from filtration and centrifugation study protocols with [^{14}C]Sch 56592 and [^{3}H]Sch-Y. (C) [^{14}C]Sch 56592 and [^{3}H]Sch-Y dpm counts per ml following 3 or 24 h equilibration. Error bars represent SEM for $n = 3$. Where not seen, the error bar is smaller than the size of the symbol.

pound by the total concentration (μg/ml) of radiolabeled and non-radiolabeled drug charged in the donor gave estimates of the 'apparent solubility' for Sch 56592, Sch-X and Sch-Y as 11.98, 0.0363 and 4.02 μg/ml. As reference, the solubility of Sch-Y in pH 7.4 phosphate buffer using LC–MS assay was estimated to be 5.2 μg/ml, which agrees well with the value estimated by radioactivity.

Table 1
Soluble concentration of radiolabeled Sch 56592, Sch-X and Sch-Y in modified Ringer's solution (MRS), pH 7.4

Condition	Soluble drug concentration (μg/ml) in MRS (pH 7.4)[a]		
	Sch 56592	Sch-X	Sch-Y
Control	1.36	0.0003	0.22
+ 1% povidone	2.0 (1.47)[b]	0.0014 (4.7)	0.40 (1.8)
+ 0.1% pluronic F68	2.0 (1.47)	ND[c]	ND
+ 1% pluronic F68	3.0 (2.2)	0.003 (10)	0.37 (1.7)
+ 1% gelucir 44/14	16.0 (11.8)	0.04 (133)	0.44 (2)
+ 1% 3:2 propylene glycol/Tween-80	15.0 (11)	0.06 (200)	0.44 (2)
+ 0.4% methyl cellulose + 0.5% Tween-80	20.0 (14.7)	0.04 (133)	ND
+ 0.005% Tween-80	ND	ND	0.42 (1.9)
+ 1% polyethylene glycol (PEG) 300	ND	ND	0.30 (1.4)

[a] Mean value of three separate determinations.
[b] Numbers within parentheses indicate the -fold increase by excipients.
[c] ND, not determined.

3.2. Transport of Sch 56592, Sch-X and Sch-Y

Fig. 2 illustrates the permeation time profile in typical transport experiments. In this case, povidone improved Sch 56592 flux by 40% over the baseline. Fig. 3 summarizes the results obtained with all excipients for the three compounds. In sharp contrast to the effect of povidone, 1% gelucir 44/14 or 3:2 propylene glycol/Tween-80 significantly reduced Sch 56592 flux to 30 and 60% of baseline value, respectively ($P < 0.05$). The use of 1% pluronic F68, gelucir 44/14 or 3:2 propylene glycol/Tween-80 as a solu-

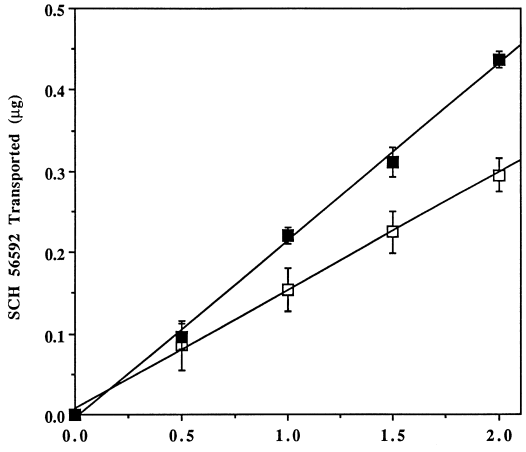

Fig. 2. Time course of Sch 56592 apical–basal transport across Caco-2 monolayers in the absence (□) or presence (■) of 1% povidone. Error bars represent SEM for $n = 3$–4. Where not seen, the error bar is smaller than the size of the symbol.

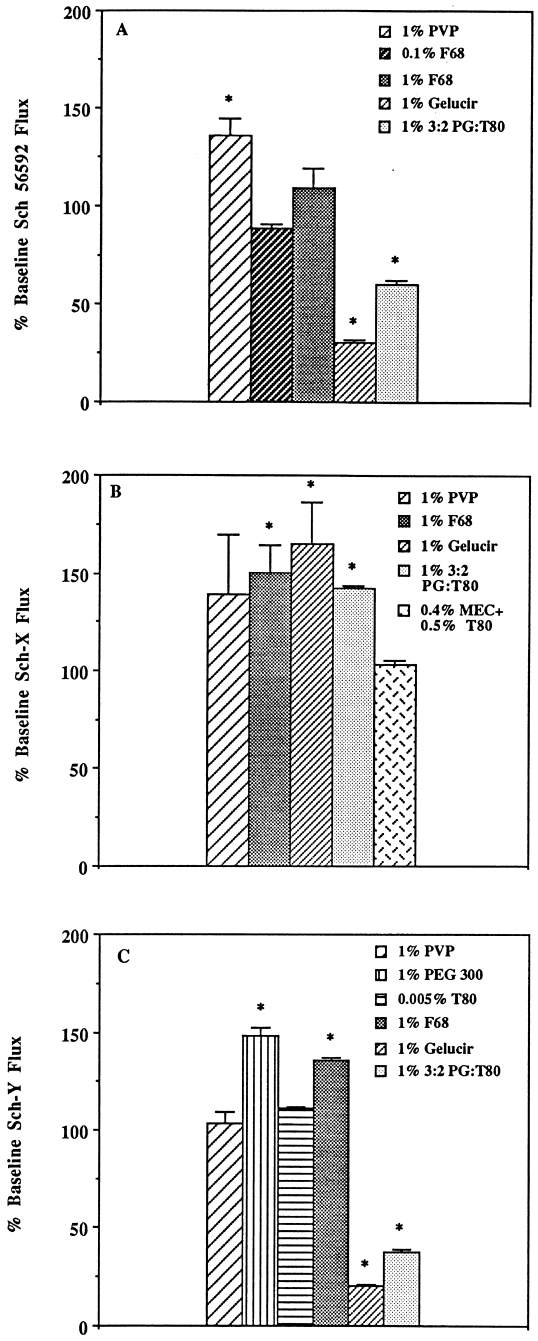

Fig. 3. Effect of excipients on apical–basal flux (expressed as percent baseline flux) of Sch 56592 (A), Sch-X (B) and Sch-Y (C) across Caco-2 monolayers. Error bars represent SEM for $n = 3$–4. Where not seen, the error bar is smaller than the size of the symbol. Asterisk indicates statistical significance ($P < 0.05$) compared with baseline value.

bilizing vehicle improved Sch-X flux by 51, 65 and 43%, respectively ($P < 0.05$). For Sch-Y, a 50 and 36% flux increase ($P < 0.05$) was observed with polyethylene glycol (PEG) 300 and pluronic F68, respectively. However, Sch-Y flux was drastically decreased by 1% gelucir 44/14 and 1% 3:2 propylene glycol/Tween-80.

From the flux profiles and soluble radiolabeled drug concentration in donor in the absence of excipients, the baseline apparent permeability coefficients (P_{app}, cm/s) for Sch 56592, Sch-X and Sch-Y were calculated as 3.4, 2.3 and 1.6×10^{-5} cm/s, respectively (Table 2).

3.3. Effect of excipients on Caco-2 monolayers

The effect of solubilizing excipients on the integrity of cell monolayers during Sch 56592, Sch-X and Sch-Y flux studies was monitored by mannitol permeability, and TEER measurements. The P_{app} values of [^{3}H]mannitol (during Sch 56592 flux) and [^{14}C]mannitol (during Sch-X, Sch-Y flux) in absence or presence of excipients are shown in Table 3. The corresponding TEER (initial, final and percent initial) values are also summarized (Table 3).

3.4. Transport of reference compounds

The apparent permeability coefficients (P_{app}, cm/s) of the reference compounds are summarized in Table 2. The P_{app} of mannitol (paracellular marker, log PC $= -3.1$), and hydrophilic PEG 4000 (log PC $= -5.1$) were 1.9×10^{-6} and 2.1×10^{-7} cm/s, respectively. Higher P_{app} values ($\geq 3 \times 10^{-5}$ cm/s) were obtained for propranolol (log PC $= 3.6$), diltiazem (log PC $= 2.7$) and phenytoin (log PC $= 2.5$). Cimetidine (log PC $= 0.4$) exhibited a permeability value (3.09×10^{-6} cm/s) comparable to that of mannitol.

The TEER of Caco-2 monolayers during flux studies with reference compounds remained stable ($P > 0.05$) over the experimental period. The TEER as a percentage of the initial values for mannitol, PEG 4000, cimetidine, propranolol, diltiazem and phenytoin were $86 \pm 7\%$, $91 \pm 5\%$, $101 \pm 1\%$, $97 \pm 7\%$, $111 \pm 4\%$ and $111 \pm 7\%$, respectively.

Table 2
Apparent permeability coefficient (P_{app}) values for apical–basal transport of Sch 56592, Sch-X, Sch-Y, and reference compounds across Caco-2 monolayers

Solute	Log PC[a]	P_{app} ($\times 10^{-5}$ cm/s)[b]
Sch 56592	2.4	3.4 ± 0.2
Sch-X	ND[c]	2.3 ± 0.3
Sch-Y	4.0	1.6 ± 0.1
PEG 4000	−5.1	0.02 ± 0.002
Mannitol	−3.1	0.19 ± 0.01
Cimetidine	0.4	0.3 ± 0.05
Phenytoin	2.5	2.9 ± 0.4
Diltiazem	2.7	4.9 ± 0.3
Propranolol	3.6	8.6 ± 0.4

[a] Logarithm of the *n*-octanol/pH 7.4 buffer partition coefficient. Log PC of Sch 56592 and Sch-Y was determined by the Sirius PCA101/GLpK$_a$ instrument. Log PC values of reference compounds were from the literature [6,7].

[b] Mean ± SEM for $n = 4$.

[c] ND, not determined.

Table 3
Mannitol permeability coefficient (P_{app}) and TEER during apical–basal transport of Sch 56592, Sch-X, Sch-Y across Caco-2 monolayers in absence or presence of excipients

Drug	Condition	Mannitol P_{app}[a] ($\times 10^{-6}$ cm/s)	TEER[a]		
			Initial (Ω cm^2)	Final (Ω cm^2)	% Initial value
Sch 56592	Control	1.55 ± 0.38	343 ± 10	352 ± 28	102 ± 5
	+ 1% povidone	2.57 ± 0.23	341 ± 12	339 ± 22	100 ± 8
	+ 0.1% pluronic F68	1.99 ± 0.28	330 ± 12	339 ± 21	104 ± 9
	+ 1% pluronic F68	2.52 ± 0.25	310 ± 8	316 ± 12	103 ± 7
	+ 1% gelucir	1.70 ± 0.32	339 ± 3	339 ± 40	100 ± 12
	+ 1% 3:2 propylene glycol/Tween-80	2.01 ± 0.09	306 ± 1	345 ± 25	113 ± 8
Sch-X	Control	2.43 ± 0.05	260 ± 20	295 ± 32	115 ± 15
	+ 1% povidone	2.66 ± 0.08	284 ± 15	284 ± 18	101 ± 9
	+ 1% pluronic F68	2.20 ± 0.28	286 ± 4	214 ± 3	75 ± 0
	+ 1% gelucir	2.51 ± 0.10	275 ± 2	232 ± 3	84 ± 1
	+ 1% 3:2 propylene glycol/Tween-80	2.20 ± 0.07	269 ± 4	225 ± 15	84 ± 7
	+ 0.4% methyl cellulose + 0.5% Tween-80	1.90 ± 0.13 *	269 ± 7	274 ± 10	102 ± 5
Sch-Y	Control	1.47 ± 0.22	292 ± 3	359 ± 3	123 ± 2
	+ 1% povidone	0.93 ± 0.07	313 ± 3	452 ± 10	145 ± 2*
	+ 1% PEG 300	0.85 ± 0.10	304 ± 3	505 ± 7	166 ± 1*
	+ 0.005% Tween-80	1.24 ± 0.03	309 ± 7	367 ± 11	119 ± 2
	+ 1% pluronic F68	0.82 ± 0.20	358 ± 13	512 ± 24	143 ± 6*
	+ 1% gelucir	2.49 ± 0.22 *	319 ± 22	119 ± 6	38 ± 3*
	+ 1% 3:2 propylene glycol/Tween-80	0.91 ± 0.05	362 ± 14	311 ± 14	86 ± 1*

[a] Mean ± SEM for $n = 3$–4, $*P < 0.05$, significantly different from control.

4. Discussion

The present study highlights the use of Caco-2 transport studies in formulation research and drug development. Combined with standard solubility and in vitro dissolution studies, this model would be useful in optimizing the selection of solubilizing excipients for solubility-limited NCEs (new chemical entities), as well as in classifying NCEs under the BCS guidance (Biopharmaceutics Classification System).

The correlation of human oral absorption data from literature with Caco-2 permeability values for our reference compounds is shown in Fig. 4. The Caco-2 permeability of reference compounds determined in this study showed good correlation with their extent of human oral absorption data, suggesting their predictive utility for assessing oral absorption. Rubas et al. [9] and Pade and Stavchansky [8], amongst other groups, have reported similar results. This would be useful in assigning a permeability ranking to new drug entities (such as Sch 56592, Sch-X, Sch-Y) based on their Caco-2 permeability values. The proposed BCS guidance defines a high permeability drug as one with > 90% extent of human absorption (based on administered dose). As shown in Table 2, Sch 56592, Sch-X and Sch-Y all exhibited high baseline Caco-2 permeability (3.4, 2.3 and 1.6×10^{-5} cm/s, respectively), and should be well absorbed in humans.

Sch 56592, Sch-X and Sch-Y were solubility-limited NCEs based on available preformulation data that indicated the compounds to have poor solubility in water and pH 7.4 buffer. This is evident to some extent by our studies on assessing soluble radiolabeled drug concentration, and esti-

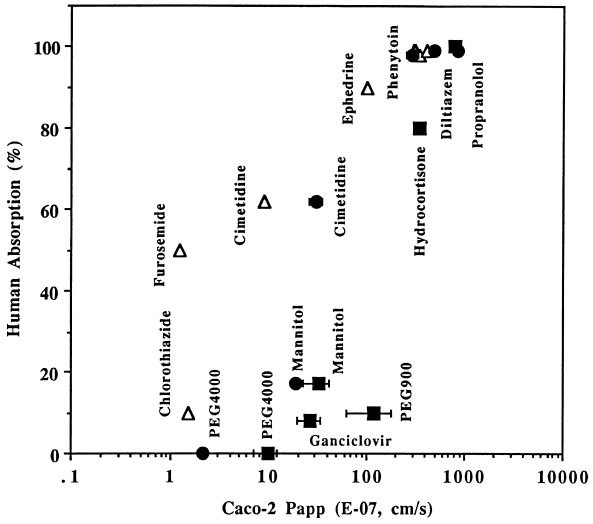

Fig. 4. Correlation of apparent permeability coefficient (P_{app}) values across Caco-2 monolayers with human oral absorption data (from the literature) for reference compounds. Error bars represent SEM for $n = 4$. Where not seen, the error bar is smaller than the size of the symbol. The correlation was compared with those of Rubas et al. [7], and Pade and Stavchansky [6]. (●) Saha and Kou; (■) Rubas et al; (△) Pade and Stavchansky.

mate the total soluble donor drug concentration (radiolabel + non-radiolabel) based on the charged amount (per ml) of radiolabeled and non-radiolabeled drug; this affords a reasonable albeit approximate estimate of 'apparent solubility'. Under the proposed BCS guidance, a high-solubility drug is defined as one, which at the highest human dose is soluble in 250 ml water throughout the physiological pH range (pH 1–8) [5]. For our test compounds to meet this criteria, the clinical dose would have to be low; otherwise solubility and dissolution can be the rate-limiting steps in their overall oral absorption process. For this class of compounds, a rational formulation strategy to develop bioavailable dosage forms will require screening of solubilizing excipients not only for their ability to enhance solubility or dissolution but also for their effect on the overall drug absorption process.

An important finding in this study is that enhancement in soluble donor concentration of a drug by solubilizing excipients does not translate in all cases to a similar extent of flux improvement across Caco-2 monolayers. The excipients not only improve soluble drug concentration in donor through their effect on solubility, but may also effect the membrane transport and drug partitioning process across intestinal barrier (in this case, Caco-2 monolayers). The relative contribution of the excipient effect on soluble drug concentration and transport parameters will determine the net effect on oral drug absorption. In turn, this will depend on the excipient-drug interaction based on the drug's intrinsic physiochemical properties (solubility, log PC). For instance, 1% gelucir 44/14 improved the soluble donor concentration of radiolabeled Sch 56592 (11.8-fold), Sch-X (133-fold), and Sch-Y (2-fold) (Table 1); however, only Sch-X flux was improved by 65%, while Sch 56592 and Sch-Y flux decreased to 30 and 20% of baseline value, respectively (Fig. 3). A similar effect was observed with 1% 3:2 propylene glycol/Tween-80 which elicited a marked increase in soluble donor concentration of Sch 56592, Sch-X and Sch-Y (Table 1); only Sch-X flux increased by 43%, while Sch 56592 and Sch-Y flux was drastically reduced (Fig. 3). Other vehicles improved the soluble donor concentration of Sch 56592 and Sch-Y as well. Only 1% povidone increased Sch 56592 flux by 40%, while Sch-Y flux was improved with only 1% PEG 300 or pluronic F68 (Fig. 3).

The effect of excipients on Caco-2 monolayers during Sch 56592, Sch-X and Sch-Y flux studies was assessed by concomitant mannitol (a paracellular marker) [13] permeability, and TEER measurements. A significant drop in monolayer TEER (percent initial value) associated with an increase in mannitol permeability may suggest that integrity of the epithelial barrier is compromised. As shown in Table 3, there was no significant ($P > 0.05$) drop in Caco-2 TEER (percent initial value) or increase in mannitol permeability during Sch 56592 and Sch-X flux in the presence of excipients (compared with control). In contrast, both gelucir and propylene glycol/Tween-80 significantly ($P < 0.05$) decreased TEER (by 62 and 14%, respectively) during Sch-Y flux. Only in the case of Sch-Y with gelucir though, was there an associated increase in permeability of mannitol

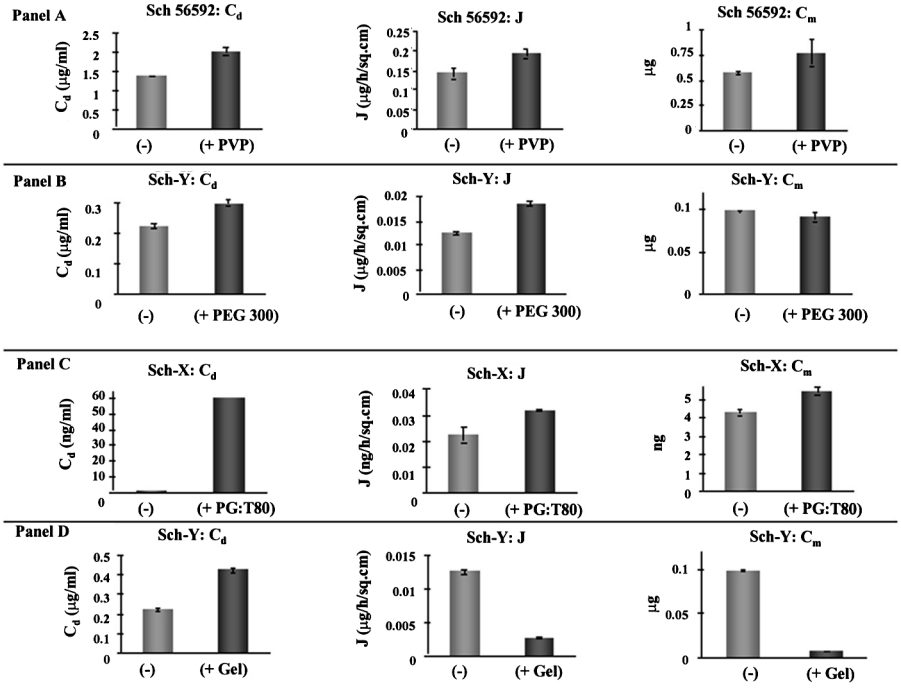

Fig. 5. Effect of 1% povidone (A), 1% PEG 300 (B), 1% propylene glycol/Tween-80 (C), and 1% gelucir 44/14 (D) on soluble donor radiolabeled drug concentration (C_d), flux (J) and cell-internalized drug amount (C_m) of Sch 56592 (A), Sch-X (C), and Sch-Y (B,D). Error bars represent SEM for $n = 3$–4. Where not seen, the error bar is smaller than the size of the symbol.

Table 4
Case studies of excipient effect on transport parameters J, C_d, K and P_m [a]

Condition	J	C_d	K	P_m
Sch 56592 (+PVP)	↑	↑	∅	∅
Sch-Y (+PEG 300)	↑	↑	↓	↑
Sch-X (+PG:T80)	↑	↑↑	↓	∅
Sch-Y (+Gelucir)	↓	↑	↓↓	∅

[a] ↑, increase; ↓, decrease; ∅, no change.

(paracellular marker) [13] through intercellular tight junctional spaces. Therefore, it appears that the integrity of Caco-2 monolayers may be compromised with Sch-Y and gelucir. The TEER increased (43–66%) during Sch-Y permeation in presence of povidone, PEG 300 or pluronic F68; however, that did not effect mannitol permeability ($P > 0.05$) either, and may be attributed to an excipient-drug interaction characteristic of Sch-Y based on its physiochemical properties. Nevertheless, for a hydrophobic molecule as Sch-Y (log $P = 4.0$) that is likely to opt for the transcellular diffusion pathway, the paracellular permeation will be minimal under all circumstances.

In order to delineate the net effect of an excipient on oral absorption of solubility-limited candidates, its individual effect on soluble donor radiolabeled drug concentration, Caco-2 flux (transport rate) and drug partitioning (across Caco-2 monolayers) is illustrated in Fig. 5. The analysis follows from the equation

$$J = P_m \times C_m = P_m \times (K \times C_d) \quad (2)$$

where J is flux, P_m is drug permeability across Caco-2, C_m is monolayer drug concentration, K is the drug partition coefficient, and C_d the soluble drug concentration in donor phase. For poorly soluble compounds, the use of solubilizing vehicles in donor phase not only improves C_d but may also affect K. The amount of drug accumulated within Caco-2 monolayers (cell accumulation, C_m) at the end of flux studies was taken as a reasonable estimate of the drug's ability to partition into the intestinal cells from the apical donor (K). Fig. 5A illustrates the effect of 1% povidone on Sch 56592 transport parameters. This is the most ideal case where an increase (1.47-fold) in Sch 56592 soluble donor concentration (C_d) translates approximately to a proportional increase in flux and cell accumulation (C_m), whereby K remains unchanged. A second case study is depicted in Fig. 5B. PEG 300 increased Sch-Y soluble donor concentration (C_d) 1.4-fold, but C_m remains unchanged; this can be explained by a proportional decrease in K ($C_m = K \times C_d$). However, Sch-Y flux (J) increased 50%, thereby suggesting an effect of PEG 300 on membrane permeability, P_m. Fig. 5C illustrates the effect of 1% propylene glycol/Tween-80 on Sch-X: C_d increased 200-fold while J and C_m increased only 40%; this can be explained by a marked decrease in K. In Fig. 5D, gelucir 44/14 decreased cell accumulation (C_m) of Sch-Y by 90%, despite a 2-fold increase in C_d. In this case, the decrease in K reduced Sch-Y flux (J) to 20% of baseline value.

The diverse excipient effects on the transport parameters, J, C_d, K and P_m, from the above case studies are summarized in Table 4. It is likely that solubilizing excipients may affect other transport parameters (like diffusion coefficient) as well. Nevertheless, such an analysis can begin to dissect the complex nature of excipient effect on drug transport and optimize the rational screening of solubilizing excipients for poorly soluble NCEs.

In summary, a rational formulation development strategy for oral dosage forms based on Caco-2 monolayer as an in vitro screening model was proposed. It was demonstrated that the Caco-2 model could provide permeability information to classify new entities based on a solubility/permeability paradigm according to BCS. This would allow the identification of the rate-limiting step in the overall drug absorption scheme. More importantly, it was demonstrated that for low-solubility compounds, an assessment of the impact of solubility enhancing excipients on drug permeation would be critical for developing bioavailable formulations.

References

[1] 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 Del. Rev. 23 (1997) 3–25.
[2] G.L. Flynn, R.W. Smith, Membrane diffusion III: influence of solvent composition and permeant solubility on membrane transport, J. Pharm. Sci. 61 (1972) 61–66.
[3] I.J. Hidalgo, T.J. Raub, R.T. Borchardt, Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability, Gastroenterology 96 (1989) 736–749.
[4] A.R. Hilgers, R.A. Conradi, P.S. Burton, Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa, Pharm. Res. 7 (1990) 902–910.
[5] S. Yee, In vitro permeability across Caco-2 cells (colonic) can predict in vivo (small intestinal) absorption in man – fact or myth, Pharm. Res. 14 (1997) 763–766.
[6] P. Artursson, K. Palm, K. Luthman, Caco-2 monolayers in experimental and theoretical predictions of drug transport, Adv. Drug Del. Rev. 22 (1996) 67–84.
[7] 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.
[8] V. Pade, S. Stavchansky, Link between drug absorption solubility and permeability measurements in Caco-2 cells, J. Pharm. Sci. 87 (1998) 1604–1607.
[9] W. Rubas, N. Jezyk, G.M. Grass, Comparison of the permeability characteristics of a human colonic epithelial (Caco-2) cell line to colon of rabbit, monkey, and dog intestine and human drug absorption, Pharm. Res. 10 (1993) 113–118.
[10] A. Avdeef, pH-metric log P. 2. Refinement of partition coefficients and ionization constants of multiprotic substances, J. Pharm. Sci. 82 (1993) 183–190.
[11] B. Slater, A. McCormack, A. Avdeef, J.E.A. Comer, pH-metric log P. 4.

Comparison of partition coefficients determined by shake-flask, HPLC and potentiometric methods, J. Pharm. Sci. 83 (1994) 1280–1283.

[12] S.D. Kramer, J.-C. Gautier, P. Saudemon, Considerations on the potentiometric log P determination, Pharm. Res. 15 (1998) 1310–1313.

[13] A. Adson, T.J. Raub, P.S. Burton, C.L. Barsuhn, A.R. Hilgers, K.L. Audus, N.F.H. Ho, Quantitative approaches to delineate paracellular diffusion in cultured epithelial cell monolayers, J. Pharm. Sci. 83 (1994) 1529–1536.