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RESEARCH PAPER

In Vitro and *Ex Vivo* Investigation of the Impact of Luminal Lipid Phases on Passive Permeability of Lipophilic Small Molecules Using PAMPA

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ABSTRACT

Purpose Evaluate the impact of luminal micellar phase on passive permeability of five lipophilic ($1.9 \leq \text{clogP} \leq 9.0$) small molecules using biorelevant media and evaluate the impact of luminal coarse lipid particles on danazol permeability after oral administration of a triglyceride solution to fed adults using PAMPA.

Methods Permeability of carbamazepine, furosemide, danazol, and Compound A was evaluated using Prisma™ HT, FaSSIF-V2, and FeSSIF-V2 in the donor compartment. Compound B could not be tested using Prisma™ HT, due to negligible solubility. Individual intestinal aspirates collected after administration of danazol solution in the olive oil portion of a meal and corresponding micellar phases were subjected to PAMPA. Commercially available Acceptor Sink Buffer was used in all cases.

Results Unlike with furosemide (under constant pH) and Compound B, permeability of carbamazepine, danazol, and Compound A steadily decreased in the presence of increasing micelle concentration of media. Danazol permeability from aspirates was reduced compared to that from micellar phases; fluxes were similar.

Conclusions Using PAMPA, the impact of luminal micellar phase on passive permeability of lipophilic molecules varies with the molecule. After administration of a triglyceride solution of danazol, high danazol concentrations in coarse lipid particles balance in terms of drug flux the reduced permeability.

KEY WORDS biorelevant media · human intestinal contents · human intestinal micellar phases · PAMPA · passive permeability

INTRODUCTION

It is known almost 40 years now, that mixed bile salt micelles enhance the solubilization of lipophilic compounds but, primarily due to their size, their diffusion towards the intestinal epithelium is slower than that of the single lipophilic drug molecules (1). Depending on their capacity, solubilization in mixed bile salt micelles can enhance mass transport of lipophilic compounds (2). More recently, Caco-2 data have confirmed that passive permeability of lipophilic compounds may be decreased in presence of micelles (3–5) and lipophilicity of the compound as well as composition and concentration of mixed micelles are two factors affecting the extent of decrease (Markopoulos *et al.*, under revision). It has further been proposed that the presence of micelles may modify the unstirred water layer properties (6) and the possibility of direct interaction of micelles with the apical cell membrane cannot be excluded (7). These data raise concerns on the usefulness of data collected by using the Caco-2 model and conventional transport media especially if one additionally considers that compound ranking in regard to the permeability characteristics may also be affected by the presence of micelles in the donor compartment (Markopoulos *et al.*, under revision).

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The first objective of the present investigation was to confirm the importance of micellar phase of intestinal fluids on passive permeability characteristics of small molecules by using biorelevant media and an alternative *in vitro* technique, the parallel artificial membrane permeability assay (PAMPA). PAMPA is a high throughput screening technique that is used for the assessment of the passive permeability characteristics of new molecules with potential pharmacological activity and, typically, experiments are performed by using simple aqueous media (e.g. 8,9 and references therein). Based on one relevant abstract presented by Lu *et al.* at the 2007 AAPS Annual Meeting in San Diego (http://abstracts.aapspharmaceutica.com/ExpoAAPS07/CC/forms/attendee/index.aspx?content=sessionInfo&sessionId=1429) effects of concentration and composition of mixed micelles on passive permeability characteristics of small molecules in PAMPA are modest, but, the impact of ionization or of increased lipophilicity were not addressed.

On the other hand, although it has been suggested that lipophilic APIs are transported to the epithelial surface via the micellar phase, the contribution of other phases cannot be excluded (10). We have recently suggested that luminal coarse lipid particles that are formed after administration of triglyceride solution of danazol to adults in the fed state can directly contribute to the flux of danazol across the intestinal mucosa (11). That study was performed by using the Caco-2 cell model and, therefore, due to cell toxicity issues, relevant experiments had to be performed by using diluted human intestinal aspirates and diluted micellar phases of aspirates (11). The second objective of the present investigation was to evaluate the impact of coarse lipid particles that are formed after administration of a triglyceride solution of danazol to adults on intestinal permeation of danazol by using PAMPA, a technique that could be applied without prior dilution of the luminal samples.

MATERIALS AND METHODS

Materials

Physicochemical characteristics of the five model lipophilic compounds ($1.9 \leq \text{clogP} \leq 9.0$) tested in the present investigation are shown in Table I. Carbamazepine, furosemide, and danazol were donated by Novartis Hellas (Metamorphosis, Greece), Uni-Pharma (Kifissia, Greece), and Sanofi-Aventis (Fawdon, UK), respectively, while Compounds A and B were kindly provided by F. Hoffmann-La Roche (Basel, Switzerland and Nutley, NJ, USA). Both Compounds A and B are classified as high permeability compounds that are absorbed passively, but Compound B is also substrate for P-gp (F. Hoffmann-La Roche data in file).

Acceptor Sink Buffer (ASB), Prisma™ HT System Solution, GIT-0 Lipid Solution, 96-well plates with magnetic stirrers, and Gut-Box® were purchased from Pion Inc. (MA, USA).

Table I Physicochemical Characteristics of Model Compounds Tested in the Present Study

	clogP	MW	pK_a	Aqueous solubility (μg/mL)
Carbamazepine	1.9	236.3	N.A.	220 104.9[a]
Furosemide	2.3	330.7	3.04 (acidic)	130 18.25[b]
Danazol	4.2	337.5	N.A.	2.5 0.42[c]
Compound A	2.1	302.3	N.A.	3
Compound B	9.0	727.8	4.0 and 5.4 (both basic)	Not quantifiable at pH 7

Unless a specific reference is provided, data for carbamazepine, furosemide, and danazol come from https://scifinder.cas.org and data for Compounds A and B come from F. Hoffmann-La Roche. *N.A.* not applicable for physiologically relevant pH range ($1 < \text{pH} < 8$)

[a] 14

[b] 21

[c] 20

Phosphatidylcholine (Lipoid E PC S, egg, 96%) was donated by Lipoid (Ludwigshafen, Germany) and glyceryl monooleate (RYLO MG 19 Pharma) was donated by Danisco (Brabrand, Denmark). Sodium taurocholate was purchased from Prodotti Chimici e Alimentari (Basaluzzo, Italy). Sodium oleate and sodium chloride were purchased from Riedel-de-Haën AG (Seelze, Germany).

Lucifer Yellow was purchased from Sigma Aldrich Chemie GmbH (Germany).

All chemicals used for the optimization and application of analytical methods were of HPLC grade and purchased from Sigma Aldrich Chemie GmbH or E. Merck (Germany).

Methods

Experiments Using Buffers and Simulated Micellar Phases of Intestinal Fluids as Donor Solutions

Assays were performed using all five model compounds: carbamazepine, furosemide, danazol, Compound A, and Compound B. Carbamazepine and furosemide were tested as potentially high and low permeability standards, respectively (12).

Donor Solutions. Each compound was studied by using commercially available Prisma™ HT buffer (a universal buffer, 43 mOsm/kg at pH 7.4), a medium simulating the micellar phase composition in the fasted upper small intestine (FaSSIF-V2) (13), and a medium simulating the micellar

phase composition in the fed upper small intestine (FeSSIF-V2) (13), as donor media. The latter two media were considered to provide a basis for investigating the importance of mixed bile salt micelles on the transport of lipophilic APIs towards the membrane of intestinal epithelial cells. For carbamazepine, initial concentration in Prisma™ HT (pH 7.4) was 100 μg/mL, i.e. just below its water solubility (14). Since no solubility data in FaSSIF-V2 or FeSSIF-V2 were available, the same initial donor concentration was used for experiments in FaSSIF-V2 or FeSSIF-V2. For furosemide, initial donor concentration was based on its therapeutic dose, assuming a total gastrointestinal fluid volume of 250 mL and 800 mL in the fasted and the fed state, respectively. One half of this concentration was used in the experiments with FaSSIF-V2 (i.e. 80 μg/mL) and FeSSIF-V2 (i.e. 25 μg/mL). Due to its ionization properties, experiments with Prisma™ HT were performed at three different pH values, 5.8, 6.5, and 7.4. Initial donor concentrations of furosemide in Prisma™ HT were the same with that used for the experiments in FeSSIF-V2. To prepare donor solutions of danazol, Compound A, and Compound B stock solutions in DMSO were prepared firstly. Those solutions were diluted with pH 7.4 Prisma™ HT (1:100), FaSSIF-V2 (1:1000), and with FeSSIF-V2 (1:1000). DMSO never exceeded 1% in the donor compartment. Quantities of compounds dissolved in DMSO were such that final concentrations approached but did not exceed the solubility in Prisma™ HT, FaSSIF-V2, and FeSSIF-V2, i.e. final concentrations were 1, 5, and 17 μg/mL, respectively, for danazol, and 2, 5, and 15 μg/mL, respectively for Compound A. Due to its minimal solubility, experiments with Compound B in Prisma™ HT were not possible; handling restrictions due to toxicity issues led to the preparation of relatively low initial donor concentration (i.e. 1 μg/mL) for experiments in FaSSIF-V2 and in FeSSIF-V2. For all compounds, solutions in Prisma™ HT, were obtained after vortexing, centrifuging (14,000 rpm, 15 min, 25°C), and using the supernatant in PAMPA experiments. Measured initial donor concentrations in Prisma™ HT (used in permeability estimation) deviated from nominal values by up to 0.4, 5.2, 56 and 10% for furosemide, carbamazepine, danazol and Compound A, respectively.

Acceptor Solutions. In all cases, commercially available ASB was used as acceptor solution. ASB (45 ± 2 mOsm/kg) contains surface active agent(s). Prior to using it, its osmolality was adjusted with NaCl so that it was equal to that of the specific sample in the donor compartment.

Evaluation of Integrity of Lipid Membrane. Damage to the lipid membrane due to donor solutions was evaluated by testing membrane integrity at various time points after initiation of an experiment based on a protocol note by Millipore (15). At various time points after the initiation of an experiment, the donor solution and the ASB from the two compartments was removed, a 5% solution (v/v) of DMSO in phosphate buffered saline (pH 7.4) containing 100 μg/mL of Lucifer Yellow was added to the donor compartment, while 5% DMSO in phosphate buffered saline (pH 7.4) was added to the acceptor compartment. The plates (Corning Costar 96-well polystyrene plate, black with clear bottom) were stirred in Gut-Box® for 4 h and then the presence of Lucifer Yellow in the acceptor compartment was evaluated using a fluorescence plate reader (Tecan Infinite M200 Pro, excitation 425 nm—emission 528 nm). A lipid membrane that is intact will result in complete rejection of Lucifer Yellow i.e. fluorescence readings comparable to background readings of buffer only should be observed. Acceptable fluorescence levels are less than three times the average background fluorescence (15). Data revealed that lipid membrane integrity is maintained for at least 8 h when pH 5.8 and pH 6.5 Prisma™ HT is used as donor solution and for at least 20 h when pH 7.4 Prisma™ HT, FaSSIF-V2 or FeSSIF-V2 is used as donor solution.

Assay Methodology. Prior to each assay, the PVDF supporting filter of the well was impregnated with 5 μL of commercially available GIT-0 Lipid Solution (20% w/v lecithin in n-dodecane). A volume of 200 μL of donor solution was added in the donor compartment and a volume of 200 μL of ASB was added into the acceptor compartment. The plates were put into Pion's Gut-Box®, a device used for the continuous stirring of the donor solution and in all experiments in this investigation the speed of the stirring was set such that an unstirred water layer of 40 μm was maintained (16,17). Concentration in the acceptor compartment was measured at various time points, after initiation of the experiment and by taking into account the time period during which lipid membrane maintains its integrity. Each well was used for collecting one sample from the acceptor compartment at a specific time point. Concentration at each sampling time point was measured in three different wells ($n=3$). Concentrations in the donor and/or the acceptor compartment were measured with HPLC-UV. Table II summarizes the chromatographic conditions used for each model compound.

Experiments Using Aspirates and Their Micellar Phases as Donor Solutions

Donor Solutions. From a recent human study (11), samples of the contents of the upper small intestine of healthy adults had been collected at various time points, after administration of 150 mg of danazol dissolved in the olive oil content of a heterogenous liquid meal [volume 500 mL, 73% fat, 13% carbohydrates, 14% proteins, total of 750 Kcal (11)]. Upon

Table II Chromatographic Conditions for the Analysis of Model Compounds Tested in the Present Investigation

	Column	Mobile phase	Flow (mL/min)	UV wavelength (nm)	Retention time (min)	LOD (μg/mL)	LOQ (μg/mL)
Carbamazepine	Fortis C18 (5 μm, 250 × 4.6 mm)	acetonitrile:water 45:55 v:v	1	285	8	0.076	0.231
Furosemide	Fortis C18 (5 μm, 150 × 3.0 mm)	methanol:water:acetic acid 55:45:0.5 v:v:v	0.4	280	6.5	0.031	0.094
Danazol	Fortis C18 (5 μm, 150 × 3.0 mm)	acetonitrile:water 70:30 v:v	0.5	286	6.5	0.012	0.036
Compound A	Fortis C18 (5 μm, 150 × 3.0 mm)	methanol:water:acetic acid 60:40:1 v:v:v	0.5	210	8.8	0.111	0.337
Compound B	Eclipse XDB-C8 (5 μm, 150 × 4.6 mm)	ammonium formate (10 mM, pH = 7.9): acetonitrile 63:37 v:v	0.4	250	8.7	0.030	0.090

LOD and LOQ were evaluated using $\mathrm{LOD} = \frac{3.3 s_{y/x}}{b}$ and $\mathrm{LOQ} = \frac{10 s_{y/x}}{b}$, respectively, where b is the slope and $s_{y/x}$ is the standard deviation of the residuals of the regression line (22).

collection, a portion of each sample had been ultracentrifuged (11). Samples of intestinal contents and of their micellar phases had been stored at −70°C until used, i.e. only one thawing was applied prior to PAMPA experiments. Five aspirated samples (each representing a specific volunteer and aspiration time) and their corresponding micellar phases were used as donor solutions (Table III). It is worth mentioning that concentrations of danazol in micellar phases of aspirates (Table III) are similar to the concentrations of danazol used for performing the experiments in FeSSIF-V2 (17 μg/mL). It should be acknowledged that collection of pure micellar phase may not be possible (18) but for the sake of simplicity in this manuscript the more precise description "primarily micellar phase" will be simply referred to as the "micellar phase".

Table III Concentration of Danazol and Lipids and Physicochemical Characteristics of the Aspirated Samples (*upper rows*) and of their Corresponding Micellar Phases (*lower rows*) Collected at Specific Time Points Post Administration of a Triglyceride Based Heterogenous Liquid Meal (11) and Tested in the Present Study

Volunteer #—time (min)	#2–180	#2–210	#4–120	#6–180	#7–180
Danazol (μg/mL)	23.45	46.12	139.8	48.2	91.24
	8.29	13.37	20.96	2.64	9.09
FAs (mM)	24.43	48.11	81.06	11.22	108.67
	6.46	7.34	13.95	0.95	9.27
MGs (mM)	6.93	2.62	9.42	0.50	8.74
	1.24	1.14	0.62	0.12	0.92
DGs (mM)	0.41	4.38	12.94	1.48	8.69
	0.01	0.02	0.22	<0.002	0.10
TGs (mM)	0.22	2.54	4.30	2.53	2.16
	<0.004	<0.004	<0.004	<0.004	<0.004
Lyso-PC (mM)	2.22	3.21	2.01	1.04	1.27
	1.74	2.64	1.61	0.58	0.73
PC (mM)	0.26	0.23	4.43	0.51	3.45
	<0.012	<0.012	0.14	<0.012	<0.012
CHO (mM)	0.56	0.47	0.53	0.21	0.87
	0.26	0.28	0.42	<0.001	0.16
BS (mM)	12.32	13.68	5.62	2.80	5.62
	12.22	9.50	3.62	3.16	3.06
pH	6.93	6.71	6.83	6.21	6.62
Osmolality (mOsm/kg)	214	86	291	n.a.	216
	219	191	n.a.	384	251

FAs fatty acids, *MGs* monoglycerides, *DGs* diglycerides, *TGs* triglycerides, *Lyso-PC* lysophosphatidylcholine, *PC* phosphatidylcholine, *CHO* cholesterol, *BS* bile salts, *n.a.* not available

Likewise, all lipids that are present in dispersed form in the aspirate are referred to as "coarse lipid particles".

Acceptor Solutions. ASB was used as acceptor solution. Its osmolality was adjusted with NaCl so that it was equal to that of the specific sample in the donor compartment (Table III).

Evaluation of Integrity of Lipid Membrane. Damage to the lipid membranes by aspirates and their micellar phases was evaluated by testing membrane integrity at various time points after initiation of an experiment based on the protocol described above (15). Data revealed that lipid membrane integrity is maintained for at least 8 h when aspirates are used and for at least 40 min when micellar phases of aspirates are used as donor solutions. Apparently, presence of coarse lipid particles has a protective role on the lipid membrane.

Assay Methodology. Prior to each assay, the PVDF supporting filter of the well was impregnated with 5 μL of GIT-0 Lipid Solution and experiments were run as described above with the buffers and the biorelevant media. However, due to limited availability of volumes of intestinal aspirates, concentration at each sampling time point was estimated by using data from one well ($n=1$). Initial danazol concentration in the donor compartment just prior to the initiation of stirring (Table III) and concentrations in the acceptor compartment were measured with HPLC-UV (Table II).

Data Treatment

For data collected previously with biorelevant media and intestinal aspirates using the Caco-2 model (11; Markopoulos *et al.*, under revision), a five compartment kinetic model comprising the donor and the receiver solution, the cell monolayer and the apical and the basal plastic wall of the plate was used for data analysis and parameter estimation. For the PAMPA data on the other hand collected in this study, a simpler, two compartment model (with donor and acceptor compartments being the two compartments) was initially applied, assuming that membrane (lipid and filter) and plastic walls retention completes rapidly whereas transport from the donor compartment is complete (due to the presence of ASB in the acceptor compartment) and occurs according to first-order kinetics, i.e.

$$C_A = \frac{M_{D(0)} - M_B}{V_A} \times \left(1 - e^{-\frac{P_{app} \times A}{V_D} \times t}\right) \quad (1)$$

where C_A is the compound concentration in the acceptor compartment, $M_{D(0)}$ is the initial compound amount in the donor compartment, M_B is the compound amount bound on the membrane and the plastic walls, V_D and V_A are the volume of the donor and acceptor compartment respectively, A is the membrane surface area, P_{app} is the apparent

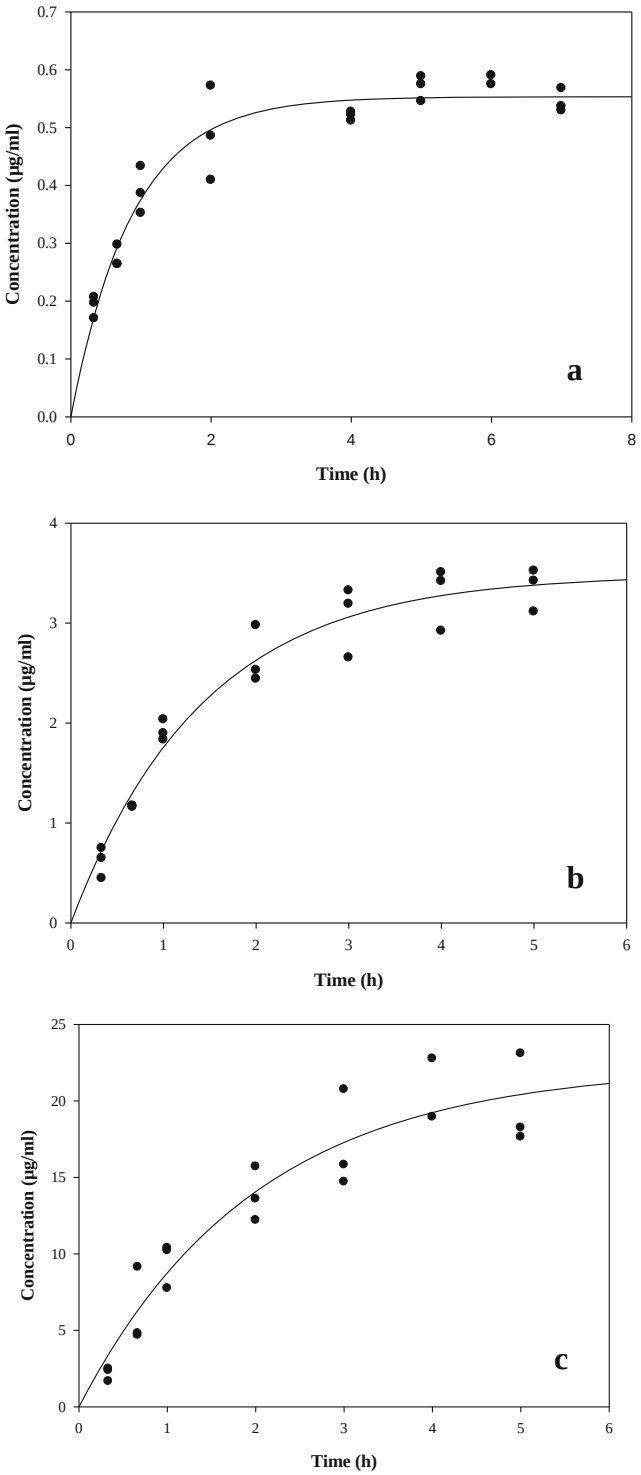

Fig. 1 Danazol concentration in acceptor compartment vs. time plots (experimental data and best fitted lines) during PAMPA experiments performed by using as donor solution (**a**) danazol in Prisma™ HT, (**b**) danazol in FaSSIF-V2, and (**c**) danazol in FeSSIF-V2. The R^2 values for the fits to the data were 0.94, 0.96, and 0.91, respectively.

permeability coefficient, and t is the time after the initiation of the experiment.

The two-compartmental model (Eq. 1) was successfully fitted to most of the data collected using Prisma™ HT, FaSSIF-V2, and FeSSIF-V2 (R^2: 0.91–0.990; $p<0.0001$ and $p<0.001$ for estimated $(M_{D(0)}\text{-}M_B)/V_D$ and $(P_{app}\times A)/V_D$ values, respectively, e.g. Fig. 1). However, this model could not be applied to all data, because membrane integrity was lost early during the permeation process, and, thus, fitting was not possible (danazol in micellar phases of aspirates and furosemide) and/or retention of drug in the donor compartment was significant (danazol in aspirates and furosemide).

In this study apparent permeability coefficients were estimated with Eq. 2 and the initial slope of a diagram depicting cumulative amount of compound in the acceptor compartment vs. time:

$$P_{app} = \frac{dM_A}{dt} \times \frac{1}{A \times C_{D(0)}} \quad (2)$$

where M_A is the compound amount transferred into the acceptor compartment and $C_{D(0)}$ is the initial measured concentration in the donor compartment. In order to estimate the initial slope, Eq. 2 was applied to all data that were collected under sink conditions and within a timeframe that lipid membrane remains intact. Equation 2 was successfully fitted to the data collected when buffers or simulated micellar phases of intestinal fluids were used as solutions in the donor compartment ($n=3$–$4/R^2$: 0.8–0.998). Also, Eq. 2 was successfully fitted to the data collected when aspirates or their micellar phases were used as solutions in the donor compartment (for data using aspirates: $n=3/R^2$: 0.95–0.9999; for data using micellar phases of aspirates: $n=3/R^2$: 0.99–0.999). Subsequently, danazol flux, J, was estimated using Eq. 3.

$$J = P_{app} \times C_{D(0)} \quad (3)$$

Comparisons of apparent permeability coefficients from Prisma™ HT and biorelevant media were performed by using the standard errors [estimated by taking into account the propagation of error in estimating the initial slope (Eq. 2)] and, after confirming the normality of data, by applying analysis of variance with Tukey's compromise as post hoc test. Comparisons of apparent permeability coefficients and of fluxes estimated using aspirates and micellar phases were performed with paired *t*-test (after confirming the normality of data). Significance level was 0.05 in all cases.

RESULTS AND DISCUSSION

Impact of Micellar Phase on Permeability Using PAMPA

Table IV shows values of (passive) permeability coefficients of the model compounds when using Prisma™ HT, FaSSIF-V2, and FeSSIF-V2 as donor solutions.

Previously published values of permeability coefficients exist only for carbamazepine (highly permeable standard) in pH 7.4 Prisma™ HT [1.58×10^{-4} cm/s (14)], and for furosemide (used as low permeability standard) in pH 7.4 Prisma™ HT [90×10^{-8} cm/s and 1.58×10^{-8} cm/s (8,17)]. Those values have been estimated using the single point measurement method (19) and are close to the values estimated in this study using pH 7.4 Prisma™ HT (Table IV).

As expected, permeability of furosemide decreases with increasing the pH (Table IV, data using Prisma™ HT), due to its ionization properties. Based on carbamazepine data both danazol and Compound A can be classified as high

Table IV Permeability Coefficients (± SE) for Carbamazepine, Furosemide, Danazol, Compound A, and Compound B Estimated using PAMPA and Buffers or Simulated Micellar Phases of Intestinal Fluids as Donor Solutions

	$P_{app}\times10^4$ (cm/s)				
	Carbamazepine	Furosemide	Danazol	Compound A	Compound B
pH 5.8 Prisma™ HT	n.e.	$0.01781\pm0.00032^{b, c, d}$	n.e.	n.e.	n.e.
pH 6.5 Prisma™ HT	n.e.	0.003088 ± 0.000076	n.e.	n.e.	n.e.
pH 7.4 Prisma™ HT	0.556 ± 0.026	0.000871 ± 0.000050	1.75 ± 0.17	0.759 ± 0.034	n.e.
FaSSIF-V2 (pH = 6.5)	0.522 ± 0.018	0.00428 ± 0.00017	0.873 ± 0.051^{c}	0.492 ± 0.011^{c}	0.139 ± 0.012
FeSSIF-V2 (pH = 5.8)	$0.388\pm0.019^{c, d}$	$0.0267\pm0.0020^{a, b, c, d}$	0.79 ± 0.15^{c}	$0.366\pm0.015^{c, d}$	0.2140 ± 0.0091^{d}

n.e. not estimated, due to lack of substantial ionization at this pH (carbamazepine, danazol, Compound A) or due to negligible solubility (Compound B)

[a] Significantly different from pH 5.8 Prisma™ HT

[b] Significantly different from pH 6.5 Prisma™ HT

[c] Significantly different from pH 7.4 Prisma™ HT

[d] Significantly different from FaSSIF-V2

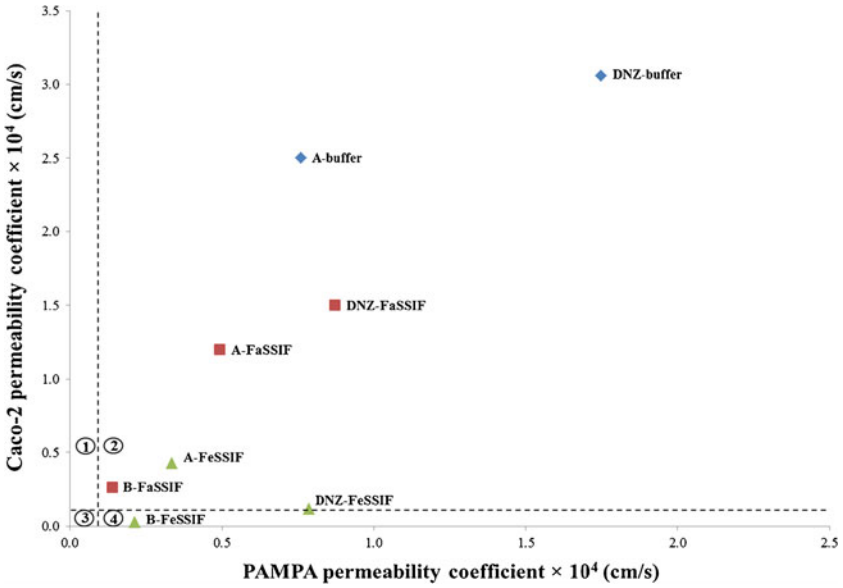

Fig. 2 Permeability coefficients estimated with PAMPA by using Prisma™ HT, FaSSIF-V2 and FeSSIF-V2 (this study) vs. permeability coefficients estimated with the Caco-2 model by using aqueous buffer (aq-TM_{Caco}), FaSSIF-TM_{Caco} and FeSSIF-TM_{Caco} (Markopoulos *et al.*, under revision). A: Compound A, B: Compound B, DNZ: danazol. The lines drawn at 0.1×10^{-4} cm/s are cutoff values for classifying molecules as high or low permeability compounds based on Balimane et al. (8). Compounds that fall in quadrants 2 and 3 are characterized by both methods as having high or low permeability, respectively. For compounds that fall in quadrants 1 and 4 there is a disagreement about the ranking between the two *in vitro* methods (8).

permeability compounds in accordance with previous published (20) and in house F. Hoffmann-La Roche data, respectively (Table IV).

Permeability of carbamazepine, danazol, and Compound A (nonionizable compounds) decreased in the presence of micelles and the effect was statistically significant (Table IV). Such decrease may affect compound flux via the intestinal mucosa. Indeed, the increased solubility of lipophilic molecules in the presence of micelles has been previously shown to be accompanied by a decreased permeability using the rat jejunal perfusion model (6). In contrast, P_{app} values of furosemide were higher from micelles containing media (pH 6.5 Prisma™ HT vs. FaSSIF-V2 and pH 5.8 Prisma™ HT vs. FeSSIF-V2). For Compound B data from Prisma™ HT could not be collected. Based on data in FaSSIF-V2 and FeSSIF-V2, permeability is higher than furosemide but lower than carbamazepine. Also, based on ionization properties and data in Table IV it may further be argued that permeability may also be increased in presence of micelles. Therefore, unlike with the Caco-2 model (Markopoulos *et al.*, under revision), for ionizable compounds the effect of micelles on permeability cannot be generalized when using PAMPA.

In agreement with Prisma™ HT data, FaSSIF-V2 or FeSSIF-V2 data also suggest that danazol and Compound A are highly permeable compounds (Table IV, comparisons with FaSSIF-V2 and FeSSIF-V2 data of carbamazepine). Interestingly, however, Compound B (a highly lipophilic compound, Table I) has been classified as low permeability compound based on FeSSIF-TM_{caco} data and the Caco-2 model (Markopoulos *et al.*, under revision), whereas in the present study data in FeSSIF-V2 indicate a high permeability compound (Table IV; P_{app} values of Compound B and carbamazepine are similar). This is more clearly presented in Fig. 2 where permeability coefficients estimated with PAMPA are contrasted with permeability coefficients estimated recently with the Caco-2 model by taking into account cutoff values for classifying molecules as high or low permeability compounds. It should be noted that for all other

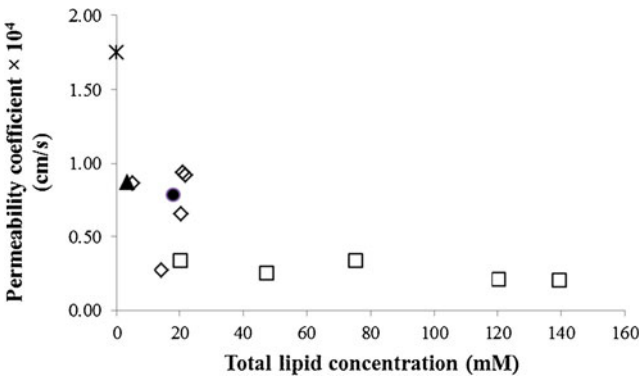

Fig. 3 Permeability coefficients for danazol estimated from experiments with PAMPA by using Prisma™ HT (*asterisk*), aspirates from the upper small intestine of adults in the fed state (*squares*), micellar phases of the aspirated samples (*diamonds*), FaSSIF-V2 (*triangle*) and FeSSIF-V2 (*circle*) vs. the corresponding total lipid concentration, i.e. the sum of monoglycerides, diglycerides, triglycerides, free fatty acids, phosphatidylcholine, lysophosphatidylcholine, and bile salts concentrations.

compounds and media the classification based on PAMPA measurements was in agreement with that based on Caco-2 experiments (Markopoulos *et al.*, submitted).

Based on the above data it may be argued that, to the extent that presence of lipid formulation components in the micellar phase of luminal contents can be simulated *in vitro*, PAMPA (like Caco-2 cell lines) might be useful to test effects of formulation composition on intraluminal transport of lipophilic small molecules.

Permeability vs. Flux of Small Molecules from Coarse Lipid Particles of Intestinal Aspirates Using PAMPA

Mean ± SD values of permeability coefficients of danazol from five aspirates and from their corresponding micellar phases were $(0.270\pm0.065)\times10^{-4}$ cm/s and $(0.73\pm0.28)\times10^{-4}$ cm/s, respectively ($p=0.012$). A similar finding has been recently reported using the Caco-2 model, diluted aspirates and diluted micellar phases of aspirates (11). It appears that permeability values decrease nonlinearly with increasing lipid concentration (Fig. 3). Figure 3 also confirms that the dependence of P_{app} on the lipid content of the sample holds likewise for the aspirates and for their micellar phases, as it was observed in previous experiments with the Caco-2 model. It is interesting to note that P_{app} values from experiments with danazol in FaSSIF-V2 and in FeSSIF-V2 are in line with the P_{app} values measured by using micellar phases of luminal contents (Fig. 3). Furthermore, P_{app} reaches a plateau value at high total lipid concentration, an observation that could not be previously made by Caco-2 data due to the use of diluted samples and insufficient data at high lipid concentrations.

It would be valuable to those involved in lipid formulation development if a relationship between permeability coefficients estimated using aspirates and permeability coefficients estimated using a biorelevant medium that simulates the supersaturation level observed in total aspirates was defined. However, unlike with the micellar phase, simulating total aspirates is not easy, because matching danazol concentrations would need to be accompanied by matching total lipid concentrations.

In terms of flux, high danazol concentrations in coarse lipid particles balance the reduced permeability coefficient values. Mean±SD danazol transport rates per unit surface area for the aspirates and for their micellar phases were $(1.73\pm0.85)\times10^{-3}$ and $(0.83\pm0.48)\times10^{-3}$ $(\mu g/s)/cm^2$, respectively; the difference did not reach significance ($n=5$ paired samples, $p=0.065$). Yet the trend of these data confirms previous observations, made with the use of the Caco-2 model and diluted aspirates and micellar phases (11), according to which micellar lipids are not the sole contributors to drug flux through the mucosa when the drug is administered as a triglyceride solution; coarse lipid particles play a role due to their high drug content. It seems therefore, that in addition to bypassing luminal dissolution issues, administration of drug solutions in digestible lipids can make them directly available to the mucosa for uptake.

CONCLUSIONS

Compared with data collected using the Caco-2 cell model, the impact of luminal micellar phase on passive permeability of lipophilic compounds estimated using PAMPA is less clear. For highly lipophilic compounds, permeability classification should be done cautiously. In line with previously collected data with the Caco-2 cell model, following the administration of a triglyceride solution of danazol, high concentrations in coarse lipid particles can balance in terms of drug flux the reduced permeability.

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