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Simulated intestinal fluid as transport medium in the Caco-2 cell culture model

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Abstract

The Caco-2 model is widely used as a predictive tool for the oral absorption of drug candidates. Presently, transport experiments in the Caco-2 system are usually performed in 'HBSS-like' buffers. In this paper, we investigate the possibility of using simulated intestinal buffers as donor solvent during Caco-2 experiments. Toxicity assessment of these buffers on the monolayer showed that FASSIF was compatible with the Caco-2 model for at least 2 h. On the other hand, FESSIF was toxic to the monolayer. The functionality of the Caco-2 cells was assessed by determination of the transport of model compounds and the metabolic activity of hydrolases in presence of these buffers. Similar P_{app} values for the (passive) theophyllin transport as well as for the (active) phenylalanine transport were obtained in TM and FASSIF. It was demonstrated that NaTC (present in FASSIF) had a P-gp inhibitory activity, as inclusion of NaTC in the apical compartment resulted in an increased absorptive and decreased secretory transport of CsA. The activity of the aminopeptidase enzyme was similar in both models. These results suggest that FASSIF can be used as an apical medium in the Caco-2 system. Since bile salts are also present in physiological conditions, the use of FASSIF may increase the relevance for the prediction of oral absorption using Caco-2 experiments.

Keywords: Caco-2; Fasted State Simulated Intestinal Fluid (FASSIF); Fed State Simulated Intestinal Fluid (FESSIF); Permeability

Abbreviations: A–B, apical to basolateral; B–A, basolateral to apical; CsA, cyclosporin A; FASSIF, Fasted State Simulated Intestinal Fluid; FESSIF, Fed State Simulated Intestinal Fluid; HBSS, Hanks, Balanced Salt Solution; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulphonic acid; LNAA, Large Neutral Amino Acid; MES, 2-(*N*-morpholino)ethane sulphonic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NaTC, sodium taurocholate; P_{app}, apparent permeability coefficient; SDS, sodium dodecyl sulfate; TEER, Transepithelial Electrical Resistance; TM, transport medium.

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1. Introduction

After its introduction in the early 90s as an in vitro model of the intestinal mucosa, the Caco-2 cell culture system has become a useful tool for the selection of drug candidates during the development of oral drug delivery systems (Hidalgo et al., 1989; LeCluyse and Sutton, 1997). Experiments with Caco-2 cells allow us to explore the mechanisms of drug transport, the metabolism during absorption, the modulation of transport by carriers and the mucosal toxicity (Gan and Thakker, 1997). In addition, the Caco-2 permeability data of various model drug compounds have been shown to correlate with the fractional oral absorption data in humans (Artursson and Karlsson, 1991). For the realization of permeability and transport studies with the Caco-2 cell culture model, classical buffered salt solutions are commonly used. Hanks' Balanced Salt Solution (HBSS) buffered with HEPES (10 mM) at pH 7.4 and supplemented with glucose, is a salt solution which guarantees the viability and the maintenance of the ionic equilibrium at the cell membrane during the experiment. Most transport studies performed in high-throughput screening or during drug development are done with this transport medium (TM) or a similar aqueous saline solution.

Nevertheless, many shortcomings are associated with the use of such saline solution as TM for Caco-2 experiments. One of the disadvantages of this medium is the pH, which is often buffered at 7.4 with HEPES. As the pH of the intestinal lumen has been reported to be around 6.5 (Gray and Dressman, 1996), use of solutions buffered at pH 7.4 can give erroneous results of permeation, as the pH of the solution will have an impact on the ionization grade of an ionizable compound. The feasibility to modify the pH range of the apical transport medium from 5.0 to 8.0 (with MES 20 mM or HEPES 10 mM) while keeping a good integrity of the monolayer has been reported (Palm et al., 1999) and was confirmed in our laboratory.

Another major drawback of the use of classical buffers in Caco-2 transport experiments is the low solubility often encountered with many of the compounds to be tested. The high hydrophilicity of the medium as well as the presence of counterions can negatively influence the solubility of the compounds. In this regard, efforts have been made to evaluate the possibility of using co-solvents or other components such as surfactants or bile salts (Ginski et al., 2000; Saha and Kou, 2000; Yamashita et al., 2000) (e.g. the addition of $\leq 1\%$ DMSO is commonly used to enhance the solubility of compounds to be tested in the transport medium).

But still, the use of a plain aqueous salt solution is far from the physiological conditions and one can question whether potential permeability problems encountered when using the Caco-2 cell culture model will be representative for the situation observed in vivo. Therefore, we investigated the possibility to use simulated intestinal fluid buffers as a vehicle for the solubilization of the compound applied at the donor side. Fasted State Simulated Intestinal Fluid (FASSIF) and Fed State Simulated Intestinal Fluid (FESSIF) were introduced by the group of Professor Dressman in 1998, as dissolution media to simulate the in vivo dissolution behavior of compounds (Galia et al., 1998; Dressman et al., 1998). The composition of SIF-buffers as published by Dressman et al. is

Table 1
Composition of the Fasted State Simulated Intestinal Fluid (FASSIF) and Fed State Simulated Intestinal Fluid (FESSIF)

	[a]	[b]
FASSIF		
NaTC	3 mM	5 mM
Lecithin	0.75 mM	1.5 mM
KH_2PO_4	3.9 g	3.9 g
KCl	7.7 g	16.4 g
NaOH	ad pH 6.5	ad pH 6.8
Water	ad 1 l	ad 1 l
FESSIF		
NaTC		15 mM
Lecithin		3.75 mM
KH_2PO_4		8.65 g
KCl		15.2 g
NaOH		ad pH 5.0
Water		ad 1 l

[a] Published by Galia (Galia et al., 1998).
[b] Published by Dressman (Dressman et al., 1998).

given in Table 1. The use of such buffers in the Caco-2 cell culture model would permit one to perform permeability studies in conditions that are closer to physiological conditions.

In this study, we demonstrate the compatibility and the absence of toxicity in 2 h experiments when using the FASSIF buffer in the Caco-2 system, the implication of using FASSIF buffers in the evaluation of the transport of different model compounds (for passive and active transport) and the effect of these buffers on the functionality of P-gp related efflux carriers and metabolism. We also demonstrate the toxicity of FESSIF and the influence of the different constituents of FASSIF on the integrity of the Caco-2 monolayer.

2. Materials and methods

2.1. Materials

Sodium taurocholate (NaTC) was purchased from Fluka (Bornem, Belgium). Phospholipon 90G was provided by Nattermann Phospholipid Gmbh (Köln, Germany). ^{3}H-Cyclosporin A (7 Ci/mmol) was from Amersham Life Science (Gent, Belgium), while unlabelled cyclosporin A (CsA) was obtained from Glaxo Wellcome (North Carolina, USA). Culture medium consisted of Dulbecco's Modified Eagle Medium (DMEM) containing 100 IU/ml penicillin–100 μg/ml streptomycin, 1% MEM Nonessential Amino Acids and 10% Fetal Bovine Serum. Subculturing of cells was performed using a Trypsin–EDTA solution containing 0.5 g Trypsin and 0.2 g EDTA per liter of Modified Puck's Saline A.

TM consisted of HBSS supplemented with glucose (final concentration 25 mM) and HEPES (10 mM) adjusted with NaOH 0.2 N to pH 7.4 or 6.5. All other chemicals were of the highest purity and were used as received.

2.2. Methods

2.2.1. Preparation of cell monolayers

Caco-2 cells were purchased from ATCC (Rockville, MD) and grown in 75 cm^2 Nunc flasks in an incubator at 37 °C with controlled atmosphere containing 5% CO_2 and 90% relative humidity. Caco-2 cells were seeded in Nunc's Tissue Culture Inserts with Anopore™ (Whatman Scientific Ltd.) membranes of 25 mm diameter and a pore size of 0.2 μm or on Costar Transwell polycarbonate membranes of 12 mm diameter and a pore size of 0.4 μm at a density of 90,000 cells/cm^2. The maintenance and seeding of the Caco-2 cells were performed following previously published procedures (Augustijns et al., 1998). Passage numbers from 110 to 145 were used for experiments. The confluence and integrity of the cell monolayer were controlled by measuring the transepithelial electrical resistance (TEER) and fluxes of a hydrophilic marker (sodium fluorescein). Only monolayers having TEER values above 200 Ω cm^2 were used in these experiments. All volumes added to the apical or basolateral compartment during experiments amounted to 2 ml for the 25 mm inserts and to 0.5 ml apically and 1.5 ml basolaterally for the 12 mm inserts.

2.2.2. Compatibility studies

The toxicity of the FASSIF buffer was studied for a period of incubation up to 180 min. TEER values were measured after 0, 5, 15, 30, 45, 60, 90, 120, 150 and 180 min of incubation with FASSIF. Sodium fluorescein flux was measured after 3 h of incubation.

To test the integrity of the Caco-2 monolayer in presence of FESSIF and modified compositions of FASSIF buffers, cells were incubated with 2 ml of the buffer at the donor side and TEER measurements were performed after 15, 30 and 60 min. TEER values of cells incubated with TM were taken as reference.

2.2.3. Colorimetric MTT (tetrazolium) toxicity assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) is a tetrazolium salt that is cleaved by mitochondrial dehydrogenase in living cells to give a dark blue product (Mosmann, 1983; Tada et al., 1986; Scudiero et al., 1988). For the toxicity experiment, confluent Caco-2 monolayers cultured on 12 mm diameter inserts were used. After a preincubation of 60 min with TM,

TEER values were measured and the monolayers were incubated for an additional 1, 2 or 3 h with FASSIF or for 2 h with TM or FESSIF at the apical side. In all cases TM was used as basolateral solvent. After the incubation period, the apical solutions were replaced by TM. A MTT stock solution of 5 mg/ml in TM (filtered through Gelman 0.45 μm) was prepared and added to the apical (100 μl) and basolateral (300 μl) side and plates were incubated for 4 h at 37 °C. The reaction product was solubilized with a SDS solution (10% SDS in TM). Samples were measured for absorbance at 590 nm.

2.2.4. Histological examination

Confluent Caco-2 monolayers cultured on 25 mm diameter membranes were assessed for their integrity by measuring the TEER value after a preincubation of 60 min with TM. Cell monolayers were then incubated for 2 h with 2 ml of FASSIF, TM (as reference) or FESSIF at the apical side. After the incubation period, the apical solutions were replaced by 2 ml of TM and samples were fixed in 10% neutral buffered formalin. After dehydratation, the monolayer was embedded in methacrylate, sectioned at 4 μm and stained with periodic acid Schiff (PAS) by conventional methods.

2.2.5. Transport experiments

For the determination of the transepithelial flux of the different marker molecules, Caco-2 monolayers (grown on 25 mm inserts) were first rinsed twice with TM. Inserts were then preincubated with TM for 30 or 60 min, after which TEER values were measured. The medium was replaced by TM or FASSIF containing the test compound at the donor side. After incubation, samples were collected and TEER values were measured. Samples were analyzed by HPLC or by UV spectrophotometry. After each experiment, an incubation step with sodium fluorescein (0.1% w/v) was performed for 60 min, followed by TEER measurement. The amount of sodium fluorescein appearing in the acceptor compartment was measured by UV spectrophotometry at 490 nm. Sodium fluorescein flux values across the monolayers were below 0.5%/h cm^2.

2.2.5.1. Theophyllin. Theophyllin was used as a marker molecule to assess passive transcellular transport. Transport was initiated by adding 2 ml of a solution with test compound (200 μM) to the donor side. The transport was studied in the apical to basolateral direction as well as in the basolateral to apical direction. To maintain sink conditions during the experiment, inserts were transferred every 15 min to wells containing fresh TM; when assessing the basolateral to apical transport, the whole apical compartment was withdrawn every 15 min and replaced by fresh TM. Transport was studied for a period of 45 min.

Samples were analyzed by HPLC using a Novapak C18 3.9 × 150 mm column, with KH_2PO_4 50 mM–MeOH–ACN (90:8:2; v/v/v) as mobile phase. The flow rate was 1.5 ml/min and the injection volume amounted to 100 μl. A UV spectrophotometer (270 nm) was used for detection (Waters, Belgium).

2.2.5.2. Phenylalanine. Phenylalanine is transported by the Large Neutral Amino Acid (LNAA)-carrier. To assess the polarity in transport of phenylalanine, cell monolayers were incubated with a 1 mM phenylalanine solution at the apical or at the basolateral side. In order to study the effect of competition for the carrier, transport of phenylalanine was also studied in the presence of lysine (10 mM); in this case, the preincubation step was also performed in the presence of lysine (10 mM). The absorptive transport of phenylalanine was also studied in presence of NaTC (0.3, 1 and 3 mM), in order to assess the effect of NaTC on this carrier. Samples were withdrawn from the acceptor side and analyzed by HPLC on a Novapak C18 3.9 × 150 mm column with sodium acetate 0.02 M pH 4.5–MeOH (97:3; v/v) as mobile phase. Detection occurred using a UV spectrophotometer at 215 nm (Waters, Belgium). Flow rate amounted to 1.8 ml/min and 50 μl was injected.

2.2.5.3. Cyclosporin A (CsA). In order to study the presence of active efflux mechanisms, CsA (1 μM) was used as a model compound of the P-gp efflux carrier.

The effect of FASSIF and the individual components of FASSIF on this efflux mechanism were investigated by studying their influence on the polarity in transport of CsA. After rinsing the cells, transport was initiated by adding ^{3}H–CsA (0.1 μCi) and unlabelled CsA to the donor compartment to a final concentration of 1 μM. To study the effect of FASSIF, various dilutions of FASSIF with TM (pH 6.5) were applied (0, 25, 50 and 100% FASSIF) to the apical compartment. To investigate the effect of the individual components, NaTC (0.3–3 mM in TM, pH 6.5) and lecithin (0.75 mM in TM, pH 6.5) were included in the apical compartment. Transport was assessed in both directions. Following incubation (60 min), the samples in the acceptor compartment were removed via multiple pipetting with 200 μl disposable pipette tips and placed (along with the tips) in scintillation vials (16 ml scintillation liquid, Ready Safe®, Beckman, Fullerton, CA) for liquid scintillation counting (Liquid Scintillation Counter, Wallac 1410, Beckman, Fullerton, CA). All solutions of CsA were made in siliconized glass tubes to avoid adsorption of CsA. To assure total recovery of ^{3}H–marked CsA in the basolateral acceptor compartment, 10 μl of a 1 mM unlabelled CsA solution in DMSO and 200 μl DMSO was added to the wells for 1 h before collecting the samples.

2.2.6. Aminopeptidase activity

Aminopeptidase activity was investigated by measuring the release of *p*-nitroaniline from leucine-*p*-nitroanilide. Cell monolayers were incubated for 6 min at the apical side with a solution of the substrate (100 μM) in presence or absence of the specific inhibitor of the aminopeptidase bestatin (50 μg/ml) (Umezawa and Aoyagi, 1983).

2.3. Calculations

2.3.1. Apparent permeability coefficient (P_{app})

The permeability coefficient (in cm/s) was calculated as follows:

$$P_{\mathrm{app}} = \frac{\mathrm{d}Q}{\mathrm{d}t} \times \frac{1}{A \times C_0}$$

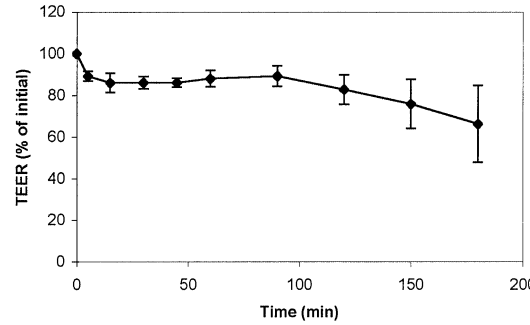

Fig. 1. Effect of FASSIF on TEER values of Caco-2 monolayers. Values represent average percentage of the initial TEER ± SD ($n = 3$).

with $\mathrm{d}Q/\mathrm{d}t$ the amount of drug appearing in the acceptor compartment in function of time (nmol/s), C_0 the initial concentration in the donor compartment (μM) and A the surface area (cm^2) across which the transport occurred.

2.3.2. Enzymatic activity (A)

The activity of the enzyme (in nmol/min cm^2) was calculated following:

$$A = \frac{V \times C}{t \times S}$$

with V the volume of the solution (ml), C the concentration of the released *p*-nitroaniline (μM), t the time of incubation (min) and S the surface of the monolayer (cm^2).

3. Results and discussion

3.1. Compatibility of simulated intestinal buffers with Caco-2

It was demonstrated that incubation of the Caco-2 monolayer with FASSIF caused an initial decrease of the TEER value (10–20%) as compared to incubation with TM (Figs. 1 and 2). However, this reduction of the TEER value was completely reversible to the initial value after rinsing with TM. This initial decrease observed can probably be attributed to a difference in ionic composition between TM and FASSIF.

As shown in Fig. 1, incubation of the Caco-2 monolayer with FASSIF for 180 min caused a continuous reduction of the TEER value. However, after 3 h incubation with FASSIF, in-house specifications for acceptable TEER values and sodium fluorescein fluxes (200 Ω cm^2 and 0.5%/h cm^2, respectively) were still met.

The impact of the different constituents of FASSIF and the toxicity of FESSIF on the integrity of Caco-2 monolayers was investigated by TEER measurement (Fig. 2). This revealed that the relative concentration of the individual components has a major influence on the observed toxicity. Indeed, increasing the NaTC concentration to 10 mM resulted in an immediate loss of monolayer integrity, while the increase of the lecithin concentration to 3 mM, elicited no toxic effects. On the other hand, FASSIF without lecithin demonstrated a high toxicity, while in absence of NaTC, no higher toxicity was observed than the parent FASSIF composition. pH and osmolarity were similar for all modified formulations. These observations demonstrate the importance of the relative concentrations of NaTC and lecithin. Bile salts and lecithin have been reported to form mixed micelles (Higuchi et al., 1987; Schwarz et al., 1997). The increased toxicity observed in the absence of lecithin or upon increasing the relative concentration of NaTC are in agreement with other studies which demonstrated that the presence of lecithin reduced the membrane toxicity caused by bile salts (Narrain et al., 1999).

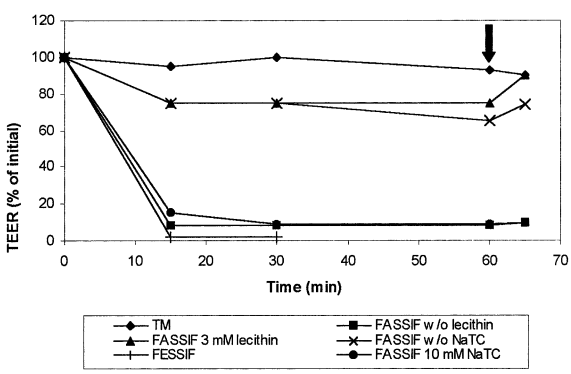

Fig. 2. Effect of FASSIF, FESSIF and modified formulations of FASSIF (based on formulation b of Table 1) on the TEER values of Caco-2 monolayers. TEER values were measured after an incubation period of 15, 30 or 60 min. After 60 min (indicated by the arrow) monolayers were rinsed with TM and TEER values were measured again.

Incubation of the Caco-2 monolayer with FESSIF showed an immediate decrease of the TEER value. The low pH of the buffer (pH 5.0) along with the high relative NaTC concentration (15 mM) as well as the high osmolarity of the solution (600 mOsm) may be responsible for the observed toxicity.

The presence of NaTC (up to 3 mM) in TM did not show any significant decrease of the TEER value as compared to an incubation with TM (data not shown).

3.2. MTT toxicity assessment

The MTT dye conversion following a 4 h incubation period is presented as the percentage of the absorbance of the control condition (TM, 2 h incubation). Absorbance at 590 nm was very low for cells treated with FESSIF ($5.4 \pm 0.3\%$) illustrating the high toxic effect of FESSIF on the cell monolayer. No significant difference in MTT transformation is observed for cells treated with FASSIF for 1 ($100.7 \pm 4.0\%$), 2 ($117.8 \pm 17.8\%$), or 3 ($96.9 \pm 20.1\%$) h, as compared with the negative control. These data suggest that cells treated with FASSIF are still viable and possess active forms of mitochondrial dehydrogenase.

3.3. Histological examination

In Fig. 3, photomicrographs of the Caco-2 monolayer are shown after a 2 h incubation period with TM (A), FASSIF (B) and FESSIF (C), respectively. No major differences are observed between the cells incubated with TM and those incubated with FASSIF. On the other hand, when incubated in presence of FESSIF, the morphology of the cells is completely altered: cells are no longer adherent to the membrane, have irregular nuclei and glycogen (dark spots) is no longer present.

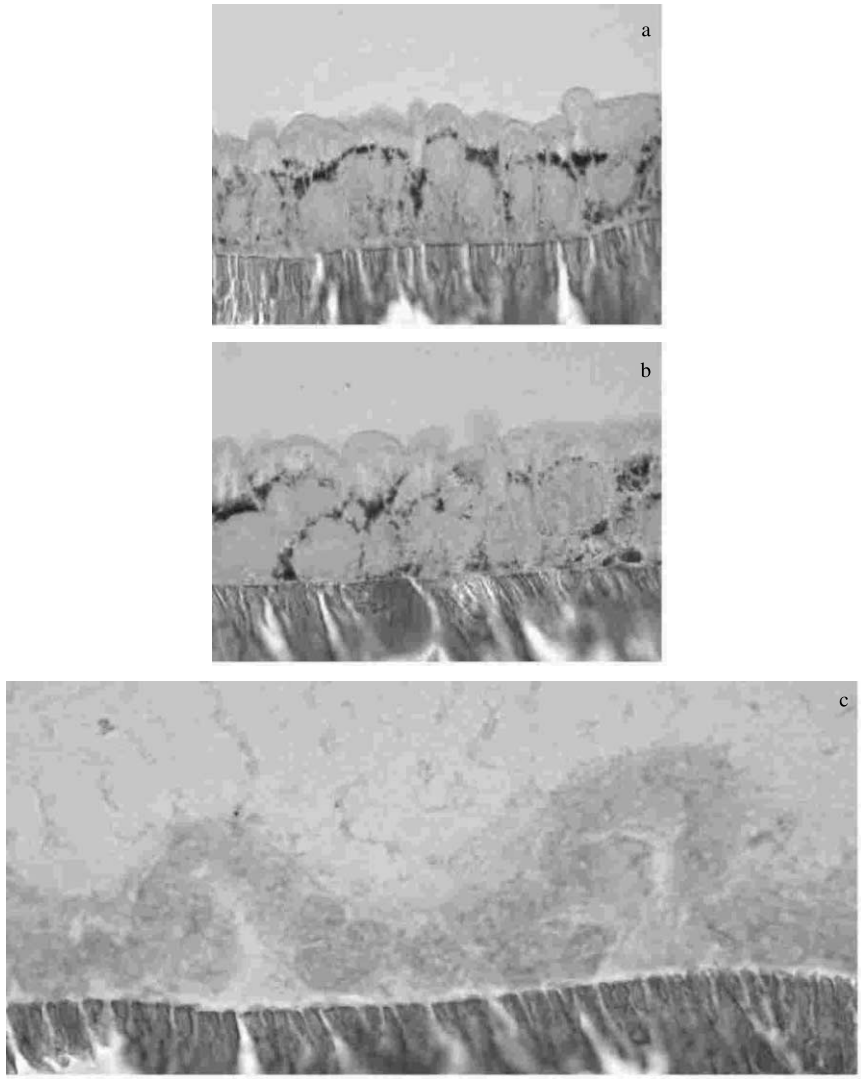

Fig. 3. Histological evaluation and comparison of the effect of FASSIF and FESSIF on the morphology of the cultured Caco-2 monolayers after PAS staining. Glycogen appears as dark spots. (A) control; (B) FASSIF 2 h; (C) FESSIF 2 h.

3.4. Transport experiments

Model compounds for passive transcellular and active (absorptive and efflux) transport were assessed for their transport characteristics when using TM or FASSIF as donor medium. The results are summarized in Table 2.

3.4.1. Theophyllin

No significant difference between TM and FASSIF was observed for the transport of theophyllin. In both cases, similar P_{app} values were obtained. No decrease of transport flux was observed when using FASSIF. For other compounds with passive transport characteristics, it

Table 2
P_{app} values for theophyllin (200 μM), phenylalanine (1 mM) [in absence and presence of lysine (10 mM)] or NaTC (0.3, 1 or 3 mM) and CsA (1 μM) and enzymatic activity of the aminopeptidase when using TM or FASSIF as donor solvent

	TM		FASSIF	
	P_{app} (A–B) ($\times 10^6$ cm/s)	P_{app} (B–A) ($\times 10^6$ cm/s)	P_{app} (A–B) ($\times 10^6$ cm/s)	P_{app} (B–A) ($\times 10^6$ cm/s)
Theophyllin (200 μM)	23.2(±0.3)	26.6(±0.6)	28.0(±0.4)	27.2(±0.4)
Phenylalanine (1 mM)	6.8(±0.4)	0.7(±0.2)	6.1(±0.4)	0.5(±0.1)
+Lysine (10 mM)	2.3(±0.3)		2.3(±0.4)	
+NaTC (0.3 mM)	7.3(±0.4)			
+NaTC (1 mM)	7.4(±0.2)			
+NaTC (3 mM)	7.3(±0.4)			
CsA (1 μM)	2.6(±0.2)	20.2(±1.2)	7.2(±0.2)	7.2(±0.6)
	A (nmol/min cm^2)		A (nmol/min cm^2)	
Aminopeptidase	1.48(±0.1)		1.60(±0.3)	

Values are average values of three experiments (±SD).

may be possible that a reduction of absorptive transport occurs when using FASSIF due to a decrease in free concentration of the compound by micellar inclusion.

3.4.2. Phenylalanine

Phenylalanine is transported by the LNAA transporter which has been reported to be functionally present in Caco-2 cells (Hidalgo and Borchardt, 1990). The observed polarity in transport was similar for the transport in TM and in FASSIF. In presence of 10 mM lysine (a competitive inhibitor of the carrier) a significant decrease of phenylalanine transport was observed in FASSIF and TM. The presence of NaTC (0.3–3 mM) in TM buffer had no effect on the absorptive transport of phenylalanine (Table 2). The observed results suggest that no major difference is present in the activity and functional expression of this carrier when performing the experiment in FASSIF, nor in presence of NaTC.

3.4.3. CsA

The functional activity of P-gp efflux mechanisms in the Caco-2 cell culture model is shown by using ^{3}H–CsA as a model compound. Evidence for a polarized efflux system in Caco-2 cells modulating the CsA transport had been demonstrated before (Augustijns et al., 1993) and was confirmed in this study as illustrated by the fact that, when using TM, the apical to basolateral transport of CsA was much lower than in the reverse direction (Fig. 4). However, when FASSIF or dilutions of FASSIF with TM (50:50 and 25:75) were used in the apical compartment, a concentration dependent increase in absorptive transport of CsA, as well as a concentration dependent decrease of the secretory transport of CsA, could be observed. These results suggest an

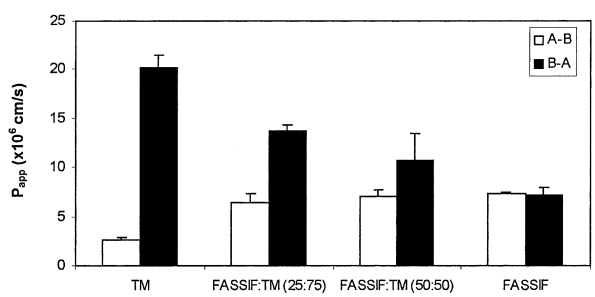

Fig. 4. Transport of CsA in the apical to basolateral or in opposite direction after addition of CsA (1 μM) to the donor side in TM, FASSIF or dilutions of FASSIF. Bars represent average P_{app} value ± SD ($n = 3$).

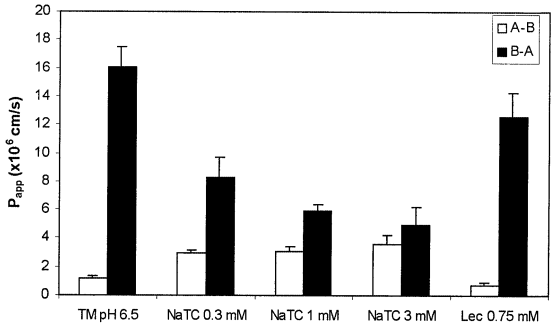

Fig. 5. Effect of lecithin (0.75 mM) and NaTC (0.3, 1 or 3 mM added at the apical side) on the absorptive and secretory transport of CsA (1 μM). Bars represent average P_{app} value ± SD ($n = 3$).

inhibitory effect of compounds present in FASSIF on the modulating function of efflux carriers.

Subsequently, the inhibitory effect of the separate constituents of FASSIF on the function of efflux carriers was assessed. Fig. 5 shows that the addition of lecithin to the apical compartment did not result in an increased absorptive transport, nor in an inhibition of the secretory activity of efflux carriers. In contrast to this observation, the application of NaTC to the apical side of the monolayers resulted in a concentration dependent increase of the absorptive and a concentration dependent decrease of the secretory transport of CsA.

3.5. Aminopeptidase activity

The family of aminopeptidases belongs to the numerous hydrolases of the intestinal brush border of the small intestine which is present in an active form in the Caco-2 cell culture model (Hauri et al., 1985; Howell et al., 1992). Aminopeptidases can contribute (along with other peptidases and proteases) to the metabolism of peptides and proteins, and thus to limited intestinal absorption of peptides. The observed aminopeptidase activity was similar when using TM as apical compartment medium as when using FASSIF (1.48 ± 0.06 and 1.60 ± 0.26 nmol/min cm^2, respectively), suggesting that use of FASSIF in the Caco-2 cell culture model does not alter the activity of this hydrolase. Bestatin (50 μg/ml) inhibited the activity of the aminopeptidase in both cases (0.99 ± 0.02 nmol/min cm^2 in TM and 0.65 ± 0.17 nmol/min cm^2 in FASSIF).

4. Conclusions

In this paper we demonstrate the compatibility of the FASSIF buffer with the Caco-2 cell monolayer for 2 h experiments. By assessing the toxicity of the FASSIF and FESSIF buffers, it was shown that FASSIF did not alter the morphology and integrity of the Caco-2 monolayer. On the other hand, FESSIF is not suitable for use in the Caco-2 system. As shown in Table 2, P_{app} values for different tested model compounds (theophyllin and phenylalanine) and the activity of the aminopeptidase were similar when using the classical TM buffer and the FASSIF buffers. However, we demonstrated a concentration dependent P-gp inhibitory activity of NaTC (present in FASSIF) when assessing the CsA transport (Fig. 5). NaTC (up to 3 mM) had no effect on the monolayer integrity, nor on the absorptive transport of phenylalanine. The results of the study illustrate that the use of FASSIF may mask the effect of efflux carriers on total drug absorption. On the other hand, considering the presence of NaTC in bile fluids, the results of the study suggest that the contribution of P-gp efflux carriers may be overestimated when using HBSS-like salt buffer solutions.

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