European Journal of Pharmaceutics and Biopharmaceutics 79 (2011) 416–422

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Effect of the non-ionic surfactant Poloxamer 188 on passive permeability of poorly soluble drugs across Caco-2 cell monolayers

Sarah Maud Fischer [a,b,1], Martin Brandl [a,1], Gert Fricker [b,*]

[a] Department of Physics and Chemistry, University of Southern Denmark, Odense, Denmark
[b] Department of Pharmaceutical Technology and Biopharmacy, University of Heidelberg, Heidelberg, Germany

ARTICLE INFO

Article history:
Received 7 January 2011
Accepted in revised form 19 April 2011
Available online 28 April 2011

Keywords:
Passive drug permeability
Micelle
Solubilisation
Pluronic
Ketoprofen
Nadolol
Trans-epithelial electrical resistance

ABSTRACT

Drug permeability of the model drugs ketoprofen and nadolol across Caco-2 cell monolayers was determined in the absence and presence of the non-ionic surfactant Poloxamer 188 (Pluronic® F68, P-188). Stringent controls confirmed that P-188 in concentrations up to 50 mg/ml did not adversely affect cell viability or monolayer integrity. Equilibrium experiments confirmed that the drugs were merely passively transported. Caco-2 permeability of both drugs was found to be decreased by the surfactant in a concentration-dependent manner. Ultrafiltration revealed that both drugs were associated with surfactant micelles. The systematic investigation of micellization on passive absorption showed that association of drugs with P-188 micelles appears to depress their passive permeability under conditions where other transport mechanisms can be neglected.

1. Introduction

Due to greater patient convenience and thus enhanced compliance, the oral route is the preferred route of drug administration. In recent years, many new chemical entities (NCEs) that are poorly soluble in aqueous medium have been identified. Such unfavourable solubility characteristics are usually accompanied by poor bioavailability with the result that modern oral dosage form design is predominantly focussing on drug delivery systems providing oral bioavailability enhancement. Besides selection of the most soluble salt form and synthesis of prodrugs with enhanced solubility, modification of the solid state [1–3] is a well-acknowledged approach. Among the advanced formulations, cyclodextrins and surfactants or lipids are most commonly used [4–8]. Literature illustrates the successful use of solubility-enhancing formulations to improve the bioavailability of poorly soluble drugs [9–11], although in some cases the opposite has been reported [12]. The solubility-enhancing effect of surfactant-containing formulations in the context of micellar solubilization of a drug has been thoroughly studied. In contrast, investigations regarding the effects of surfactants on drug permeability remain scarce and contradictory. However, more recently, a small number of solubilizing agents have been studied in *in vitro* permeability tests. Their role was partly to overcome recovery and detection limit challenges [13], while also to investigate their influence on permeability [14]. In the present literature, results are contradicting: there are indications for surfactant influences on passive drug perfusion via barrier interaction [15], via inhibition of P-glycoprotein (P-gp) [16], via micellar solubilization [17], ion-pair formation [18] or membrane fluidization [19]. A further complicating factor is that the Caco-2 cell permeation model employed in most of the aforementioned studies exhibits several parallel drug transport pathways: transcellular, paracellular, carrier-mediated and endo-/transcytotic transport. However, a systematic investigation of the interplay of such factors by which a surfactant may act on solubility as well as permeability is missing. As a result, this renders it difficult to identify precisely the direct impact of a given excipient.

Thus, for the current study, we have chosen two model drugs, ketoprofen and nadolol, which have been described to be passively absorbed [20,21], because we wanted to exclude interaction with active transport systems. As a surfactant, we have chosen Poloxamer 188 (P-188), which is commonly used in oral drug formulations, mostly as a bio-enhancer [22–25], and which has shown to have a low impact on Caco-2 cell viability [26]. For permeability experiments, stringent controls for monolayer integrity and functionality have been employed in terms of trans-epithelial electrical resistance (TEER) measurement over time in combination with permeability assessment of a paracellular marker (carboxyfluorescein). In our opinion, considerations regarding the integrity control

* Corresponding author. University of Heidelberg, Institute of Pharmacy and Molecular Biotechnology, Department of Pharmaceutical Technology and Biopharmacy, Im Neuenheimer Feld 366, 69120 Heidelberg, Germany. Tel.: +49 6221548330; fax: +49 6221545971.

E-mail addresses: smf@ifk.sdu.dk (S.M. Fischer), mmb@ifk.sdu.dk (M. Brandl), gert.fricker@uni-hd.de (G. Fricker).

1 Tel.: +45 65502525, fax: +45 66158760.

0939-6411/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2011.04.010

have not been sufficiently taken into account in other permeability studies when surfactants were present.

The aim of the current study was to systematically determine the influence of the non-ionic surfactant P-188 on passive permeability of the two poorly water soluble model drugs, ketoprofen and nadolol. In order to exclude other potential influences, we have stringently monitored Caco-2 cell viability of the surfactant, maintenance of monolayer integrity and absence of alternative pathways, such as active transport.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS) and supplements were supplied by Biochrom (Berlin, Germany). Rat tail collagen was purchased from Roche (Mannheim, Germany). The buffer used in all experiments was Hanks' Balanced Salt Solution (Sigma–Aldrich Chemie GmbH, Munich, Germany) containing KCl 0.40 g, KH_2PO_4 0.06 g, NaCl 8.0 g, Na_2HPO_4 0.048 g, D-glucose 1 g, $CaCl_2 \times 2H_2O$ 0.185 g and supplemented with $MgSO_4 \times 7H_2O$ 0.98 g, $NaHCO_3$ 0.35 g (1000 ml) and was adjusted to pH 7.4. Poloxamer 188 (Pluronic® F68, Lutrol® F68, P-188) was kindly provided by BASF SE, Ludwigshafen, Germany. Triton X-100, 5(6)-carboxyfluorescein, ketoprofen, and nadolol, rhodamine 123 as well as all other chemicals were purchased from Sigma–Aldrich Chemie GmbH, Munich, Germany.

2.2. Cell culture

Caco-2 cells were cultured as described in [27]. In brief, Caco-2 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Biochrom, Berlin, Germany), which was supplemented with 10% FBS, 1% non-essential amino acids, 1% pyruvate, 1% L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in 5% CO_2 atmosphere in equilibrium with distilled water. The medium was changed every other day, and the cells were split at 80% confluency. For experiments, cells were used at passage number 34–44.

2.3. Preparation of sample solutions

All drug and surfactant solutions were prepared in Hanks' Balanced Salt Solution (HBSS++) and were finally adjusted to pH 7.4. In cell studies, the concentrations of the paracellular marker carboxyfluorescein, the P-gp substrate rhodamine 123 and the two model drugs ketoprofen and nadolol were 0.02, 0.05, 2.6 and 7.5 mM, respectively. The carboxyfluorescein concentration of 0.2 mM was previously found to be appropriate to provide reliable permeability data [14]. Rhodamine 123 at a concentration of 0.05 mM was found to give reproducible results in equilibrium experiments (data not shown). The concentrations of the two model drugs were chosen so as to be well below the saturation limits (at pH 7.4) yet at the same time to yield reliably detectable receiver concentrations during permeation studies. For additional information regarding the model drugs, an overview of their physicochemical properties is provided in Table 1.

2.4. Viability studies of Caco-2 cells

For cell viability studies, the alamarBlue® assay was employed. The underlying principle is that alamarBlue®, a reduction–oxidation (redox) dye, monitors the reductive environment of cell growth by being transformed into the reduced form, i.e. is transformed from its blue (oxidized) to its red (reduced) form. Hereby, it offers a quantitative analysis of cell viability measured by fluorescence spectroscopy. For experiments, the Caco-2 cells were seeded on rat tail collagen-coated 96-well plates at a density of 65,000/cm^2 and were used after 14 days. Cells were maintained under the same conditions as described above. Prior to experiments, the cells were washed twice with HBSS++ and were then incubated over 6 h at 37 °C with sample solutions of different concentrations of P-188. In case of the negative control, cells were incubated only with HBSS++, while for the positive control, Triton X-100 1% dissolved in HBSS++ was used. Thereafter, the cells were washed again with HBSS++ and then the indicator dye alamarBlue® was added. After 3 h of incubation (37 °C), the change of the redox dye was determined by fluorescent spectroscopy (excitation wavelength 530 nm, emission wavelength 590 nm). Cell viability (VIAB) was then calculated according to the following equation: VIAB = SAMPLE/CONTROL × 100%, where CONTROL is the emission of the cells incubated with HBSS++ only and SAMPLE the emission of cells incubated with either P-188 or Triton X-100 solutions.

Table 1
Physico-chemical parameters for the two model compounds.

Compound	MW*	pK_a**	log p**	Aqueous solubility***	PhEur 7.0
Ketoprofen	254.3	4.6	3.12	0.051	Practically insoluble in water
Nadolol	309.4	9.4	0.71	8.33	Slightly soluble in water

* Molecular weight (MW) in g/mol.
** From [37].
*** from [38] in mg/ml (unbuffered).

2.5. Equilibrium and permeation experiments across Caco-2 cell monolayers

For equilibrium and permeation experiments, Caco-2 cells were seeded on rat tail collagen-coated polyester filters (Transwell® Permeable Supports, 12 mm, Corning Inc., New York) at a density of 80,000 cells/cm^2. The medium was changed every other day, and they were used for experiments after 14–15 days. Twelve hours prior to experiments, the supplemented DMEM was changed to phenol red-free DMEM that was unsupplemented. Before the actual experiment, the cell monolayers were washed twice with HBSS++ and incubated for ~1 h. Then, in case of equilibrium experiments, the buffer both in the apical and basolateral compartment was removed and replaced by sample solution. These experiments were performed using the same concentration of drug dissolved in HBSS++ (pH 7.4) in the apical and the basolateral compartment. After incubation over 4 h while the culture plates were shaken (≃50 rpm), the concentration of the drug in both compartments was measured and compared with the initial concentration in order to see whether a directed (active) transport was involved.

For permeation experiments, the buffer in the apical compartment was removed after washing and incubation and replaced by sample solution. These experiments were performed by applying a certain drug concentration at the apical side, whereas the basolateral side contained only HBSS++. Over time periods of 3.5 h (carboxyfluorescein and nadolol) and 3 h (ketoprofen), the inserts were moved to fresh wells containing HBSS++ at time intervals of 30–45 min in order to ensure sink conditions. The inserts containing plates were shaken throughout the experiment (≃50 rpm). The concentration at the basolateral side was measured. After the experiment, the concentration at the apical side was also quantified to check mass balance. Mass balances were in the range of 93–99%. The cumulative amount of drug that had permeated through the monolayer, i.e. measured in the basolateral compartment, was plotted against time giving the cumulative flux. When the flux reached steady state, i.e. the slope was linear, the apparent permeability coefficient (P_{app}) was calculated according the following equation. $P_{app} = dm/dt \cdot (1/A{\cdot}c^0)$, where dm is the cumulative amount of drug permeated by the time dt, A is the area of the insert used and c^0 is the initial donor concentration. Steady-state conditions, i.e. linear dependency between cumulative flux and time ($R^2 \geqslant 0.99$), was found to be achieved after 1 h for both, ketoprofen and nadolol. In the case of carboxyfluorescein, steady state of the flux was reached after 1.5 h ($R^2 \geqslant 0.98$). For calculation of the P_{app}, five remaining points of the linear parts of the flux curves were used in order to calculate the P_{app} values.

2.6. Trans-epithelial electrical resistance measurements

Trans-epithelial electrical resistance (TEER) measurements were performed in a cellZscope® apparatus (Nanoanalytics, Münster, Germany), an automated cell monitoring system, where cell inserts were placed inside, and the TEER was determined by continuously measuring the frequency-dependent impedance of the cell layer [27]. This was done in parallel (up to 24 inserts) over time. The volume at the apical and basolateral side was 0.6 ml and 1.0 ml, respectively.

2.7. Ultrafiltration experiments

Ultrafiltration experiments were performed using Amicon Ultra-15 Centrifugal Filter Units with regenerated cellulose (Ultracel-10) membranes (MWCO = 10 kDa), Millipore GmbH, Schwalbach, Germany. Prior to experiments, the filter units were filled with HBSS++ and centrifuged (25 °C, 4000*g*, 2 min) in order to wash the membrane filter. Sample solution was then poured in the filter unit, and the tube was centrifuged again (25 °C, 4000g, 2 min) to saturate the filter membrane. The ultrafiltrate was discarded. Afterwards, the tube was centrifuged a third time (25 °C, 4000*g*, 5 min), and the amount of drug in the ultrafiltrate was analysed. Preliminary experiments had been performed to ensure constant levels in the ultrafiltrate (data not shown). Subsequently, 5-min centrifugation was chosen due to a sufficient amount of sample for analysing and reproducible concentrations of compounds. Finally, the relative recovery was obtained by dividing the ultrafiltrate concentration by the initial concentration.

2.8. Analysis

The alamarBlue® indicator dye as well as carboxyfluorescein was analysed by fluorescence spectroscopy using a Fluoroskan Ascent® plate reader (excitation wavelength 530 and 485 nm, emission wavelength 590 and 520 nm, respectively). Ketoprofen and nadolol were analysed using a Dionex Ultimate 3000 HPLC with photodiode array detection (Ultimate 3000 Photodiode Array Detector). Separation was performed by employment of an Acclaim® 120 (C18, 5 μm particle size, 120 Å, 4.6 × 250 mm) column. Mobile phases, gradients and wavelengths are listed in Table 2. The software used was Chromeleon 6.80, Dionex GmbH, Idstein, Germany.

2.9. Statistical analysis

Origin 6.0 (OriginLab Corporation, Northampton, MA, USA) was used for the statistical analysis. Comparison of two means was performed by applying an unpaired *t*-test (two-tailed) where $p < 0.05$ was considered as statistical significant.

Table 2
HPLC parameters for ketoprofen and nadolol.

Compound	Mobile phase (%)			Run time (min)	Flow (ml/min)	λ (nm)
	H_2O[a]	Methanol[a]	Acetonitrile[a]			
Ketoprofen	20	80	–	0	0.08	260
	20	80	–	12		
Nadolol	90	–	10	0	1.0	220
	70	–	30	6		
	70	–	30	10		
	90	–	10	18		

[a] Mobile phases contained 0.05% trifluoroacetic acid.

3. Results

3.1. Cell viability and monolayer integrity of Caco-2 cells in the presence of P-188

Prior to performance of permeation studies across Caco-2 cell monolayers, cell viability and monolayer integrity were analysed in the presence of increasing concentrations of P-188. All P-188 concentrations used were above the CMC (0.26% w/w) [28]. HBSS++, the buffer used in all experiments, served as a (negative) control. Triton X-100, which is known to rapidly dissolve cell membranes, served as a positive control. For viability studies, the cells reductive capacity on alamarBlue® was measured in order to confirm whether metabolic activity was maintained. After incubation over 6 h with P-188 (up to 50 mg/ml), no significant change of viability as compared to the control was observed (Fig. 1). Apparently, P-188 did not negatively affect cell viability. Furthermore, in order to find out whether P-188 affects the integrity of the cell monolayer barrier, we measured the TEER before and after adding P-188 solution to the donor compartment and subsequently over a time range of 4 h. As shown in Fig. 2, the TEER was not changed in the presence of P-188 as compared to HBSS++, whereas in the positive control, Triton X-100 caused the TEER to drop rapidly and drastically. The decrease in TEER after incubation with HBSS++ was due to aspiration of buffer and adding of sample solutions ($t = 0$) and occurred when the control as well as surfactant solutions were used. This decrease was relatively low and the TEER

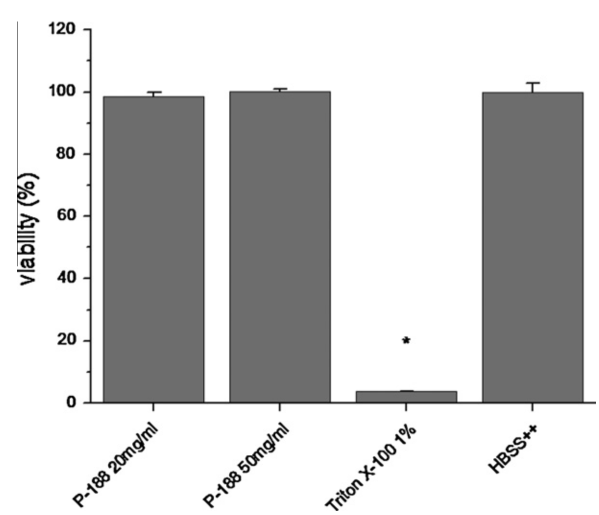

Fig. 1. Viability values obtained from alamarBlue® cytotoxicity assay after incubation (6 h) with P-188 (Poloxamer 188), Triton X-100 (positive control) and HBSS++ (negative control). Data are shown as percentage of the negative control (=100%). Values are given as mean ± SEM, $n = 8$. Significant differences with HBSS++ are marked with * ($p < 0.05$).

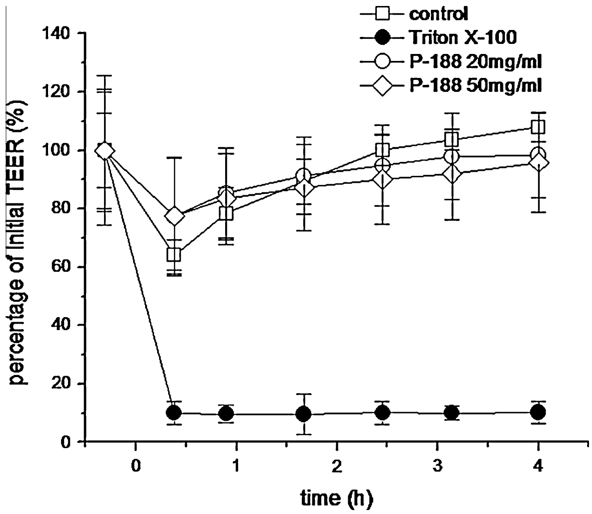

Fig. 2. Trans-epithelial electrical resistance (TEER) according to the time in the absence (control) and presence of P-188 (Poloxamer 188). Triton X-100 (1%v/v) served as positive control. Data are given as percentage of the initial TEER (prior to experiment). Values are given as mean ± SEM, $n \geqslant 8$ for the control and P-188 experiments, and n = 5 for Triton X-100.

level re-established with time. In parallel, we determined permeability of the paracellular marker carboxyfluorescein. Permeability values were not enhanced in the presence of various P-188 concentrations up to 50 mg/ml (see Fig. 3). Maintenance of high TEER values and low paracellular marker permeability, as observed here with all P-188 concentrations, is usually interpreted as an indication for closed (intact) tight junctions. This is consistent with other Caco-2 cell studies, where different poloxamers were used [29]. Surprisingly, carboxyfluorescein permeability was found to be reduced at the highest P-188 concentration, an effect, which cannot easily be explained. It can potentially be attributed to an unspecific interaction of P-188 with carboxyfluorescein.

3.2. Influence of P-188 on the permeation of poorly soluble model drugs (ketoprofen and nadolol)

Permeation of the two poorly water soluble model drugs ketoprofen and nadolol was examined both in the absence and in the presence of P-188. P-188 was used at concentrations of 10, 20 and 50 mg/ml. Each permeation study was repeated three times with four parallels each. Permeability values in relation to P-188 concentration are given in Figs. 4 and 5. The permeation of both drugs was found to be significantly decreased in the presence of P-188. For ketoprofen (Fig. 4), a highly reproducible decrease in P_{app} with increasing P-188 concentration was observed. For nadolol, a similar, yet more variable tendency could be observed (Fig. 5). This variability may be due to the fact that nadolol is more hydrophilic. Consequently, the proportion of nadolol that is paracellularly transported compared with the overall permeability is higher than when compared with ketoprofen, which is more lipophilic. Interestingly, for batches of monolayers, where the barriers were leakier, both prior to and after the permeability experiment as indicated by a relatively lower TEER, P-188 induced a stronger depression of nadolol permeability than for relatively tighter monolayers. Despite this variability, the effect was apparent in all parallels.

3.3. Equilibrium permeation of ketoprofen and nadolol

Ketoprofen and nadolol were chosen as model drugs for the passive permeability pathways. In order to see whether a directed transport was involved, Caco-2 cell monolayers were incubated over 4 h with drug solution of the same concentration at the apical and basolateral side. As expected, the equilibrium was not disturbed for ketoprofen and nadolol, while the P-gp-substrate rhodamine 123 showed the expected accumulation on the apical side (Fig. 6). This allows the assumption that ketoprofen and nadolol are passively transported at the concentrations tested in our model here and that interactions with active transporters can be neglected.

3.4. Quantitative analysis of the fraction of free drug upon separation from micelle-bound drug

In order to find out whether or not the two model drugs ketoprofen and nadolol interact with surfactant micelles, ultrafiltration across cellulose filters was employed to separate molecularly dissolved drug from its micelle-associated form. The chosen cut-off (MWCO = 10 kDa) was expected to be such that surfactant micelles would not pass. The concentration of drug in the ultrafiltrate was quantified in comparison with that prior to fractionation. The

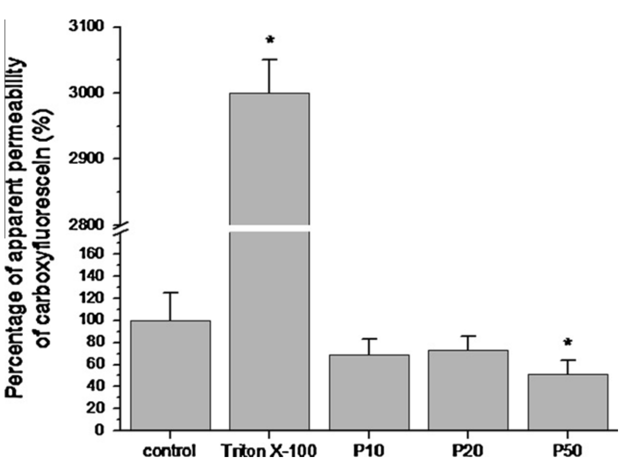

Fig. 3. Apparent permeability (P_{app}) of carboxyfluroescein in the absence and presence of different concentrations of Poloxamer 188 (P-188) (P10-P50 = 10, 20 and 50 mg/ml, respectively). Data are given as percentage of the control (concentration of P-188 = 0 mg/ml). Values are given as mean ± SEM, $n \geqslant 8$ for the control and P-188 experiments, $n = 5$ for Triton X-100 (1%v/v). Significant differences with the control are marked with * ($p < 0.05$).

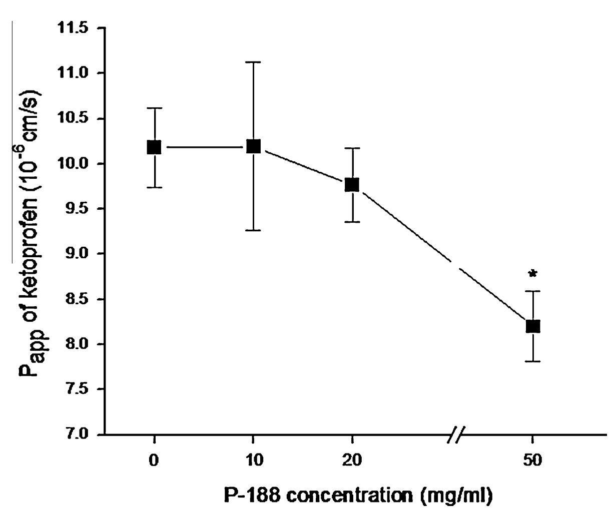

Fig. 4. Permeation of ketoprofen in the presence of different concentrations of Poloxamer 188 (P-188). Values are given as mean of three independent replicates with $n = 4$ each ±SEM, $n = 12$. Significant differences are marked with * ($p < 0.05$).

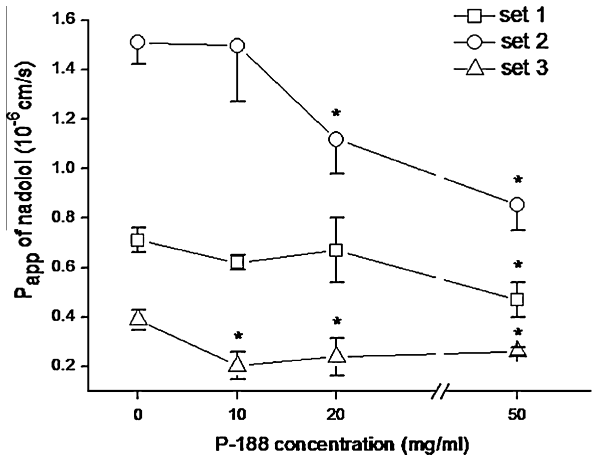

Fig. 5. Three independent sets of nadolol permeability at different concentrations of Poloxamer 188 (P-188). Values are given as mean ± SEM, $n = 4$. Significant differences are marked with * ($p < 0.05$).

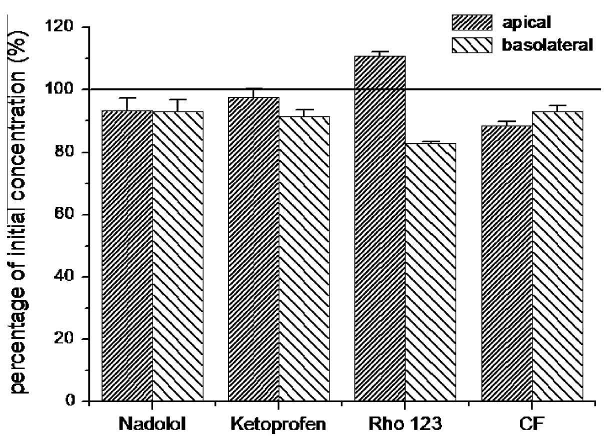

Fig. 6. Equilibrium of drug after 4 h of incubation. Concentrations of drugs were 7.5, 2.6, 0.05 and 0.02 mM for nadolol, ketoprofen, rhodamine 123 (Rho123) and carboxyfluorescein (CF), respectively.

ultrafiltration results are listed in Table 3. In the absence of surfactant, the concentration in the ultrafiltrate of both ketoprofen and nadolol was identical to the initial concentration, which enabled exclusion of unspecific loss of drug in the system. In contrast, in the presence of P-188, the concentrations of both ketoprofen and nadolol in the ultrafiltrate were found to be significantly lower. As evidenced from Table 3, there existed a correlation between surfactant concentration and fraction of micelle-bound drug. As expected, the more lipophilic ketoprofen showed a stronger tendency to associate with P-188 micelles than nadolol.

4. Discussion

Our permeability studies with the model drugs ketoprofen and nadolol showed a significant decrease in apparent permeability for both drugs in the presence of P-188. The effect was dependent on the surfactant concentration.

In contrast, the vast majority of cellular drug permeability studies report a permeability enhancing effect of both ionic and non-ionic surfactants [30–32]. However, these effects were often accompanied by a decreased trans-epithelial electrical resistance (TEER) and/or cell viability. In our case, where both impaired cell viability and/or monolayer integrity was ruled out by stringent controls, the opposite effect was seen. Under conditions where an unaffected mannitol-flux and TEER confirmed integrity of the cell monolayer, Saha et al. found for three proprietary drug compounds either no effect or a permeability enhancement with P-188 (1%) using the Caco-2 cell-model [17]. But controls on whether the drug compounds were subject for other pathways than transcellular diffusion were not reported. In another study, a complex interaction of passive and active drug transport with various non-ionic surfactants of the poloxamer type was observed [33]: poloxamers were found to be permeability enhancers, mostly due to inhibition of efflux pumps in connection with a lowering of membrane fluidity [29,34]. For the drugs in our study design, carrier-mediated transport was proven to be irrelevant, and a P-188 effect on the latter can thus be ruled out. There are few studies where comparable surfactants were shown to retard permeation: Neuhoff et al. reported a decrease in Caco-2 permeability of felodipine in the presence of Cremophor [35]. Katneni et al. reported for the poorly soluble drug diazepam an inverse correlation of excised rat jejunum permeability with micellar solubilization using polysorbate 80 and polyoxyl 35 castor oil [36]. This effect was attributed to the reduced thermodynamic activity of the drug, and/or the fact that the micelle-bound fraction of drug is not readily permeable.

We found that the micellar fraction of drug, as determined by ultrafiltration experiments (Table 3) correlated with the surfactant concentration, for both ketoprofen and nadolol. Despite the facts that the more lipophilic ketoprofen associated with P-188 micelles to a higher percentage than nadolol and ketoprofen being more permeable than nadolol, a significant P-188-induced depression of drug permeability was seen with both drugs. This may indicate that micellar encapsulation is one possible reason for suppressed permeability but not the only one.

Table 3
Free amount of drug in P-188 solutions ($n = 3$).

Compound	Medium	Mean initial concentration ± SEM (mM)	Mean concentration in ultrafiltrate ± SEM (mM)	Fraction of non-micellar drug [a] (%)
Ketoprofen	HBSS++	4.43 ± 0.03	4.36 ± 0.02	98.4 ± 0.2
	P-188 10 mg/ml	4.37 ± 0.04	4.18 ± 0.05*	95.6 ± 0.3
	P-188 20 mg/ml	4.45 ± 0.04	4.05 ± 0.08*	91.0 ± 1.8
	P-188 50 mg/ml	4.42 ± 0.10	3.69 ± 0.04*	83.5 ± 2.0
Nadolol	HBSS++	8.04 ± 0.11	8.02 ± 0.06	99.8 ± 1.2
	P-188 10 mg/ml	8.04 ± 0.04	7.91 ± 0.06	98.4 ± 0.3
	P-188 20 mg/ml	8.06 ± 0.14	7.73 ± 0.04*	95.9 ± 1.5
	P-188 50 mg/ml	8.19 ± 0.02	7.80 ± 0.11*	95.3 ± 1.0

P-188 (Poloxamer 188) was dissolved in HBSS++.
[a] The fraction of non-micellar drug is calculated as the concentration of the ultrafiltrate divided by the initial concentration and given as percentage.
* Significant differences of the ultrafiltrate concentrations are marked with ($p \leqslant 0.05$).

In total, micellar drug incorporation was relatively low, which might be due to the fact that all solutions were adjusted to pH 7.4. Hence, the drugs were (partly) charged and thus better soluble in the water phase. But it should be emphasized that drug incorporation was studied when all drug was dissolved, which was possible at the chosen pH.

Surprisingly, we observed a decrease in carboxyfluorescein permeability, at the highest P-188 concentration (50 mg/ml) used, although carboxyfluorescein due to its hydrophilicity is not expected to associate with P-188 micelles. Thus, micellar association should not be the only reason for the observed decreased permeability. One may speculate that the decrease in carboxyfluorescein permeability might be due to either a change in viscosity of the aqueous donor compartment or an unspecific interaction with P-188. At the same time, there was no increase in TEER across the monolayer seen at any P-188 concentration, indicating unaltered tight junctions.

5. Conclusions

Based on the above findings with the Caco-2 model, where stringent controls confirmed both monolayer integrity and cell viability, it could be shown that the non-ionic surfactant Poloxamer 188 depressed permeability of the two model drugs ketoprofen and nadolol. Both ketoprofen and nadolol are described in literature not to be substrates of active transport mechanisms, a conclusion that is supported by our equilibrium studies. Thus, the P-188-induced change in drug permeability observed here is not regarded to be due to an interaction of P-188 with transporters. Furthermore, the fact that P-188 does not significantly influence TEER renders a direct interference of P-188 with tight junctions unlikely. Both permeability depression and the extent of micelle association of the drugs were found to correlate with surfactant concentration suggesting that the latter is one reason for the reduced drug permeability. The innovative aspect of the present study is the investigation of micellization on passive absorption in combination with an attempt to systematically rule out other potential explanations for the observed effect. It remains to investigate whether these observations hold true for other surfactants and additional drugs. If so, micellar solubilization of a drug should reduce its ability to diffuse across Caco-2 monolayers via the transcellular and/or paracellular pathway(s).

Disclosure

The authors declare that they have no conflicts of interest to disclose.

Acknowledgements

The authors wish to thank the Phospholipid Research Center, Heidelberg, Germany, for the financial support for this work.

The authors would like to thank Paul Sogokon for assistance with ultrafiltration experiments.

We appreciate the critical reading of the manuscript and helpful suggestions by Stephen T. Buckley (University of Southern Denmark).

References

[1] A.T. Serajuddin, Salt formation to improve drug solubility, Adv. Drug Deliv. Rev. 59 (2007) 603–616.
[2] V.J. Stella, K.W. Nti-Addae, Prodrug strategies to overcome poor water solubility, Adv. Drug Deliv. Rev. 59 (2007) 677–694.
[3] D.T. Friesen, R. Shanker, M. Crew, D.T. Smithey, W.J. Curatolo, J.A. Nightingale, Hydroxypropyl methylcellulose acetate succinate-based spray-dried dispersions: an overview, Mol. Pharm. 5 (2008) 1003–1019.
[4] M.E. Davis, M.E. Brewster, Cyclodextrin-based pharmaceutics: past, present and future, Nat. Rev. Drug Discov. 3 (2004) 1023–1035.
[5] E.M. Merisko-Liversidge, G.G. Liversidge, Drug nanoparticles: formulating poorly water-soluble compounds, Toxicol. Pathol. 36 (2008) 43–48.
[6] C.W. Pouton, Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems, Eur. J. Pharm. Sci. 11 (2) (2000) S93–98.
[7] A.T. Serajuddin, Solid dispersion of poorly water-soluble drugs: early promises, subsequent problems, and recent breakthroughs, J. Pharm. Sci. 88 (1999) 1058–1066.
[8] V.P. Torchilin, Micellar nanocarriers: pharmaceutical perspectives, Pharm. Res. 24 (2007) 1–16.
[9] T. Vasconcelos, B. Sarmento, P. Costa, Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs, Drug Discov. Today 12 (2007) 1068–1075.
[10] N.S. Barakat, Enhanced oral bioavailability of etodolac by self-emulsifying systems: in-vitro and in-vivo evaluation, J. Pharm. Pharmacol. 62 (2010) 173–180.
[11] H. Tonsberg, R. Holm, J. Bisgaard, J. Jacobsen, A. Mullertz, Effects of polysorbate 80 on the in-vitro precipitation and oral bioavailability of halofantrine from polyethylene glycol 400 formulations in rats, J. Pharm. Pharmacol. 62 (2010) 63–70.
[12] F.G. Poelma, R. Breas, J.J. Tukker, D.J. Crommelin, Intestinal absorption of drugs. The influence of mixed micelles on the disappearance kinetics of drugs from the small intestine of the rat, J. Pharm. Pharmacol. 43 (1991) 317–324.
[13] F.M. Ingels, P.F. Augustijns, Biological, pharmaceutical, and analytical considerations with respect to the transport media used in the absorption screening system, Caco-2, J. Pharm. Sci. 92 (2003) 1545–1558.
[14] J. Kanzer, I. Tho, G.E. Flaten, M. Magerlein, P. Holig, G. Fricker, M. Brandl, In-vitro permeability screening of melt extrudate formulations containing poorly water-soluble drug compounds using the phospholipid vesicle-based barrier, J. Pharm. Pharmacol. 62 (2010) 1591–1598.
[15] D.S. Pisal, V.K. Yellepeddi, A. Kumar, R.S. Kaushik, M.B. Hildreth, X. Guan, S. Palakurthi, Permeability of surface-modified polyamidoamine (PAMAM) dendrimers across Caco-2 cell monolayers, Int. J. Pharm. 350 (2008) 113–121.
[16] K. Bogman, F. Erne-Brand, J. Alsenz, J. Drewe, The role of surfactants in the reversal of active transport mediated by multidrug resistance proteins, J. Pharm. Sci. 92 (2003) 1250–1261.
[17] P. Saha, J.H. Kou, Effect of solubilizing excipients on permeation of poorly water-soluble compounds across Caco-2 cell monolayers, Eur. J. Pharm. Biopharm. 50 (2000) 403–411.
[18] K.H. Song, S.J. Chung, C.K. Shim, Enhanced intestinal absorption of salmon calcitonin (sCT) from proliposomes containing bile salts, J. Controlled Release 106 (2005) 298–308.
[19] B.D. Rege, J.P. Kao, J.E. Polli, Effects of nonionic surfactants on membrane transporters in Caco-2 cell monolayers, Eur. J. Pharm. Sci. 16 (2002) 237–246.
[20] C. Hilgendorf, H. Spahn-Langguth, C.G. Regardh, E. Lipka, G.L. Amidon, P. Langguth, Caco-2 versus Caco-2/HT29-MTX co-cultured cell lines: permeabilities via diffusion, inside- and outside-directed carrier-mediated transport, J. Pharm. Sci. 89 (2000) 63–75.
[21] J. Smalley, P. Kadiyala, B. Xin, P. Balimane, T. Olah, Development of an on-line extraction turbulent flow chromatography tandem mass spectrometry method for cassette analysis of Caco-2 cell based bi-directional assay samples, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 830 (2006) 270–277.
[22] C.F. Mu, P. Balakrishnan, F.D. Cui, Y.M. Yin, Y.B. Lee, H.G. Choi, C.S. Yong, S.J. Chung, C.K. Shim, D.D. Kim, The effects of mixed MPEG-PLA/Pluronic copolymer micelles on the bioavailability and multidrug resistance of docetaxel, Biomaterials 31 (2010) 2371–2379.
[23] X. Qin, F. Yuan, D. Zhou, Y. Huang, Oral characteristics of bergenin and the effect of absorption enhancers in situ, in vitro and in vivo, Arzneimittelforschung 60 (2010) 198–204.
[24] M. Newa, K.H. Bhandari, D.X. Li, T.H. Kwon, J.A. Kim, B.K. Yoo, J.S. Woo, W.S. Lyoo, C.S. Yong, H.G. Choi, Preparation, characterization and in vivo evaluation of ibuprofen binary solid dispersions with Poloxamer 188, Int. J. Pharm. 343 (2007) 228–237.
[25] C.S. Yong, M.K. Lee, Y.J. Park, K.H. Kong, J.J. Xuan, J.H. Kim, J.A. Kim, W.S. Lyoo, S.S. Han, J.D. Rhee, J.O. Kim, C.H. Yang, C.K. Kim, H.G. Choi, Enhanced oral bioavailability of ibuprofen in rats by poloxamer gel using Poloxamer 188 and menthol, Drug Dev. Ind. Pharm. 31 (2005) 615–622.
[26] F. Seeballuck, M.B. Ashford, C.M. O'Driscoll, The effects of pluronics block copolymers and Cremophor EL on intestinal lipoprotein processing and the potential link with P-glycoprotein in Caco-2 cells, Pharm. Res. 20 (2003) 1085–1092.
[27] J. Parmentier, F.J. Hartmann, G. Fricker, In vitro evaluation of liposomes containing bio-enhancers for the oral delivery of macromolecules, Eur. J. Pharm. Biopharm. 76 (2010) 394–403.
[28] M. Greulich, Einfluss von Tensiden auf die Resorption von Substraten der ABC-Transportproteine, in: Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Heidelberg, 2003, p. 91.
[29] E.V. Batrakova, H.Y. Han, V. Alakhov, D.W. Miller, A.V. Kabanov, Effects of pluronic block copolymers on drug absorption in Caco-2 cell monolayers, Pharm. Res. 15 (1998) 850–855.
[30] B.J. Aungst, Intestinal permeation enhancers, J. Pharm. Sci. 89 (2000) 429–442.
[31] D. Dimitrijevic, A.J. Shaw, A.T. Florence, Effects of some non-ionic surfactants on transepithelial permeability in Caco-2 cells, J. Pharm. Pharmacol. 52 (2000) 157–162.

[32] Y. Takahashi, H. Kondo, T. Yasuda, T. Watanabe, S. Kobayashi, S. Yokohama, Common solubilizers to estimate the Caco-2 transport of poorly water-soluble drugs, Int. J. Pharm. 246 (2002) 85–94.
[33] E.V. Batrakova, A.V. Kabanov, Pluronic block copolymers: evolution of drug delivery concept from inert nanocarriers to biological response modifiers, J. Controlled Release 130 (2008) 98–106.
[34] E.V. Batrakova, H.Y. Han, D.W. Miller, A.V. Kabanov, Effects of pluronic P85 unimers and micelles on drug permeability in polarized BBMEC and Caco-2 cells, Pharm. Res. 15 (1998) 1525–1532.
[35] S. Neuhoff, P. Artursson, I. Zamora, A.L. Ungell, Impact of extracellular protein binding on passive and active drug transport across Caco-2 cells, Pharm. Res. 23 (2006) 350–359.
[36] K. Katneni, S.A. Charman, C.J. Porter, Permeability assessment of poorly water-soluble compounds under solubilizing conditions: the reciprocal permeability approach, J. Pharm. Sci. 95 (2006) 2170–2185.
[37] C. Hansch, P.G. Sammes, J.B. Taylor, Comprehensive medicinal chemistry: the rational design, 1st ed., Mechanistic Study & Therapeutic Application of Chemical Compounds, Pergamon Press, Oxford, New York, 1990.
[38] S. Thomas, F. Brightman, H. Gill, S. Lee, B. Pufong, Simulation modelling of human intestinal absorption using Caco-2 permeability and kinetic solubility data for early drug discovery, J. Pharm. Sci. 97 (2008) 4557–4574.