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

Fischer, Sarah; Brandl, Martin

Published in:
European Journal of Pharmaceutics and Biopharmaceutics

DOI:
10.1016/j.ejpb.2011.04.010

Publication date:
2011

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier’s archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright
Effect of the non-ionic surfactant Poloxamer 188 on passive permeability of poorly soluble drugs across Caco-2 cell monolayers

Sarah Maud Fischer, Martin Brandl, Gert Fricker

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 forms are predominantly focussed 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 is a well-acknowledged approach. Among the advanced formulations, cyclodextrins and surfactants or lipids are most commonly used. Literature has partly to overcome recovery and detection limit challenges, while also to investigate their influence on permeability. In the present literature, results are contradicting: there are indications for surfactant influences on passive drug perfusion via barrier interaction, via inhibition of P-glycoprotein (P-gp), via micellar solubilization, ion-pair formation or membrane fluidization. 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, 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, and which has shown to have a low impact on Caco-2 cell viability. 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...
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 Hank’s Balanced Salt Solution (Sigma–Aldrich Chemie GmbH, Munich, Germany) containing KCl 0.40 g, KH2PO4 0.06 g, NaCl 8.0 g, Na2HPO4 0.048 g, glucose 1 g, CaCl2·2H2O 0.183 g and supplemented with MgSO4·7H2O 0.98 g, NaHCO3 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% insulin, 100 μg/ml penicillin and 100 μg/ml streptomycin at 37 °C in 5% CO2 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 Hank’s 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 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 physico-chemical 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 fluo-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Physico-chemical parameters for the two model compounds.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>MW</td>
</tr>
<tr>
<td>----------</td>
<td>----</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>254.3</td>
</tr>
<tr>
<td>Nadolol</td>
<td>309.4</td>
</tr>
</tbody>
</table>

* Molecular weight (MW) in g/mol.
** From [37].
*** From [38] in mg/ml (unbuffered).
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 (Ultra-cel-10) membranes (MWCO = 10 kDa), Millipore GmbH, Schwabach, Germany. Prior to experiments, the filter units were filled with HBSS++ and centrifuged (25 °C, 4000g, 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, 4000g, 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 x 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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mobile phase (%)</th>
<th>Run time (min)</th>
<th>Flow (ml/min)</th>
<th>λ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoprofen</td>
<td>20 80 –</td>
<td>0</td>
<td>0.08</td>
<td>260</td>
</tr>
<tr>
<td>20 80 –</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nadolol</td>
<td>90 – 10</td>
<td>0</td>
<td>1.0</td>
<td>220</td>
</tr>
<tr>
<td>70 – 30</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70 – 30</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 – 10</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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...
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 $P_{\text{app}}$ 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
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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Medium</th>
<th>Mean initial concentration ± SEM (mM)</th>
<th>Mean concentration in ultrafiltrate ± SEM (mM)</th>
<th>Fraction of non-micellar drug (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoprofen</td>
<td>HBSS++</td>
<td>4.43 ± 0.03</td>
<td>4.36 ± 0.02</td>
<td>98.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>P-188 10 mg/ml</td>
<td>4.37 ± 0.04</td>
<td>4.18 ± 0.05</td>
<td>95.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>P-188 20 mg/ml</td>
<td>4.45 ± 0.04</td>
<td>4.05 ± 0.08</td>
<td>91.0 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>P-188 50 mg/ml</td>
<td>4.22 ± 0.10</td>
<td>3.69 ± 0.04</td>
<td>83.5 ± 2.0</td>
</tr>
<tr>
<td>Nadolol</td>
<td>HBSS++</td>
<td>8.04 ± 0.11</td>
<td>8.02 ± 0.06</td>
<td>99.8 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>P-188 10 mg/ml</td>
<td>8.04 ± 0.04</td>
<td>7.91 ± 0.06</td>
<td>98.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>P-188 20 mg/ml</td>
<td>8.06 ± 0.14</td>
<td>7.73 ± 0.04</td>
<td>95.9 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>P-188 50 mg/ml</td>
<td>8.19 ± 0.02</td>
<td>7.80 ± 0.11</td>
<td>95.3 ± 1.0</td>
</tr>
</tbody>
</table>

P-188 (Poloxamer 188) was dissolved in HBSS++.

* 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 \((p < 0.05)\).
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 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 unspecified 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


