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Research Paper

In-vitro permeability of poorly water soluble drugs in the phospholipid vesicle-based permeation assay: the influence of nonionic surfactants

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Abstract

Objectives The aim of this study was to determine the influence of nonionic surfactants on drug permeability using the phospholipid vesicle-based permeation assay (PVPA), which excludes other than *trans*-membrane diffusion pathways.

Methods Barrier integrity was monitored both by electrical resistance and permeability measurement of the hydrophilic marker calcein. Permeability of the model drugs ketoprofen and nadolol across the PVPA-barrier was measured by HPLC-UV. Micelle association of the model drugs was determined using ultrafiltration, whereby micelle-bound drug and molecular drug were separated.

Key findings The nonionic surfactant poloxamer 188 was demonstrated not to affect barrier integrity. Drug permeability was found depressed in the presence of poloxamer 188 in a concentration-dependent manner. Both drugs were found to associate with poloxamer 188 micelles. The extent of the decrease in permeability correlated mostly, but not in all cases, with the fraction of micelle-bound drug.

Conclusions Micelle association was one important but not the only factor affecting drug permeability across the PVPA-barrier.

Keywords ketoprofen; micelle; nadolol; passive drug permeability; solubilisation

Introduction

The oral route is the preferred route of drug administration due to its convenience for the patient and the resulting good compliance. Modern drug development is facing the challenge that a considerable proportion of drug compounds is poorly soluble in aqueous medium and the fraction of poorly soluble compounds among new drug entities has been continuously increasing over the past few years. Poor solubility usually implies poor bioavailability. Current oral dosage form design is thus to a large extent dealing with drug delivery systems aiming at oral bioavailability enhancement of such drugs. Overall, there are three main strategies to overcome poor solubility: one is molecular modification of the compounds in terms of choice of salt or synthesis of prodrugs; the second is modification of the solid state; and the third strategy is to employ formulation additives such as cyclodextrines, surfactants or lipids.[1–8] There are numerous examples in literature, where solubility enhancing formulations have been successfully used for improving the bioavailability of poorly soluble drugs, while in some cases the opposite is reported.[9–12] While the solubility enhancing effect of surfactant-containing formulations through micelle association of the drug is well understood and documented in literature, studies on surfactant effects on drug permeability are still scarce and contradicting. Only recently has it become more common to employ solubilising agents in in-vitro permeability tests, partly to overcome recovery and detection limit challenges, partly to investigate the influence of formulations on permeability.[13,14] However, little is known about if and how drug solubilisation may contribute to the complex scenario of bioavailability enhancement. In literature, there are indications for surfactant influences in terms of enhancement of passive diffusion via barrier impairment, efflux-pump inhibition, micellar solubilisation, ion-pair formation or membrane fluidisation.[15–19] But a systematic investigation into the interplay between the various influences, which a surfactant may exhibit on solubility as

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well as on permeability, is missing. Furthermore, the Caco-2 cell permeation model employed in most of the aforementioned studies exhibits several parallel drug transport pathways: passive transcellular, paracellular, carrier-mediated and endo-/transcytotic transport. This renders it difficult to differentiate between the different potential influences surfactants may have. Thus, for this study, we have chosen the phospholipid vesicle-based permeation assay (PVPA), an in-vitro model that mimics the intestinal absorption of drugs and has been shown to yield permeability data that correlate well with fractions absorbed in humans.[20,21] The model consists of a sandwich of phospholipid bilayers fixed on a filter-support. This model was chosen due to its suitability to exclusively monitor passive absorption, eliminating the potential impact of efflux pumps or other transport pathways. As with any in-vitro permeability model, PVPA was originally designed for high-throughput screening of new chemical entities. But, recently, we have demonstrated its suitability for permeation studies of drug formulations, such as co-solvent systems and solid dispersions.[14,22] For such purpose, stringent controls for barrier integrity and functionality have been introduced in terms of *trans*-barrier electrical resistance measurement in combination with permeability assessment of a poorly permeable hydrophilic marker (calcein). In our opinion, the integrity control aspect has not been sufficiently taken into account in other permeability studies in the presence of surfactants.

The aim of this study was to investigate the influence of several commonly used nonionic surfactants on barrier integrity and to determine their influence on passive permeability of two poorly water soluble model drugs, ketoprofen and nadolol. To this end, we were looking into four potential influences, which an addition of nonionic surfactant to the donor-medium may bring about: increase of the viscosity of the medium and thereby decrease of the activity of the drugs investigated; formation of micelles with incorporation of drug and thereby reduction of free drug concentration in the donor solution; interaction with the PVPA-barrier and thereby change of barrier properties; or change of dielectric constant of the donor medium.

Materials and Methods

Materials

Egg phosphatidyl choline, Lipoid E-80, was kindly provided by Lipoid GmbH, Ludwigshafen, Germany. Poloxamer 188 (Pluronic F68, Lutrol F68) and polyoxyl 40 hydrogenated castor oil (Cremophor RH40) were kindly provided by BASF SE, Ludwigshafen, Germany. Macrogol 15 hydroxystearate (Solutol HS15) and lauroyl macrogol-32 glycerides (Gelucire 44/14) were donated by Gattefossé, Saint-Priest, France. Triton X-100, calcein, ketoprofen and nadolol were purchased from Sigma-Aldrich Denmark A/S, Copenhagen, Denmark. Clear culture Transwell inserts (diam. 6.5 mm) and plates were obtained from Corning GmbH, Life Sciences, Wiesbaden, Germany. Clear Millicell cell culture plates (24 well) were purchased from Millipore A/S, Copenhagen, Denmark. The phosphate buffer used in all experiments contained KH_2PO_4 0.60 g, $Na_2HPO_4 \times 12H_2O$ 6.40 g, NaCl 7.42 g (1000 ml) and was adjusted to pH 7.4. All chemicals were purchased from Sigma-Aldrich Denmark A/S, Copenhagen, Denmark.

Preparation of phospholipid vesicle-based barriers

Phospholipid vesicle-based barriers were prepared as described previously.[20,21] In brief, mixed cellulose ester filters (Millipore A/S, Copenhagen, Denmark) were sealed by heat (150°C, 30 s) on clear Transwell inserts (Corning Inc., Corning, USA) or clear Millicell plates (Millipore A/S, Copenhagen, Denmark) using a custom-made sealing machine (IBR-Ingenieurbüro, Waldkirch, Germany). Liposomes were made of egg phosphatidylcholine Lipoid E-80 by film hydration and subsequent extrusion through polycarbonate filters. The liposomes were spun down successively on the filter inserts: first the smaller liposomes to allow them to enter the pores of the filter support and then the larger ones to layer on top. The inserts were frozen at −80°C and thawed at 65°C resulting in fusion of the liposomes so that tight barriers were obtained.

Preparation of sample solutions

All drug and surfactant solutions were prepared in phosphate buffer and were finally adjusted to pH 7.4. The concentrations of the hydrophilic marker calcein and the two model drugs ketoprofen and nadolol were 10, 4.6 and 7.5 mM, respectively. The calcein concentration of 10 mM had been found previously to be appropriate to provide reliable permeability data.[22] The concentrations of the two model drugs were chosen to be well below the saturation limits (at pH 7.4) and 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.[23,24]

Permeation experiments

Before the permeation experiments, the phospholipid vesicle-based barriers were incubated with phosphate buffer for 1 h. The phosphate buffer in the donor compartment was then removed and replaced by sample solution. Over a period of 5 h (for calcein and nadolol) or 4.5 h (for ketoprofen), the inserts were moved to fresh wells containing phosphate buffer at pH 7.4 at certain time intervals to ensure sink conditions. The amount of drug in the receptor phase was then quantified (for details see the section 'Analysis' below). The cumulative

Table 1 Physicochemical parameters for the two model compounds

Compound	MW*	pK_a**	logP**	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 in g/mol. **Hansch *et al*.[23]. ***Thomas *et al*.[24] in mg/ml (un-buffered).

amount of drug that had permeated through the barrier was plotted against the time giving the cumulative flux. When the flux reached steady state, meaning the slope was linear, the apparent permeability coefficient (P_{app}) was calculated according the following equation:

$$P_{app} = dm/dt \cdot (1/A \cdot C_0) \quad (1)$$

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 of the fluxes ($r^2 \geq 0.99$) were achieved after 2 h for ketoprofen and nadolol, and 2.5 h for calcein. For calculation of the P_{app}, 6, 4 and 6 points of the linear part of the flux curve were used in the case of ketoprofen, nadolol and calcein, respectively.

Electrical resistance measurements

The electrical resistance was measured after each permeation experiment i.e. after 5 h incubation with sample solution, using a Millicell-ERS (Millipore GmbH, Schwalbach, Germany). The blank resistance (= resistance of the pure filter) was subtracted from the total resistance to obtain the actual resistance of the model membrane. Finally, these resistance values multiplied by the area of an insert gave the final electrical resistance (ER) in Ω cm^2.

Analysis

Calcein was analysed by fluorescence spectroscopy using a Fluostar Omega, BMG Labtech GmbH, Offenburg, Germany (excitation 485 nm, emission 520 nm). Ketoprofen and nadolol were analysed using a Waters 2695 HPLC with UV detection (Waters 2487 Dual λ Absorbance Detector). Separation was performed using an Acclaim 120 (C18, 5 µm particle size, 120 Å, 4.6 × 250 mm) column. Run times, mobile phases (isocratic in the case of ketoprofen and with gradient in the case of nadolol), flow, wavelengths and retention times (Rt) are listed in Table 2. The lowest standard concentrations of ketoprofen and nadolol used were 1.2 and 2.0 µM, respectively, which was far above the quantification limit. All samples ranged well within the constraints of the lowest and highest standard, respectively. The standard curves yielded linear fits ($r^2 \geq 0.999$). The software used was Chromeleon 6.80 (Dionex Denmark A/S, Hvidovre, Denmark).

Ultrafiltration experiments

Ultrafiltration experiments were performed using Amicon Ultra-15 Centrifugal Filter Units with Ultracel-10 membranes (molecular weight cut-off: 10 kDa), Millipore GmbH, Schwalbach, Germany. Before the experiments, the filter units were filled with phosphate buffer and centrifuged (25°C, 4000g, 2 min) 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 for a third time (25°C, 4000g, 5 min) and the amount of drug in the ultrafiltrate was analysed. At last, the relative recovery was obtained by dividing the ultrafiltrate concentration by the initial concentration. Details regarding the validity of this technique are provided in the Results section.

Characterisation of sample solutions

The kinematic viscosities of surfactant solutions of different concentrations were measured at T = 25°C ± 0.2 using an Ubbelohde viscometer (type 501 03, Schott, Germany) with the apparatus constant k = 0.003268 mm^2/s^2. The kinematic viscosities were multiplied with the density (measured with a pycnometer) to obtain the absolute (= dynamic) viscosities (mPa·s). Furthermore, the electrical resistances of surfactant solutions on blank inserts were measured.

Statistical analysis

The Kruskal-Wallis analysis of variance by ranks test was employed. A multiple comparison was performed with $P \leq 0.05$ considered as significant. The software used was R Version 2.12.0 (The R Foundation for Statistical Computing, package pgirmess).

Results

Integrity of the phospholipid vesicle-based permeation barrier in the presence of surfactants

To find out if the chosen barrier could withstand the contact with various surfactants without significant impairment of barrier function, we chose two independent tests on integrity: measurement of electrical resistance and permeability testing using a hydrophilic model compound (calcein) which shows rather low permeability. This double approach has been found appropriate to secure the validity of the model in the presence of co-solvents.[22] In our experience, variability in electrical resistance may depend on various factors, one of them being compromised barrier integrity, whilst increased calcein permeability always indicates compromised barrier integrity. High electrical resistance is usually in accordance with low calcein permeability. Partial dissolution of the barrier phospholipids, one of the mechanisms behind changes in barrier integrity, has been demonstrated to correlate with both reduced electrical resistance and increased calcein permeability.[22]

Table 2 HPLC parameters for ketoprofen and nadolol

Compound	Chromatographic conditions	Flow (ml/min)	λ (nm)	Rt** (min)
Ketoprofen	Isocratic 20 / 80 water / methanol*	0.8	260	3.6
Nadolol	0 to 6 min gradient 90 / 10 to 75 / 25 water / acetonitrile* thereafter isocratic 75 / 25 water / acetonitrile*	1.0	220	6.0

*Mobile phases contained 0.1% formic acid. **Retention time.

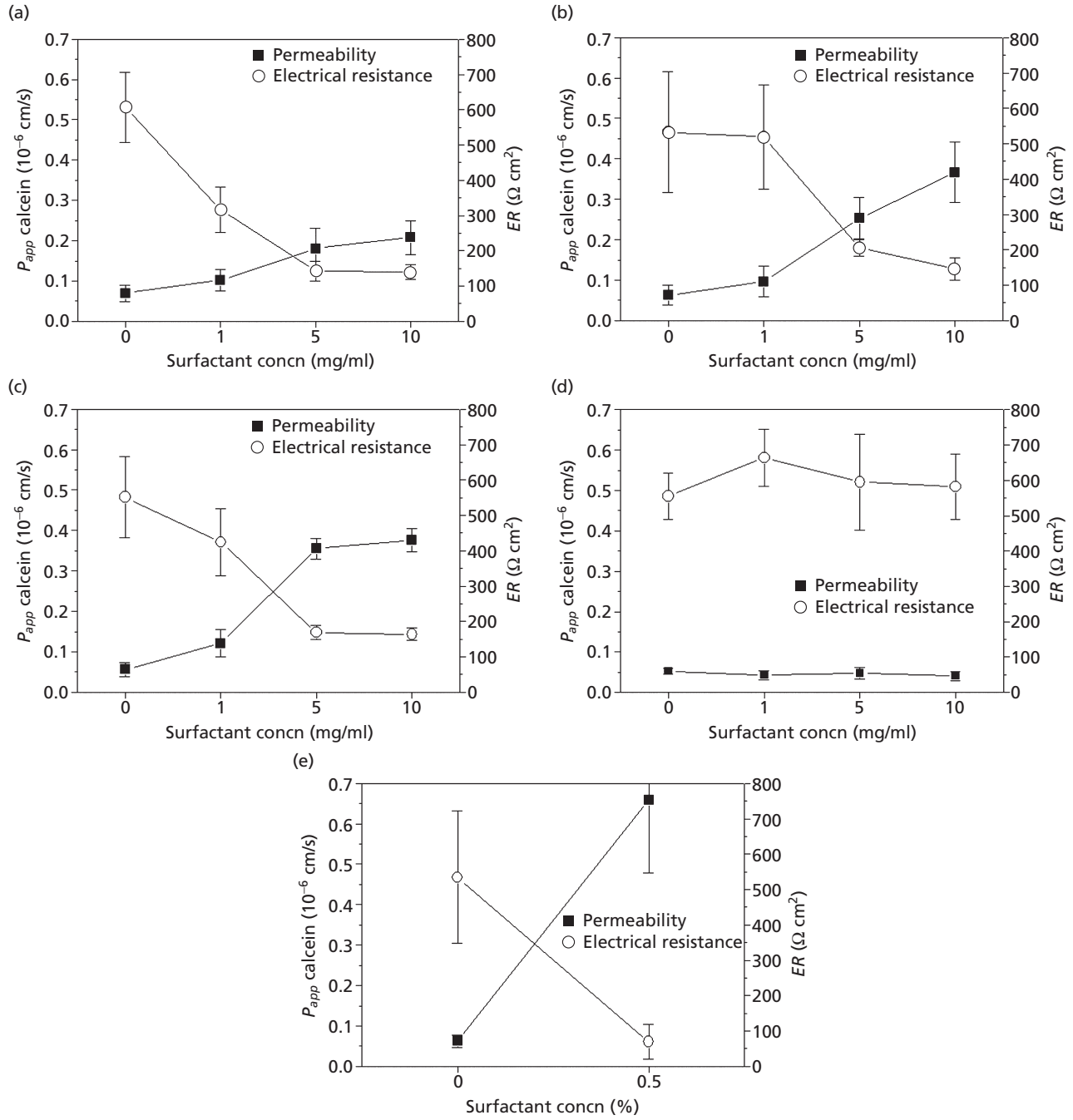

Figure 1 Calcein permeability and electrical resistance in the absence and presence of various surfactants
(a) Lauroyl macrogol-32 glycerides; (b) polyoxyl 40 hydrogenated castor oil; (c) macrogol 15 hydroxystearate; (d) poloxamer 188; (e) Triton X-100. Values are given as mean ± SEM, $n = 6$. ER, electrical resistance; P_{app}, apparent permeability.

The electrical resistance and the permeability of calcein were measured both in the absence and presence of increasing concentrations of four commonly used nonionic surfactants (Figure 1). Triton X-100 (Figure 1e) is known to readily dissolve phospholipid bilayers and thus served as a positive control. In the case of lauroyl macrogol-32 glycerides (Figure 1a), macrogol 15 hydroxystearate (Figure 1b), and polyoxyl 40 hydrogenated castor oil (Figure 1c), calcein permeability was found to be increasing and electrical resistance decreasing at relatively low concentrations when compared with the sample without surfactant (c = 0 mg/ml), which served as a negative control. In contrast, poloxamer 188 (Figure 1d) showed no influence on electrical resistance nor on calcein permeability over the whole range of concentrations used (1–10 mg/ml). In the next step, calcein permeability and electrical resistance were followed over an extended range of poloxamer 188 concentrations up to 50 mg/ml. No significant differences were seen (Figure 2a).

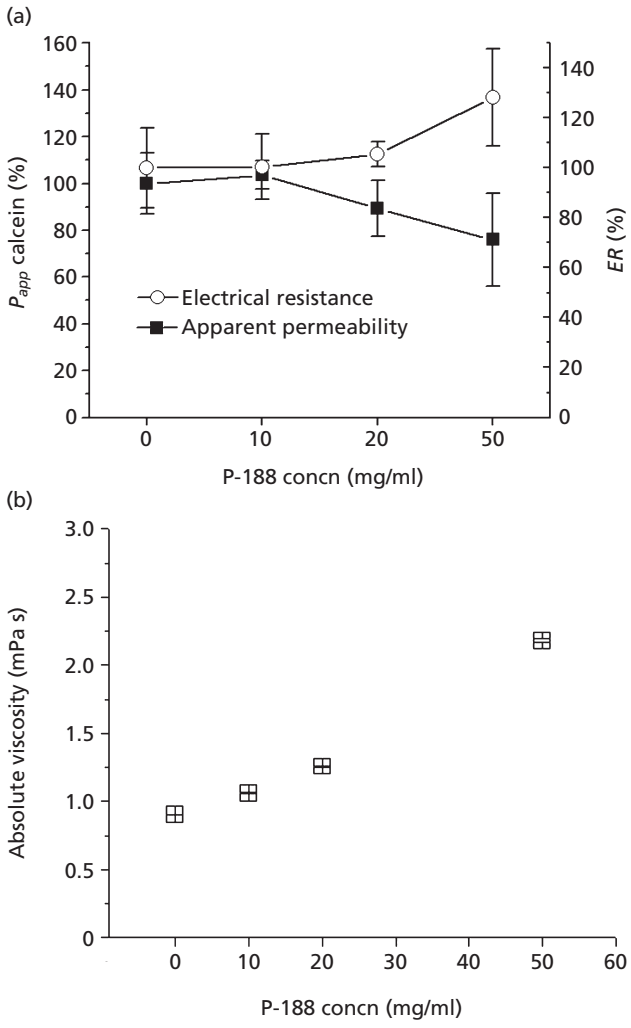

Figure 2 (a) Calcein permeability and electrical resistance in the absence or presence of different concentrations of poloxamer 188 and (b) absolute viscosities of phosphate buffer solutions containing different concentrations of poloxamer 188

For (a) permeability (P_{app}) and electrical resistance (*ER*) are given as percentage of the control (concentration of poloxamer 188 (P-188) = 0 mg/ml). Values are given as mean ± SEM, $n = 6$. No significant differences. For (b) values are given as mean ± SEM, $n = 3$.

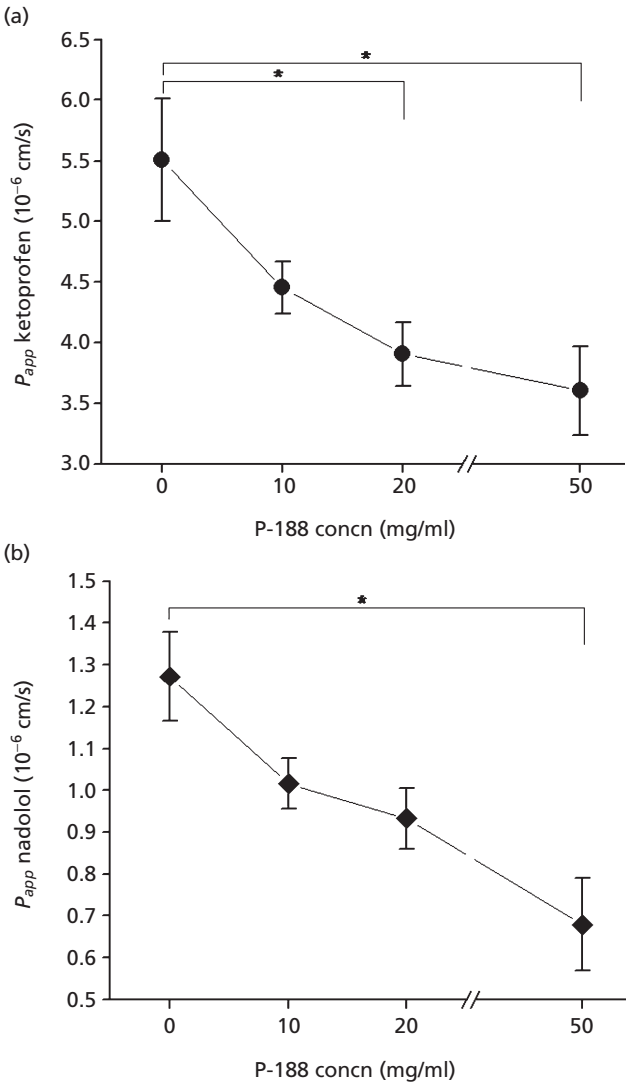

Figure 3 Permeation of two model drugs at different concentrations of poloxamer 188

(a) Ketoprofen. (b) Nadolol. Values are given as mean ± SEM, $n = 6$. $*P < 0.05$. P-188, poloxamer 188; P_{app}, apparent permeability.

Surprisingly, for an even higher concentration (50 mg/ml), electrical resistance increased slightly and calcein permeability decreased.

Influence of poloxamer 188 on viscosity and electrical resistance in the water phase

To find out if the observed changes in calcein permeability and electrical resistance over the barrier were related to a viscosity change, viscosities of surfactant solutions with increasing concentration were measured. The absolute viscosities were found to increase with surfactant concentration in the concentration range tested (Figure 2b). Furthermore, the electrical resistance over a plain membrane filter was measured with increasing surfactant concentrations (up to 100 mg/ml) in the donor phase. No significant change in electrical resistance was observed (data not shown).

Drug permeation of the two model drugs ketoprofen and nadolol

Permeation of two poorly water soluble model drugs, ketoprofen and nadolol, was examined both in the absence and presence of poloxamer 188 (10, 20 or 50 mg/ml). Each permeation study was repeated three times with six parallels each. All replicates were showing the same tendency. The permeation of both drugs was found to decrease in a concentration-dependent way when poloxamer 188 was present as shown in Figure 3. Changes were significant for the 20 and 50 mg/ml poloxamer 188 solutions compared with control for ketoprofen, and for the 50 mg/ml poloxamer 188 solution compared with control for nadolol.

Separation of micelle-associated drug from molecularly dissolved drug

Ultrafiltration using cellulose filters (molecular weight cut-off: 10 kDa) was employed to separate molecularly dissolved drug from its micelle-associated form. The chosen cut-off was expected to be considerably smaller than the expected aggregate size of the surfactant micelles (3–10 nm).[25] The dynamic light scattering (DLS) count rate of a 50 mg/ml poloxamer 188 solution as read from a DLS instrument (Brookhaven Instruments, Älvsjö, Sweden) was found considerably smaller in the ultrafiltrate, which was taken as a qualitative indication that poloxamer-188-micelles did not readily pass the ultrafiltration membrane within the time frame used here. In our experiments, we removed only 20% of the total volume by ultrafiltration. To find out if the chosen ultrafiltration was a valid approach to differentiate between molecularly dissolved and micellar drug, we followed the drug concentration in the ultrafiltrate and retentate. In Table 3, concentration values of ketoprofen at different centrifugation times are summarised when either blank phosphate buffer or poloxamer 188 50 mg/ml was used as medium. In the case of phosphate buffer, the ketoprofen concentration did not noticeably change with centrifugation time in either the retentate or the ultrafiltrate. This indicated that unspecific loss of drug did not occur. In the presence of poloxamer 188, the amount of ketoprofen was found to be very slightly increased in the retentate, while the ultrafiltrates showed constant drug concentrations over time. This was within expectation. Under the assumption that the ultrafiltration membrane lets pass through drug and water equally well, a change in the drug concentration in the water phase should not occur. In the retentate, accumulation of micelles that are in equilibrium regarding their drug content occurs, which is reflected in a slightly rising overall drug concentration in the retentate. Ultrafiltration, however, obviously does not significantly influence the equilibrium between molecularly dissolved drug and micelle-associated drug. The ultrafiltration approach was thus assumed to yield valid results in this respect.

For this study, the concentrations of both model drugs ketoprofen and nadolol, as well as the marker calcein, were quantified in the ultrafiltrate in comparison with the initial (non-fractionated) sample. In the absence of surfactant, the concentration in the ultrafiltrate of ketoprofen and nadolol was identical to the initial concentration. In contrast, in the presence of poloxamer 188, the concentrations in the ultrafiltrate of ketoprofen and nadolol were found significantly reduced in a surfactant concentration-dependent way, while calcein concentration in the ultrafiltrate was hardly changed as compared with the initial concentration (Table 4).

Corrected permeability values using the non-micellar fraction of drug

To find out if the observed reduction in permeability quantitatively correlated with the fraction of molecularly dissolved drugs, the P_{app} values were corrected employing the non-micellar (free) fraction as the initial donor concentration

Table 3 Validation of the ultrafiltration technique for separation of micellar ketoprofen and freely dissolved ketoprofen

Centrifugation time* (min)	Phosphate buffer		Poloxamer 188 50 mg/ml	
	Mean concn in retentate ± SEM (mM)	Mean concn in ultrafiltrate ± SEM (mM)	Mean concn in retentate ± SEM (mM)	Mean concn in ultrafiltrate ± SEM (mM)
Initial**	4.66 ± 0.04	–	4.29 ± 0.05	–
1	4.70 ± 0.02	4.63 ± 0.03	4.33 ± 0.03	3.42 ± 0.05
3	4.68 ± 0.02	4.61 ± 0.03	4.44 ± 0.06	3.38 ± 0.03
5	4.67 ± 0.08	4.59 ± 0.04	4.57 ± 0.11	3.34 ± 0.05

$n = 3$. *Centrifugation time was counted after washing the Ultracel-10 membranes with phosphate buffer for 2 min, followed by saturation of the membrane with sample solution for a further 2 min and removal of the first ultrafiltrate. **The initial concentration was measured before commencing the experiment.

Table 4 Free amount of drug in poloxamer 188 solutions

Compound	Medium	Mean initial concn ± SEM (mM)	Mean concn in ultrafiltrate ± SEM (mM)	Fraction of non-micellar drug (%)*
Ketoprofen	Phosphate buffer	4.6 ± 0.1	4.6 ± 0.1	100.7 ± 2.1
	P-188 10 mg/ml	4.6 ± 0.1	4.3 ± 0.0	93.3 ± 1.2
	P-188 20 mg/ml	4.5 ± 0.0	4.0 ± 0.0	87.7 ± 0.4
	P-188 50 mg/ml	4.3 ± 0.1	3.4 ± 0.1	73.3 ± 1.7
Nadolol	Phosphate buffer	7.3 ± 0.2	7.4 ± 0.1	100.5 ± 2.4
	P-188 10 mg/ml	7.4 ± 0.1	7.1 ± 0.0	96.1 ± 1.3
	P-188 20 mg/ml	7.9 ± 0.1	7.6 ± 0.1	96.0 ± 1.7
	P-188 50 mg/ml	7.6 ± 0.2	7.0 ± 0.2	92.2 ± 2.4
Calcein	Phosphate buffer	9.9 ± 0.2	10.0 ± 1.0	100.9 ± 8.7
	P-188 50 mg/ml	9.5 ± 0.4	9.4 ± 1.5	99.2 ± 13.6

$n = 3$. Poloxamer 188 (P-188) was dissolved in phosphate buffer. *The fraction of non-micellar drug was calculated as the concentration of the ultrafiltrate divided by the initial concentration and given as a percentage.

Table 5 Permeability data of model drugs in the presence of poloxamer 188 uncorrected and corrected with fraction of non-micellar drug

Compound	Medium in the donor compartment	Apparent permeability ± SEM (10^{-6} cm/s)	
		Uncorrected ($n = 6$)	Corrected* ($n = 6$)
Ketoprofen	Phosphate buffer	5.51 ± 0.50	–
	P-188 10 mg/ml	4.45 ± 0.21	4.77 ± 0.23
	P-188 20 mg/ml	3.91 ± 0.26	4.46 ± 0.29
	P-188 50 mg/ml	3.60 ± 0.36	4.92 ± 0.49
Nadolol	Phosphate buffer	1.27 ± 0.11	–
	P-188 10 mg/ml	1.02 ± 0.06	1.06 ± 0.06
	P-188 20 mg/ml	0.93 ± 0.07	0.97 ± 0.08
	P-188 50 mg/ml	0.68 ± 0.11	0.77 ± 0.11

$n = 6$. Poloxamer 188 (P-188) was dissolved in phosphate buffer. Ketoprofen and nadolol were dissolved in the medium at concentrations 4.6 and 7.5 mM, respectively. The pH was adjusted to 7.4. *Corrected values were calculated with the fraction of non-micellar drug as the donor concentration.

(Table 5). For ketoprofen, the corrected P_{app} values were more or less the same for all surfactant concentrations, yet slightly lower than the P_{app} value found without surfactant. For nadolol, the corrected P_{app} values were not constant but showed a slight decrease with increasing surfactant concentration and again did not reach the P_{app} value seen without surfactant.

Discussion

The first part of this study was to investigate if the PVPA-barrier could withstand contact with various surfactants without significant loss of barrier function. Our working hypothesis was that any significant increase in calcein permeability or drop of electrical resistance does indicate an impairment of barrier integrity. Under this presumption, only one of the four surfactants was found compatible with the permeation barrier at all concentrations tested: poloxamer 188. The other three surfactants, polyoxyl 40 hydrogenated castor oil, macrogol 15 hydroxystearate and lauroyl macrogol-32 glycerides, were found to induce a concentration-dependent decrease in electrical resistance and concomitantly an increase in calcein permeability. This was taken as an indication of incompatibility of the permeation barrier with these surfactants. The detected loss of barrier integrity in contact with these three surfactants might have been due to partial dissolution of phospholipid vesicles as it has been observed with other surfactants (polysorbate 80, polyoxyl 35 castor oil and macrogol laurylether) in a previous study.[22] The observed incompatibility with polyoxyl 40 hydrogenated castor oil was in agreement with findings of another study using melt extrudate formulations.[14] We thus excluded polyoxyl 40 hydrogenated castor oil, macrogol 15 hydroxystearate and lauroyl macrogol-32 glycerides from subsequent drug permeability tests and went ahead with poloxamer 188, which did not adversely affect barrier function. Permeability studies using the model drugs ketoprofen and nadolol in the absence and presence of increasing concentrations of the surfactant showed a significant decrease in apparent permeability for both drugs in the presence of poloxamer 188. The influence was dependent on the surfactant concentration. To our knowledge, this is the first observation of drug permeability rate reduction in the presence of surfactants, using a permeability model, where pathways other than passive diffusion could be excluded, and where barrier integrity has been confirmed. Han *et al*.[26] reported for a diverse set of microemulsion formulations in some cases permeability rate reduction and in some cases permeability rate increase using the parallel artificial membrane permeability assay (PAMPA). The lack of integrity controls in that study, however, rendered the findings difficult to assess.

For the vast majority of cellular permeability studies, a permeability enhancing effect of both ionic and nonionic surfactants has been described.[27–29] These observations were typically accompanied by a decreased transepithelial electrical resistance (TEER) or cell viability. In contrast, there are as yet only a few studies where surfactants have been shown to retard permeation. Neuhoff *et al*.[30] reported a decrease in Caco-2 permeability of felodipine in the presence of Cremophor and explained that with the presence of micelles. Under conditions where an unaffected mannitol-flux and TEER confirmed integrity of the barrier, Saha and Kou[17] found for three proprietary drug compounds either no effect or a permeability enhancement with poloxamer 188 (1%) using the Caco-2 cell model. However, controls on whether the drug compounds were subject to pathways other than transcellular diffusion were not reported. Interestingly, cyclodextrin-drug-complexes were found to lower permeability in a cellular model.[31] Indications for such an effect were also given when using a blood–brain barrier cell line, where a complex interaction of passive and active transport of the drug compound and various nonionic surfactants of the poloxamer type were observed.[32] In contrast, these studies, where poloxamers were found to be permeability enhancers, mostly referred to inhibition of efflux pumps and lowering of membrane fluidity.[33,34] The absence of carrier mediated transport pathways in our model may be one explanation why poloxamer 188 did not enhance permeability.

Katneni *et al*.[35] reported for the poorly soluble drug diazepam an inverse correlation of excised rat jejunum permeability with micellar solubilisation using polysorbate 80 and polyoxyl 35 castor oil. This effect was attributed to the reduced thermodynamic activity of the drug or the fact that the micelle-bound fraction of drug was not readily permeable. A corrected apparent permeability was suggested taking into account the micelle-association constant of the drug, which was experimentally determined separately.

In our case, the micellar fraction of drug, as determined by ultrafiltration experiments (Table 4) was found to correlate with the surfactant concentration, both for ketoprofen and nadolol. Yet, the more lipophilic ketoprofen was found to associate with poloxamer 188 micelles to a higher extent than nadolol, while the highly hydrophilic calcein apparently did not associate with poloxamer 188 micelles at all. In the next step, we corrected the permeability values in terms of the free (non-micellar) fraction of drug as suggested.[35] For ketoprofen, the corrected P_{app} values at different surfactant concentrations were found constant (as expected), but in general

lower than the P_{app} value obtained in absence of surfactant. For nadolol, the corrected P_{app} values were found to decrease slightly, yet significantly (at 50 mg/ml), with increasing surfactant concentration. This may suggest that the decrease of ketoprofen permeability with increasing poloxamer 188 content may be explained by micelle formation, as indicated by constant corrected P_{app} values. In contrast, the decrease from 5.5×10^{-6} cm/s down to the level of 4.7×10^{-6} cm/s (corrected) cannot be explained by the micelle hypothesis. A certain discrepancy between corrected P_{app} values and theory was reported also by Katneni *et al*.[35] for excised rat jejunum permeability, where alternative pathways may have interfered. But, in our case, i.e. under circumstances where pathways other than transcellular diffusion could be excluded and under the assumption that the ultrafiltration experiment reported relevant micelle-association data, it could be concluded that the decay in drug permeability did not strictly correlate with its extent of micelle association. Furthermore, we observed a decrease in calcein permeability at the highest poloxamer 188 concentration (50 mg/ml) used, although calcein apparently does not associate with poloxamer 188 micelles. Micellar association could thus be ruled out as an explanation for decreased permeability. It could be speculated that the decrease in calcein permeability might either have been due to a change in viscosity of the aqueous donor compartment or, alternatively, due to a change in the dielectric constant of the donor medium affecting apparent pK_a of the compound.[36] This hypothesis may also help to explain the above discrepancies between corrected P_{app} values of ketoprofen and nadolol. Since a slight increase in electrical resistance across the barrier was seen with higher poloxamer 188 concentrations, while the electrical resistance within the surfactant solution as well as across a plain filter was found unchanged as compared with buffer, a more complex interplay between surfactants and barrier could not be ruled out. In summary, it can be hypothesized that four different mechanisms may have affected permeability across PVPA barriers: nonionic surfactants may have influenced the barrier properties of PVPA; poloxamer 188, at high concentrations, due to a viscosity change of the donor medium was suggested to decrease the P_{app} of calcein (as well as the drugs); poloxamer 188 is suggested to form micelles and incorporate drugs (most obvious for ketoprofen) and thus reduce the concentration gradient of free drug across the barrier resulting in reduced P_{app}; and finally poloxamer 188 may have changed the dielectric constant of the donor medium resulting in changed apparent pK_a values and thus P_{app} values.

Conclusions

Based on the above findings with the phospholipid vesicle-based permeation assay (PVPA), where exclusively passive diffusion is measured, and under application of stringent controls for barrier integrity, it could for the first time unanimously be demonstrated that association of model drugs with nonionic surfactant micelles significantly reduced drug permeability. Detailed quantitative analysis, however, revealed that a deduction of the micellar fraction of drug from donor concentration could not fully account for the decrease seen in experimental permeability. Furthermore, the permeability of calcein was found slightly reduced at high surfactant concentrations as well, although it could be ruled out that calcein associated with poloxamer 188 micelles. These observations indicated that micelle association was one important but not the only aspect influencing drug permeability by passive transcellular transport, especially in the case of hydrophilic compounds. Other effects may have been a change of barrier properties, change of viscosity or dielectric constant of the donor medium. Whether the observed effects hold true for more complex permeability models or the in-vivo situation remains to be investigated.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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