ELSEVIER

International Journal of Pharmaceutics 207 (2000) 21–30

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

A comparison of the permeation enhancement potential of simple bile salt and mixed bile salt:fatty acid micellar systems using the CaCo-2 cell culture model

C.M. Meaney [a], C.M. O'Driscoll [b,*]

[a] *OctoPlus BV, Zernikedreef 12, 2333 CL Leiden, The Netherlands*
[b] *Department of Pharmaceutics, School of Pharmacy, Trinity College, Dublin 2, Ireland*

Received 16 March 2000; received in revised form 5 July 2000; accepted 18 July 2000

Abstract

The aim of this study was to compare the permeation enhancing potential and toxicity of simple bile salt and bile salt:fatty acid mixed micellar systems using the CaCo-2 cell culture model. The effects of micellar systems of sodium cholate, (NaC), and sodium taurocholate, (NaTC), on the permeability of the hydrophilic markers, mannitol (182) and polyethylene glycols (PEGS) 900 and 4000, were assessed. Simple micelle systems of the unconjugated bile salt, NaC, caused greater enhancement of the hydrophilic markers than the conjugated bile salt, NaTC. In the case of NaC systems the enhancement was coincident with excess membrane disruption and toxicity as indicated by altered TEERs, TEMs, MTT values, and, the lack of recovery following removal of the enhancer. In contrast, the NaTC systems were less toxic, and, in the simple micelle form the likely mechanism of enhancement of the hydrophilic markers is via a transient effect on the tight junctions. Formation of mixed micellar systems with linoleic acid (LA) accentuated the toxic effects of NaC. In comparison, NaTC:LA mixed micelles showed superior permeability enhancement versus simple micelles without increasing membrane toxicity. The mechanism of enhancement of NaTC:LA appears more complex and involves a possible combination effect on both the paracellular and transcellular routes.

Keywords: CaCo-2; Sodium cholate; Sodium taurocholate; Linoleic acid; Permeability enhancement; Micellar systems

1. Introduction

The mechanism by which bile salts influence the transfer of solutes across the gastrointestinal membrane has been the subject of many studies. Conflicting results have been reported in the literature. Bile salts may decrease absorption by decreasing the thermodynamic activity of the drug as a result of incorporation into micelles (Kakemi et al., 1970; Kimura et al., 1972; Feldman et al., 1973; Amidon et al., 1982; Poelma, 1989; Poelma et al., 1990). They may increase absorption by altering the barrier function of, the cell membrane

* Corresponding author. Tel.: +353-1-6082441.
E-mail address: kdrscoll@tcd.ie (C.M. O'Driscoll).

0378-5173/00/$ - see front matter
PII: S0378-5173(00)00526-3

(Kakemi et al., 1970; Feldman et al., 1973; Muranishi, 1985; O'Reilly et al., 1994b), the mucus layer (Martin et al., 1978; Lewin et al., 1979; Poelma et al., 1990) or the paracellular route (Yamashita et al., 1990; O'Reilly et al., 1994a; Werner et al., 1996; Lane et al., 1996). The mechanism of absorption enhancement by bile salts appears to be complex, it also depends on the physiochemical properties of the drug under investigation and the interaction of the micelle with the physiological environment.

In this study the CaCo-2 cell culture model was used to study the absorption enhancement potential of micellar systems of the trihydroxy unconjugated bile salt, sodium cholate, (NaC), (CMC = 4 mM, Small (1971)) and the conjugated bile salt, sodium taurocholate, (NaTC), (CMC = 8 mM, Small (1971)). The effects of simple bile salt micellar systems and mixed micelles of bile salt:linoleic acid (LA), on the permeability of a range of hydrophilic markers of different molecular weights, mannitol (MW182), polyethylene glycol (PEG) 900 and PEG 4000 were compared. The potential mechanism of enhancement and the relative toxicities were explored using transepithelial electrical resistance measurements, (TEER), and transmission electron microscopy, (TEM), and the MTT assay. In addition, recovery experiments were carried out to examine the reversibility of the absorption enhancing effect following removal of the bile salt micellar system.

2. Materials and methods

2.1. Materials

All tissue culture media and plastics were obtained from Gibco (Biosciences, Ireland). ^{3}H Mannitol was obtained from Sigma, St. Louis, ^{3}H-PEG 900 from NEN and ^{14}C-PEG 4,000 was obtained from Amersham International, UK. All other chemicals were obtained from Sigma.

2.2. Cell culture

CaCo-2 cells (passage 30) were obtained from ECACC (Porton Down, UK). Mycoplasma free cells of passage 30–50 were used throughout. The cells were cultivated in Dulbecco's modified Eagle's medium supplemented with 10% v/v foetal calf serum, 1% v/v nonessential amino acids, 1% w/v L-glutamine, and gentamicin 100 mg/ml. The cells were maintained at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity. For transport studies, CaCo-2 cells were seeded at a density of 6.3×10^4 cells per cm^2 (Hidalgo et al., 1989) on uncoated Transwell inserts (Costar, 0.4 μm pore size, 4.71 cm^2 surface area).

2.3. Transport studies

On the days of the transport experiment, the culture medium was replaced with Hank's balanced salt solution (HBSS) containing 25 mM glucose and 10 mM HEPES buffer, pH 7.35 (transport medium) and the cell monolayers were allowed to equilibrate for 30–60 min. The TEER was measured following equilibration using a voltmeter (EVOM, World Precision Instruments) fitted with 'chopstick' electrodes. Transport experiments were carried out after 18–21 days in culture using established procedures (Hidalgo et al., 1989). The amount of radionuclides transported at each time interval was determined by liquid scintillation counting in a Packard Tri-Carb 2500 TR liquid scintillation analyser. Quench correction was carried out using the method of external standardisation. The integrity of the cell monolayers was checked at the end of the transport studies by measuring the TEER which was then expressed as a percentage of the initial value.

Apparent permeability coefficients (P_{app}) for each marker molecule were calculated according to the following equation:

$$P_{app} = \frac{dQ}{dt}\frac{1}{AC_0} \quad (1)$$

where P_{app} is the apparent permeability coefficient (cm/s), dQ/dt the steady state flux (mol/s), A, the surface area of membrane (cm^2), C_0 the initial concentration in donor chamber (mol/cm^3).

2.4. Electron microscopy

Cell monolayers grown on Transwell inserts were rinsed with phosphate buffered saline and fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 containing 5% w/v sucrose for 1 h at 25°C. Post-fixation was carried out in 2% osmium tetraoxide in 0.1 M sodium cacodylate (pH 7.4) for 1–2 h at room temperature followed by dehydration of the cell monolayers in a graded series (10–100% v/v) ethanol. After dehydration TEM samples were embedded in Emix resin, and thin sections were stained with uranyl acetate and lead citrate and viewed using a Hitachi 7000 electron microscope.

2.5. MTT assay

MTT is a tetrazolium salt that is cleaved by mitochondrial dehydrogenase in living but not dead cells to give a dark blue product (Mossman, 1983; Tada et al., 1986). Calibration experiments showed that the dehydrogenase activity was linearly correlated with cell number in the range 5000–50 000 cells per well (data not shown). A cell concentration of 35 000 cells per well was used in all experiments. CaCo-2 cells were seeded in DMEM maintenance medium onto 96 well plates and cultured for 24–48 h. Subsequently 100 μl of the test substance in HBSS was added to the wells and incubated for 10 min at 37°C, 20 μl of MTT solution (5 mg/ml in PBS) was then added to each well and the cells were incubated for a further 4 h at 37°C. The reaction product was then solubilised with 100 μl of 10% sodium dodecyl sulphate in 0.01 N HCl overnight before quantifying the colour of the reaction product using a Dynatec plate reader at 590 nm. The concentration of reagent that produced a 50% inhibition of the dehydrogenase enzyme activity was obtained from the concentration–absorbance curves.

2.6. Recovery experiments

Initially the monolayers were exposed to HBSS for 20–60 min containing the radiolabelled markers. The medium was then changed to HBSS containing the test system and radiolabelled markers for 20 min. Finally, the medium was replaced by DMEM containing 10% v/v foetal calf serum, 1% v/v non-essential amino acids, penicillin (100 U/ml)/streptomycin (100 mg/ml), and 10 mM HEPES.

The apparent permeability of the marker molecules was measured throughout the experiments. Similar experiments without the addition of the bile salt system were used as controls.

2.7. Statistics

Unpaired Student's t-test (two-tailed) was used to test the significance of the difference between two mean values. ANOVA was used where more than two means were compared.

3. Results

3.1. Transport of hydrophilic markers

Control experiments indicated that the $P_{app} \times 10^{-6}$ cm/s of mannitol (1.13), PEG 900 (0.55) and PEG 4000 (0.05) across CaCo-2 monolayers decreased with increasing molecular weight. Initial transport studies were carried out with a range of concentrations, above the CMC, of NaC (5–30 mM) to determine the concentration that resulted in increased transport without excessive damage to the cell monolayer. These experiments revealed that the effect of NaC on the transport of the marker molecules, mannitol and PEG 4000 was dependent on concentration and time (Fig. 1a and b). The time dependence was studied by determining P_{app} values at consecutive time intervals during exposure to NaC. At 5 and 8 mM NaC concentrations no significant effect ($P > 0.05$) on marker permeability relative to the control was observed. However, at 10 mM and higher concentrations significant ($P < 0.05$) time dependent effects were observed. Between 20 and 40 min exposure to NaC 10 mM, the P_{app} value for mannitol ($6.7 \pm 0.4 \times 10^{-6}$ cm/s) and PEG 4000 (0.6×10^{-6} cm/s) had increased by approximately 6- and 12-fold, respectively, relative to the control. During the 40–60 min time period the P_{app} of mannitol and PEG 4000 increased by 19- and

43-fold, respectively. For the NaC systems an average P_{app} 0–60 was calculated from the values at consecutive time intervals. The absorption enhancements in P_{app} 0–60 relative to control for mannitol, PEG 900 and PEG 4000 in the presence of NaC and NaTC micellar systems are shown in Fig. 2a and b.

With NaTC systems (10–20 mM) a concentration dependent effect on marker transport was observed. However, in contrast to the NaC systems a steady absorption rate was obtained and maintained over 4 h. Exposure to 10 mM NaTC did not result in a statistically significant ($P > 0.05$) increase in marker permeability. However, following exposure to 20 mM NaTC for 4 h the P_{app} of mannitol, PEG 900 and PEG 4000 were enhanced by a factor of 1.4, 9 and 7, respectively, relative to the control (Fig. 2b) with the latter two being statistically significant ($P < 0.05$). With the simple micellar systems of NaC and NaTC the order of absorption enhancement was PEG 900 > PEG 4000 > mannitol.

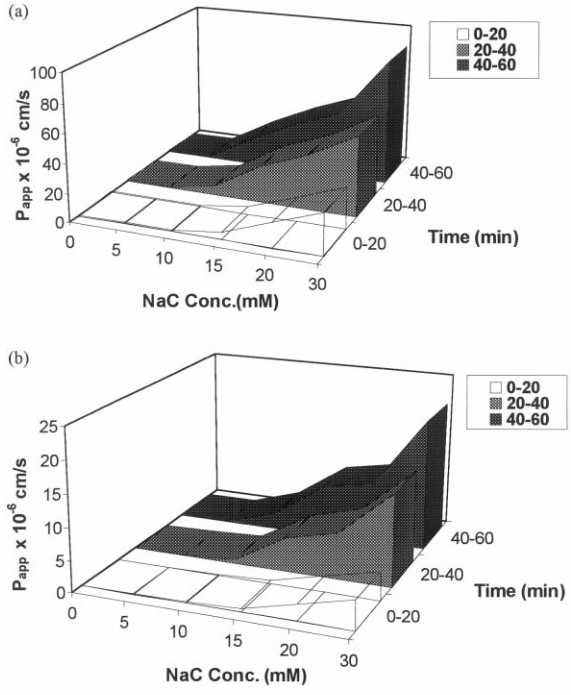

Fig. 1. Concentration and time dependent effects of NaC on the permeability of (a) mannitol and (b) PEG 4000 across CaCo-2 monolayers.

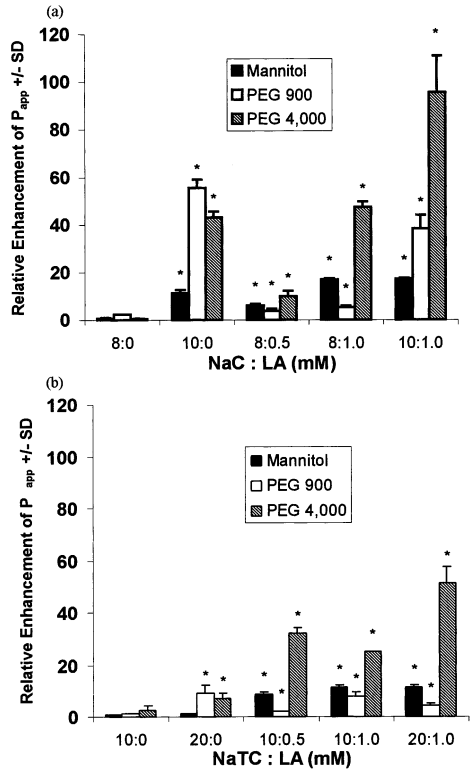

Fig. 2. Absorption enhancement (P_{app} 0–60) relative to control for mannitol, PEG 900 and PEG 4000 by (a) NaC and NaC:LA mixed micellar systems and (b) NaTC and NaTC:LA mixed micellar systems. Values represent mean ± S.D. of at least $n = 3$ observations, $^*P < 0.05$ vs. control).

Mixed micelles were formed by incorporating varying amounts of the unsaturated long chain fatty acid, linoleic acid, ($C_{18:2}$), (LA) into NaC and NaTC systems. In contrast to 8 mM NaC simple micelles, mixed micelle systems of NaC with low fatty acid loading; 8:0.5, 8:1 significantly ($P < 0.05$) increased the absorption of mannitol, PEG 900 and PEG 4000 relative to control. Further enhancements were seen with NaC:LA 10:1 mM (Fig. 2a).

In the case of NaTC mixed micellar systems consisting of NaTC:LA, 20:1, 10:1 and 10:0.5 mM were prepared. Although the simple NaTC 10 mM micelles had no significant effect, the mixed micellar system NaTC:LA 10:0.5 mM significantly ($P < 0.05$) increased the P_{app} of mannitol, PEG 900 and PEG 4000 relative to control. The rank

order of absorption enhancement observed with the mixed micellar systems was PEG 4000 > mannitol > PEG 900 with the sole exception of the NaC:LA 10:1 mM system.

3.2. TEER

As a qualitative measure of paracellular permeability TEER was monitored during the transport experiments. The simple micelles of NaC and NaTC caused a dose dependent reduction in TEER (Table 1). In the case of NaC this reduction was also time dependent. These changes in TEER corresponded to the changes observed in the marker permeability data. After exposure to NaC 10 mM for 1 h extensive disruption was caused to cell morphology as indicated by transmission electron microscopy (Section 3.3). In contrast, the conjugated bile salt, sodium taurocholate caused a significant reduction in TEER only at the higher concentration of 20 mM.

Following exposure to the mixed micellar systems of NaC and NaTC, large variability in the TEER data was observed. With the NaC:LA systems 8:0.5 and 8:1 mM, the TEER decreased significantly ($P < 0.05$) relative to the NaC 8 mM simple micelle system. The decrease in TEER increased with fatty acid loading. Although the TEER value at 1 h for the NaC:LA 10:1 mM system (50%) was higher relative to the 10 mM simple micellar system (40%) both values were extremely low relative to the control indicating that the monolayer was in both cases severely compromised and therefore non-viable. In contrast, at the high fatty acid loading of 1 mM mixed micellar systems of NaTC:LA did not result in significant changes ($P > 0.05$) in TEERs relative to the simple micelles.

3.3. Transmission electron microscopy

The increased transport of the hydrophilic markers together with the changes in TEER measurements, in the presence of the micellar systems, suggest an increase in paracellular permeability due to an effect on the tight junction. Transmission electron microscopy (TEM) was used to complement and extend these studies. Treatment with NaC 10 mM for up to 20 min resulted in no significant changes to the cell monolayer, however after 1 h exposure severe disruption to the cell monolayer occurred (Fig. 3a). In contrast, with the conjugated bile salt NaTC exposure to a higher concentration of 20 mM for up to 1 h resulted only in slight dilatations at the tight junction (Fig. 3b). With mixed micellar systems, exposure to NaC:LA, 10:1 mM, for times up to 40 min resulted in significant loss of monolayer structure (Fig. 3c). In contrast, following exposure to the higher concentration of NaTC:LA (20:1 mM) mixed micellar systems for up to 1 h the cell monolayer remained intact (Fig. 3d). Thus the increased enhancement observed with this system does not appear to be related to increased membrane damage. This is consistent with the data published by Muranishi (1985) which indicated no damage to rat colon following exposure to NaTC:oleic acid 40:40 mM mixed micelles.

Table 1
The effects of exposure to simple and mixed micellar systems of NaC and NaTC for 1 h on the transepithelial electrical resistance (TEER), across CaCo-2 cell monolayers[a]

NaC systems	% TEER ($\pm$S.D.) (n)	NaTC systems	% TEER ($\pm$S.D.) (n)
Control HBSS	98.8 $\pm$ 6.8 (20)	Control HBSS	98.8 $\pm$ 6.8 (20)
NaC 8 mM	99.7 $\pm$ 7.5 (7)	NaTC 10 mM	84.9 $\pm$ 11.0 (10)
NaC:LA 8:0.5 mM	75.0 $\pm$ 13.8 (7)**	NaTC:LA 10:0.5 mM	58.9 $\pm$ 3.5 (3)**
NaC:LA 8:1	59.3 $\pm$ 15.7 (7)**	NaTC:LA 10:1.0 mM	78.5 $\pm$ 18.7 (3)
NaC 10 mM	39.7 $\pm$ 1.3 (3)*	NaTC 20 mM	61.8 $\pm$ 11.9 (9)*
NaC:LA 10:1	50.2 $\pm$ 4.2 (4)	NaTC:LA 20:1.0 mM	83.0 $\pm$ 8.1 (7)

[a] *, $P < 0.05$ vs. control; **, $P < 0.05$ vs. simple.

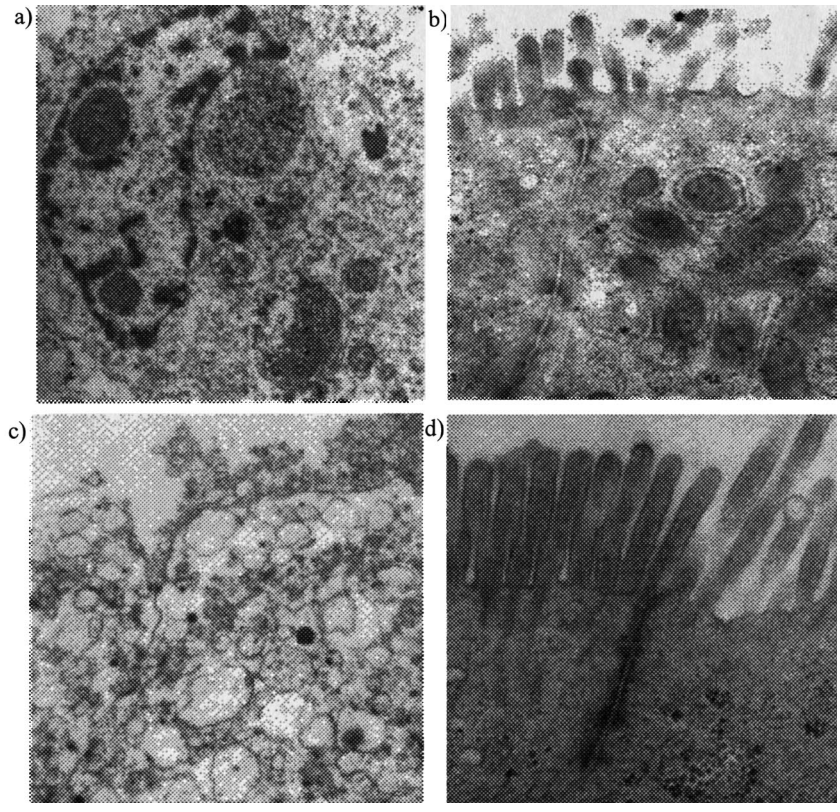

Fig. 3. Transmission electron micrographs of CaCo-2 cell monolayers exposed to (a) NaC 10 mM for 1 h; (b) NaTC 20 mM for 1 h; (c) NaC:LA, 10:1 mM for 40 min and (d) NaTC:LA, 20:1 mM for 1 h.

In general, TEM indicated that micellar systems of NaC caused significant changes in membrane integrity at lower concentrations compared with NaTC. These results are consistent with the TEER data.

3.4. Intracellular enzyme activities

The effect of NaC and NaTC micellar systems on mitochondrial dehydrogenase activity was determined using the MTT method (Mossman, 1983; Tada et al., 1986). A dose dependent reduction was observed with both simple micellar systems. The concentration that inhibited 50% of the enzyme activity (IC_{50}) was 6 mM for NaC and 10 mM for NaTC. The value for NaTC is in general agreement with the value of 9 mM observed by Anderberg et al. (1992).

The effect of mixed micellar systems of NaC:LA and NaTC:LA on intracellular dehydrogenase activity was also determined (Fig. 4). Exposure to lower concentrations of the NaC:LA mixed micelles produced a greater decrease in intracellular dehydrogenase activity compared with the NaTC:LA mixed micellar systems, indicating that they are more toxic. Increasing the proportion of fatty acid within the same concentration of bile salt also tended to decrease intracellular dehydrogenase activity, this effect was greater for the NaC:LA systems.

3.5. Recovery experiments

Enhancers may increase permeability by affecting membrane integrity. Consequently, the reversibility of this effect is an important consideration when screening penetration enhancers. The ability

of the CaCo-2 cells to recover following exposure to 10 mM NaC and 20 mM NaTC for 20 min was investigated. Similar experiments in the absence of bile salt used were used as controls. Recovery in the CaCo-2 monolayers was assessed by monitoring the transport of the hydrophilic markers, mannitol and PEG 4000 and TEER measurements during exposure and after removal of the bile salt. The absorption enhancement was quantified by calculating the area under the curve (AUC) with the trapezoidal rule (Eq. (2)) (Anderberg et al., 1993) where t is the exposure time (s):

$$\mathrm{AUC} = P_{\mathrm{app}} t \quad (2)$$

Following exposure to NaC 10 mM for 20 min no recovery in the permeability of mannitol or PEG 4000 was observed for up to 6 h (data not shown). In this experiment, P_{app} continued to increase rapidly for at least 2 h following removal of the NaC. From 2 to 6 h following exposure the P_{app} plateaued at 14×10^{-6} cm/s for mannitol and at 2.6×10^{-6} cm/s for PEG 4000. However, the permeabilities remained below that of the markers across the filter alone indicating that the cell monolayer was still contributing resistance to the transport of the hydrophilic markers.

In the case of the conjugated bile salt, following exposure to 20 mM NaTC for 20 min, recovery was observed in the monolayers within 6 h after exposure (Fig. 5a and b). The transport of mannitol was enhanced by a factor of 1.2 relative to the control ($P > 0.05$), for PEG 4000 a larger relative enhancement of 11-fold ($P < 0.05$) was observed.

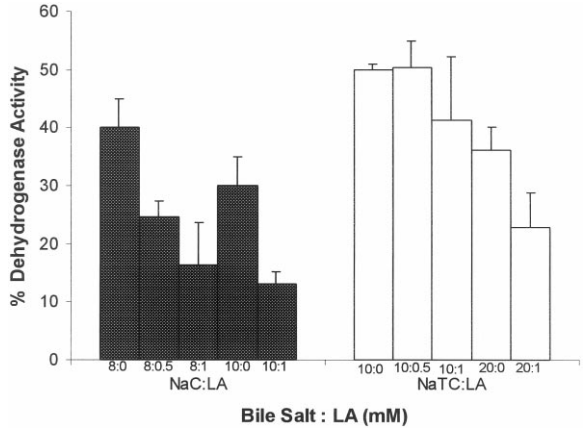

Fig. 4. Effect of simple and mixed micellar systems of NaC, and NaTC on intracellular dehydrogenase activity in CaCo-2 cells.

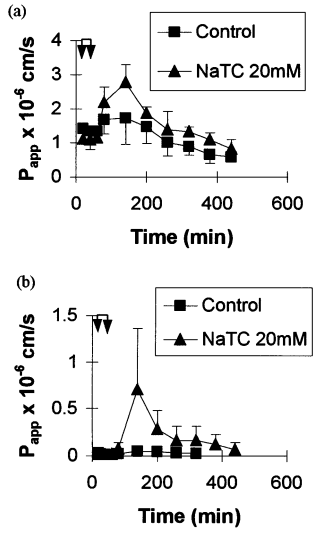

Fig. 5. Recovery of (a) the P_{app} of mannitol and (b) P_{app} of PEG 4000 across CaCo-2 monolayers after 20-min exposure to NaTC 20 mM. The arrows indicate the time during which the cells were exposed to the bile salts.

The TEER was monitored throughout the recovery experiments. In the 10 mM NaC system the TEER was approximately 57% of its original value 6 h after exposure. Following exposure to 20 mM NaTC for 20 min the TEER dropped to $77.7 \pm 7.8\%$ of its original value after exposure. However, after a further 6 h, the TEER had been restored to $88.1 \pm 9.3\%$ of its original value. These results are consistent with the marker transport data.

4. Discussion

Initial studies carried out to compare the permeation enhancing potential of the unconjugated, NaC, and the conjugated, NaTC, trihydroxy bile salts revealed that simple micellar systems of both bile salts enhanced the transport of the hydrophilic markers, mannitol (MW182), PEG 900 and PEG 4000 across CaCo-2 monolayers in a concentration dependent manner. The larger per-

meation enhancing capacity of NaC compared with NaTC appears to be associated with increased membrane damage and toxicity, as indicated by the TEM, TEER and MTT results. This is in agreement with Martin et al. (1992) who observed that NaC was more toxic than the conjugated NaTC to the epithelial cell membrane using erythrocyte haemolysis as a model parameter. In addition, the contact time between bile salt and erythrocyte appeared to be of greater significance in the NaC system. This is consistent with our results which indicate that the effect of NaC was time dependent. The difference in polarity between the unconjugated NaC and the conjugated NaTC may account for the greater damage observed after exposure to NaC systems. Conjugation reduces the overall hydrophobicity of the bile salt and Murakami et al. (1984) observed that the absorption-promoting efficacy of bile salts in rats increased with hydrophobicity.

Formation of mixed micellar systems with NaC and the fatty acid, linoleic acid caused additional increases in the P_{app} of the hydrophilic markers, relative to the simple micellar systems. However, the potential benefit of the fatty acid in these systems is compromised by the further increase in toxicity.

In contrast, more promising results were obtained with the NaTC mixed micellar systems. The CaCo-2 monolayers recovered following exposure to the simple micellar 20 mM NaTC for 20 min. This is in agreement with Werner et al. (1996) who also observed, using TEER measurements and actin staining, that exposure to concentrations of up to 20 mM NaTC resulted in reversible changes in tight junction structure. Werner et al. (1996) reported that systems consisting of NaTC and oleic acid caused permeation enhancement without causing significant damage to the CaCo-2 cell monolayer as indicated by the MTT and LDH assays. Only the TEER was affected by exposure to these mixed systems. However, Werner et al. (1996) used much lower concentrations of NaTC (0.25–1 mM) compared with our study. These concentrations are well below the reported concentrations required for micelle formation (Small, 1971). In our study, exposure to the mixed micellar system NaTC:LA 20:1 mM resulted in significant permeation enhancement of the hydrophilic markers (mannitol and PEG 4000) relative to the simple NaTC 20 mM micellar system. There appeared to be no increased toxicity associated with this enhancement, as indicated by the TEER and TEM results. The TEM indicated no adverse effects on membrane integrity following exposure to NaTC:LA, 20:1 mM for 1 h. This suggests that the increased permeability is not a consequence of increased transcellular transport of the hydrophilic markers resulting from excessive membrane damage or wounds in the monolayer, which can be visualised by TEM.

Transport of hydrophilic compounds via the paracellular route is influenced by the size and shape of the solutes. In the control monolayer the permeabilities of the markers decreased with increasing molecular weight. Cross sectional diameters have previously been reported for mannitol (6.3 Å), PEG 900 (8.3 Å) and PEG 4000 (12 Å) (Lane et al., 1996). As the P_{app} and the molecular radii are known, by using the Renkin function and a process of successive approximation (Adson et al., 1994) with all three markers, an apparent radius of the junctional pore in the CaCo-2 model was estimated as 8.43 Å $\pm$ 1.4 S.D. This value compares well with the value of 12.0 Å $\pm$ 1.9 S.D. previously reported by Adson et al. (1994).

With the simple NaTC 20 mM micellar system the relative enhancements for both the larger molecules PEG 900 and PEG 4000 are of similar order of magnitude, and both were enhanced to a greater extent relative to the smaller molecule mannitol (MW 182). This is consistent with an effect on the paracellular route. In the control monolayer the smaller molecule mannitol was less restricted by the pores than the larger molecules. However, if larger pores were produced by the enhancer then a greater relative increase in permeability would be expected for the bigger molecules. This phenomenon has previously been reported by Adson et al. (1994).

The pore radius for the perturbed monolayer in the presence of NaTC 20 mM was therefore calculated using the P_{app} of all three markers. The value obtained was 15.3 Å $\pm$ 7.5 S.D., this represents an increase in the effective pore radius of

1.8-fold. Applying the same approach to similar hydrophilic markers in a rat intestinal model, Lane et al. (1996) predicted that simple and mixed bile salt micellar systems caused between 1.2- and 1.8-fold increase in effective pore radius relative to the control.

Attempts to calculate the average pore radius in the presence of the NaTC:LA 20:1 mM mixed micellar system using all three makers resulted in an unrealistically high value. In addition, the rank order of permeability enhancement with the mixed micelles cannot be explained on the sole basis of molecular diameter. These results may indicate a different mechanism of action with the mixed micellar systems which includes a combination effect on both the transcellular and paracellular routes. In a previous study NaTC:LA, 20:1 mM increased the permeability of the lipophilic compound dextropropoxyphene across CaCo-2 monolayers, in contrast, no enhancement was observed with the simple NaTC 20 mM micellar system (Meaney and O'Driscoll, 1999). The passive transcellular transport of hydrophilic macromolecules, including dextrans with molecular weights upto 40 kDa, in the presence of fatty acid mixed micellar systems has previously been reported (Muranishi, 1985, 1989). The proposed mechanism of enhancement reported was an interaction between the mixed micelles, and, both the membrane lipid and integrated proteins, thus making the membrane more fluid and more permeable to both hydrophilic and lipophilic compounds.

In conclusion, the stronger permeation enhancement achieved with the NaC micellar systems is directly linked to excessive membrane damage. In contrast, with NaTC simple and mixed micellar systems significant permeability enhancement was reported without membrane damage, making these systems potentially more useful as safe enhancers.

Acknowledgements

This work was supported by BioResearch Ireland at the National Pharmaceutical Biotechnology Centre, TCD, Dublin, Ireland.

References

Adson, A., Raub, T.J., Burton, P.S., Barsuhn, C.L., Hilgers, A.R., Audus, K.L., Ho, N.F.H., 1994. Quantitative approaches to delineate paracellular diffusion in cultured epithelial cell monolayers. J. Pharm. Sci. 83, 1529–1536.

Amidon, G.E., Higuchi, W.I., Ho, N.F.H., 1982. Theoretical and experimental studies of transport of micelle-solubilized solutes. J. Pharm. Sci. 71 (1), 77–84.

Anderberg, E.K., Nystrom, C., Artursson, P., 1992. Epithelial transport of drugs in cell culture VII. Effects of pharmaceutical surfactant excipients and bile acids on transepithelial permeability in monolayers of human intestinal epithelial (CaCo-2) cells. J. Pharm. Sci. 81, 879–887.

Anderberg, E.K., Lindmark, T., Artursson, P., 1993. Sodium caprate elicits dilatations in human intestinal tight junctions and enhances drug absorption by the paracellular route. Pharm. Res. 10, 857–864.

Feldman, S., Reinhard, M., Wilson, C., 1973. Effect of sodium taurodeoxycholate on biological membranes: release of phosphorous, phospholipid, and protein from everted rat small intestine. J. Pharm. Sci. 62, 1961–1964.

Hidalgo, I.J., Raub, T.J., Borchardt, R.T., 1989. Characterisation of the human colon carcinoma cell line (CaCo-2) as a model system for intestinal epithelial permeability. Gastroenterology 96, 736–749.

Kakemi, K., Sezaki, H., Konishi, R., Kimura, T., Murakami, M., 1970. Effect of bile salts on the gastrointestinal absorption of drugs. Chem. Pharm. Bull. 18, 275–280.

Kimura, T., Sezaki, H., Kakemi, K., 1972. Effect of bile salts on the gastrointestinal absorption of drugs. IV. Site of intestinal absorption of sodium taurocholate and its consequence on drug absorption in the rat. Chem. Pharm. Bull. 20, 1656–1662.

Lane, M.E., O'Driscoll, C.M., Corrigan, O.I., 1996. The relationship between rat intestinal permeability and hydrophilic probe size. Pharm. Res. 13, 1552–1556.

Lewin, M.R., El Massri, S.H., Clark, C.G., 1979. Effects of bile salts on mucus secretion in the dog colon. Eur. Surg. Res. 11, 392–398.

Martin, G.P., Marriott, C., Kellaway, I.W., 1978. Direct effects of bile salts and phospholipids on the physical properties of mucus. Gut 19, 103–107.

Martin, G.P., El-Hariri, L., Marriott, C., 1992. Bile salt- and lysophosphatidylcholine-induced membrane damage in human erythrocytes. J. Pharm. Pharmacol. 22, 646–650.

Meaney, C., O'Driscoll, C., 1999. Mucus as a barrier to the permeability of hydrophilic and lipophilic compounds in the absence and presence of sodium taurocholate micellar systems using cell culture models. Eur. J. Pharm. Sci. 8, 167–175.

Mossman, T.J., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Murakami, T., Sasaki, Y., Yamajo, R., Yata, N., 1984. Effect of bile salts on the rectal absorption of sodium ampicillin in rats. Chem. Pharm. Bull. 32, 1948–1955.

Muranishi, S., 1985. Modification of intestinal absorption of drugs by lipoidal adjuvants. Pharm. Res. 3, 108–118.

Muranishi, S., 1989. Absorptions enhancers: mechanisms and applications. In: Prescott, L.F., Nimmo, W.S. (Eds.), Novel Drug Delivery and its Therapeutic Application. Wiley, New York, pp. 69–77.

O'Reilly, J.R., Corrigan, O.I., O'Driscoll, C.M., 1994a. The effect of simple micellar systems on the solubility and intestinal absorption of clofazimine (B663) in the anaesthetised rat. Int. J. Pharm. 105, 137–146.

O'Reilly, J.R., Corrigan, O.I., O'Driscoll, C.M., 1994b. The effect of mixed micellar systems, bile salt/fatty acids, on the solubility and intestinal absorption of clofazimine (B663) in the anaesthetised rat. Int. J. Pharm. 109, 147–154.

Poelma, F.G.J., 1989. The influence of taurocholate on the absorption of drugs in the small intestine of the rat. Ph.D. Thesis, Utrecht University, The Netherlands.

Poelma, F.G.J., Breas, R., Tukker, J.J., 1990. Intestinal absorption of drugs. IV. The influence of taurocholate and L-cysteine on the barrier function of mucus. Int. J. Pharm. 64, 161–169.

Small, D.M., 1971. The physical chemistry of cholanic acids. In: Nair, D.M., Kritchevsk, D. (Eds.), The Bile Acids, Chemistry, Physiology and Metabolism, vol. 1. Plenum Publishing, New York, pp. 249–356.

Tada, H., Shoho, O., Kuroshima, K., Koyama, M., Tsukamoto, K., 1986. An improved colorimetric assay for interleukin 2. J. Immunol. Methods 93, 157–165.

Werner, U., Kissel, T., Reers, M., 1996. Effects of permeation enhancers on the transport of a peptidomimetic thrombin inhibitor (CRC 220) in a human intestinal cell line (CaCo-2). Pharm. Res. 13, 1219–1227.

Yamashita, S., Masada, M., Nadai, T., Kimura, T., 1990. Effect of adjuvants on charge-selective permeability and electrical resistance of rat jejunal membrane. J. Pharm. Sci. 79 (7), 579–583.