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# Influence of monocaprin on the permeability of a diacidic drug BTA-243 across Caco-2 cell monolayers and everted gut sacs

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**Abstract**

This study explores the potential of the monoglyceride monocaprin as an enhancer of the epithelial permeability of the beta$_3$-adrenoceptor agonist BTA-243, as an approach to improving the bioavailability of this drug. The permeabilities of BTA-243 and mannitol (paracellular marker) in Caco-2 cell monolayer and everted gut sac models in aqueous buffer (pH 6.8) in the presence of 1.3 and 2.0 mM monocaprin were compared with control (monocaprin-free) solutions over a period of 1 h. The transepithelial electrical resistance (TEER) of the Caco-2 cell monolayers was measured at regular time intervals throughout the experiment and after a recovery period of 30 h. Toxicological damage to the biological models associated with exposure to monocaprin was assessed by scanning electron microscopy and by the measurement of lactate dehydrogenase (LDH) release from everted gut sacs. The permeability of BTA-243 in epithelial monolayers was enhanced in the presence of 1.3 and 2.0 mM monocaprin. Measurements of TEER and mannitol permeability showed partial recovery of barrier properties after a 30 h period following exposure to 1.3 mM monocaprin. No structural damage was evident in these monolayers. Enhancement of Caco-2 permeability to BTA-243 by 2.0 mM monocaprin was significantly greater than by 1.3 mM but was irreversible; monolayers failed to recover their barrier properties after 30 h and changes in their gross morphology were observed. The mucosal to serosal transfer of BTA-243 in everted gut sac was enhanced but to a lesser extent than in the Caco-2 model. LDH release from everted gut sacs exposed to monocaprin was significantly less than that after exposure to Triton X-100, a nonionic surfactant known to cause membrane disruption. 

*Keywords:* Epithelial permeability; Monocaprin; Caco-2 cells; Transepithelial electrical resistance; BTA-243; Everted gut sac

## 1. Introduction

BTA-243 (see below) is a pharmacologically active beta$_3$-adrenoceptor agonist of potential use in the treatment of diabetes. The drug is associated with a low oral bioavailability following administration in a solid oral dosage form. In solution at intestinal pH the molecule is surface active and

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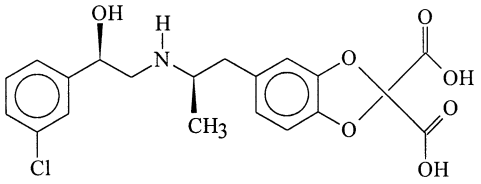
possesses two negative charges and a single positive charge associated with p$K_a$s of $<4$ (carboxylic acids) and 8.29 (amine) (Brown et al., 1998). It has been concluded from investigations into the physicochemical and biopharmaceutical properties of BTA-243 (Brown et al., 2001) that the bioavailability of this drug in vivo is likely to be limited predominantly by its very low epithelial permeability.




BTA-243

Glycerides of medium chain fatty acids have been studied as permeation enhancers of hydrophilic drug molecules. Enhancement of the permeability of penicillin V in the presence of monocaprin, a monoglyceride of a saturated $C_{10}$ fatty acid, and dicaproin, a diglyceride of a $C_8$ fatty acid, has been reported in the Caco-2 model (Shima et al., 1998). A mixture of medium chain monoacylglycerols and phosphatidylcholine (3:1 ratio) has been reported to enhance the permeability of Fragmin in the same cell culture model (Lohikangas et al., 1994). Glycerides of medium chain fatty acids have also been included in absorption enhancing formulations administered to animals. The absorption of sodium ceftriaxone in rodents and monkeys was reported to be enhanced following intraduodenal administration in Capmul 8210 ($C_8/C_{10}$ mono-/di-glycerides) (Beskid et al., 1988). The bioavailability in rats of a water soluble peptide, SK&F 106760, was reported to be enhanced from a microemulsion formulation containing Capmul MCM ($C_8/C_{10}$ mono-/di-glycerides) and Captex 255 ($C_8/C_{10}$ triglycerides) relative to an aqueous formulation (Constantinides et al., 1994).

The overall aim of the work reported here was to investigate the formulation of drug with the $C_{10}$ monoglyceride monocaprin, as an approach to improving the bioavailability of this drug and others with physicochemical properties similar to those of BTA-243. The influence of monocaprin on the permeability of BTA-243 from simple solutions of monoglyceride and drug has been investigated in Caco-2 monolayers and everted gut sacs. The Caco-2 model has been used previously in studies investigating the influence of a number of pharmaceutical additives and absorption enhancers on epithelial permeability (Hochman et al., 1994). The cells are of human origin and experiments can be performed over relatively long periods of time permitting the reversibility of any effects to be assessed (Anderberg and Artursson, 1993). Caco-2 monolayers may, however, be more sensitive to the action of enhancers of paracellular transport than human epithelial tissue. Electrophysiological and permeability data reported in the literature indicate that the permeability of the tight junctions in Caco-2 monolayers is lower than the average permeability observed in the human intestine in vivo (Artursson et al., 1996). The everted gut sac model (Barthe et al., 1998) has been employed in the reported work as a 'leakier' epithelial model complementary to the cell culture model. The influence of monocaprin on the Caco-2 permeability of the recognised paracellular marker mannitol has also been investigated to assist in the elucidation of a potential mechanism of enhancement.

Many studies have associated the action of absorption enhancers with membrane damaging effects (Lohikangas et al., 1994; Yamamoto et al., 1996). An ideal enhancer would produce a rapidly reversible change in epithelial permeability at concentrations that do not alter normal mucosal cell morphology. The potential toxicity of monocaprin as a permeability enhancer has been assessed in this study by measurement of the reversibility of changes in Caco-2 barrier properties and the release of intracellular enzymes from everted gut sacs during exposure to enhancer solutions, and by scanning electron microscopy (SEM).

## 2. Materials and methods

### 2.1. Materials

BTA-243 disodium salt was donated by Wyeth-Ayerst Research. Hanks' Balanced Salt Solution (HBSS) without calcium and magnesium ions was purchased from Bio-Whittaker. All other chemicals including monocaprin (99% pure 1-monodecanoyl-rac-glycerol), TC199 culture medium (which contains calcium and magnesium ions), Triton X-100, and $^{14}$C-mannitol were purchased from Sigma–Aldrich Co. Ltd. (UK).

### 2.2. Methods

#### 2.2.1. Assay methods

*2.2.1.1. Assay of BTA-243.* HPLC analysis was performed using a Hewlett Packard 1090 Series II HPLC system and a C8 analytical column with a particle size of 5 μm (Phenomonex, prodigy column). The mobile phase consisted of 80% aqueous phase and 20% organic phase (acetonitrile). The aqueous phase was prepared by adding 20 ml triethylamine to 1400 ml water and adjusting to pH 2.5 with concentrated phosphoric acid. BTA-243 was detected at a wavelength of 287 nm, its retention time at a flow rate of 1 ml $min^{-1}$ was 6–8 min. Two guard columns ($4 \times 3.0$ $mm^2$ diameter, Securiguard, Phenomonex) were positioned in series up stream of the analytical column in the assay of solutions from everted gut sac experiments.

*2.2.1.2. Assay of $^{14}$C-mannitol.* Samples containing $^{14}$C-mannitol were mixed with 5 ml Ecolite (ECN) scintillation fluid and the radioactivity measured in a liquid scintillation counter (Beckman LS 6000).

*2.2.1.3. Assay of lactate dehydrogenase activity in incubation medium.* Lactate dehydrogenase (LDH) was assayed using a diagnostic kit supplied by Sigma–Aldrich Co. Ltd. (UK). BTA-243, monocaprin and Triton X-100 were shown not to interfere with the LDH activity of the standard solutions of LDH (Multi-enzyme Lin-trol, Sigma–Aldrich Co. Ltd.) as determined by the assay.

#### 2.2.2. Caco-2 cell culture model

*2.2.2.1. Cell culture.* Caco-2 cells (American Tissue Culture Collection Rockville, MD, USA) were maintained at 37 °C in 95% relative humidity with 5% $CO_2$ in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated foetal calf serum, 1% nonessential amino acids and 2 mM glutamine.

For transport experiments, cells of passage 30–40 were used throughout. The cells were seeded at a density of $2 \times 10^5$ $ml^{-1}$ and permitted to multiply and differentiate on 24 mm diameter polycarbonate membranes with a 0.4 μm pore size in Transwell$^{TM}$ culture plate inserts (Costar). Monolayers were used on days 23–28 following seeding onto the Transwell$^{TM}$ inserts.

*2.2.2.2. Drug transport in Caco-2 cell monolayers.* Solutions of 2 mM BTA-243 and 0.004 mM $^{14}$C-mannitol (used as a paracellular marker) were prepared in HBSS (without calcium and magnesium ions to prevent potential precipitation of the enhancer). Monocaprin was added to the solutions in 1.3 and 2.0 mM concentrations; 2.0 mM monocaprin was the highest concentration evaluated because of the limited solubility of the monoglyceride within the donor solutions. These donor solutions were warmed to 37 °C and placed in a sonicator to facilitate complete dissolution of the lipid components. The pH of the solutions was adjusted to pH 6.8 by the addition of 1 M HCl or 1 M NaOH.

Prior to assay, differentiated, confluent Caco-2 monolayers on Transwell$^{TM}$ inserts were equilibrated in HBSS (pH 7.3) for 15 min at 37 °C. Transport experiments were started by positioning the inserts into clean wells containing 2.5 ml of fresh, complete (containing calcium and magnesium ions) HBSS (pH 7.3). 2.5 ml of the previously prepared and pre-warmed donor solution containing drug alone or drug and enhancer were added onto the apical side of the monolayer. The monolayers were incubated at 37 °C on a plate

shaker operated at 20 r.p.m. to avoid aqueous boundary layer contributions to drug transport.

Samples were withdrawn from the receiving chamber at regular time intervals and immediately replaced by an equivalent volume of fresh HBSS. Samples were also taken from the donor chamber at the end of the experiment. Transepithelial electrical resistance (TEER) was recorded using a 'chopstick' electrode (EVOM Electrode Ltd.) throughout the experiment.

*2.2.2.3. Assessment of Caco-2 cell monolayer recovery from exposure to applied enhancer.* Caco-2 monolayers were exposed to the enhancer solution for 60 min. Following this exposure the enhancer solution was removed and the inserts, upon which the cell monolayers were established, were rinsed once with HBSS. The inserts were placed into fresh wells containing 2.5 ml of pre-warmed DMEM, 2.5 ml of the same medium was added to the apical side of the cell monolayers, which were then incubated at 37 °C in the presence of 5% $CO_2$ for 30 h. At the end of this 30 h recovery period the well inserts were removed from the DMEM, washed once in HBSS and then allowed to equilibrate in pre-warmed HBSS for 30 min. At the end of the equilibration period the TEER was recorded and the transport rate of $^{14}$C-mannitol across the monolayer was determined over 40 min.

*2.2.2.4. SEM of monolayers.* The cell monolayers attached to polycarbonate filters were fixed by immersion in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for up to 2 days and then washed in sodium cacodylate buffer to remove excess glutaraldehyde. The monolayers were dehydrated in ethanol before extraction of ethanol from the samples by critical point drying. Immediately prior to viewing, specimens were coated with gold particles using an Edwards S150 sputter coater. Specimens were viewed using a Cambridge S360 Scanning Electron Microscope.

*2.2.3. Everted gut sac model*

*2.2.3.1. Preparation of gut sacs.* Male Sprague–Dawley rats weighing between 300 and 400 g were starved overnight and then killed by cervical dislocation. A 25 cm portion of the jejunum was removed from a position 10 cm distal to the pyloric sphincter, flushed through several times with normal saline at room temperature and placed into a bath of aerated TC199 medium maintained at 37 °C. The intestine was carefully everted over a steel rod (2.5 mm diameter) and one end of the tissue was closed using a steel clip to create a sac. The sac was filled with freshly oxygenated TC199 medium (pH 7.4) following the method of Barthe et al. (1998) to give a physiological distension and then sealed using a second clip. The resulting large gut sac was divided into smaller gut sacs of 2.5 cm length using braided silk.

*2.2.3.2. Drug transfer in everted gut sacs.* Each everted gut sac was placed in a 50 ml plastic beaker containing 10 ml of BTA-243 (2.0 mM) dissolved in either oxygenated TC199 medium or oxygenated TC199 medium containing monocaprin (1.3 or 2.0 mM). The pH of the bathing solution was pre-adjusted to a pH of 6.8 using 1 M HCl and 1 M NaOH. The sacs were maintained at 37 °C in a shaking water bath operating at 60 strokes per minute. At selected time intervals, a gut sac was removed from its incubation medium, washed four times in saline, dried on a paper towel and weighed. After weighing, the sacs were cut open and the contents of each serosal cavity were drained into a vial for subsequent HPLC analysis. Each sac was then reweighed to permit accurate calculation of the volume of fluid associated with the sac, and the surface area of the gut sac was measured using a grid system.

## 3. Results and discussion

### *3.1. The influence of monocaprin on the transport of BTA-243 and mannitol across Caco-2 monolayers*

The transport of BTA-243 across Caco-2 cell monolayers in solutions containing 0, 1.3 and 2.0 mM monocaprin is shown in Fig. 1. In the presence of monocaprin there was a rapid transient increase of transport during the first 10 min

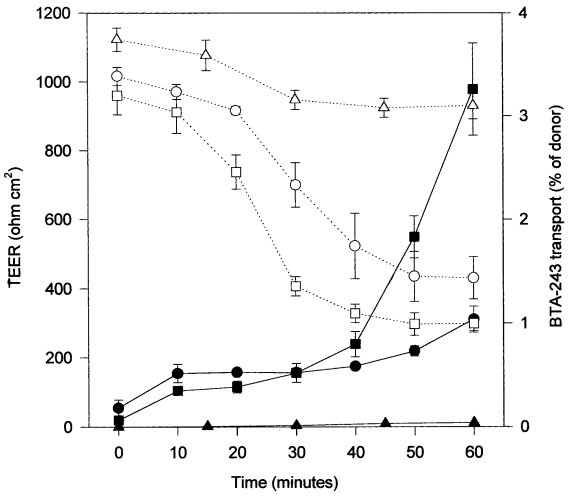



Fig. 1. Cumulative BTA-243 transport across Caco-2 monolayers (filled symbols), and TEER of Caco-2 monolayers exposed to 2.0 mM solutions of BTA-243 (open symbols), in the presence of: 0 (triangles); 1.3 (circles); and 2.0 (squares) mM monocaprin. Error bars represent SE, $n = 3$.

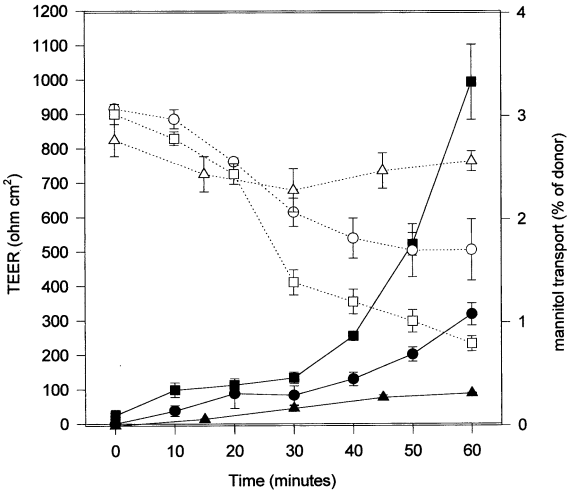



Fig. 2. Cumulative mannitol transport across Caco-2 monolayers (filled symbols), and TEER of Caco-2 monolayers exposed to 0.004 mM solutions of mannitol (open symbols), in the presence of: 0 (triangles); 1.3 (circles); and 2.0 (squares) mM monocaprin. Error bars represent SE, $n = 3$.

of exposure, little or no transport of drug between 10 and 40 min, followed by a further increase of transport up to 1 h, which was most pronounced in 2.0 mM monocaprin. The cumulative BTA-243 transport over 60 min (expressed as a percentage of BTA-243 in the donor compartment) was 1.0 and 3.3% in the presence of 1.3 and 2.0 mM monocaprin, respectively; virtually no BTA-243 transport could be detected in the absence of monocaprin. Fig. 2 shows a similar enhanced transport of mannitol (paracellular marker) across the Caco-2 cell monolayers by 1.3 and 2.0 mM monocaprin. The differences in initial (zero time) transport seen in Figs. 1 and 2 may be a consequence of a difference of 1–2 min in the time at which the first sample was taken from each of the three wells.

### 3.2. The influence of monocaprin on transepithelial electrical resistance (TEER)

The electrical conductivity measured across Caco-2 monolayers is almost entirely a result of paracellular ion flux. Consequently, TEER may be used to describe cell monolayer integrity (Delie and Rubas, 1997), a reduction in the TEER recorded across a monolayer indicating dilation of tight junctions or a disruption of the monolayer.

The changes in TEER observed after exposure of monolayers to BTA-243 (2.0 mM) solutions containing monocaprin are superimposed on the drug transport plots of Fig. 1. Differences in the initial TEER values in these samples are presumably due to the variability of the monolayers. During the first 10 min of exposure the mean TEER of these monolayers remained close to 100% of the initial value. The TEER of monolayers exposed to 1.3 and 2.0 mM monocaprin decreased by 69 and 43%, respectively over the following 30–40 min, the attainment of these threshold values coinciding approximately with the observed onset of the sustained increase in drug transport. Similar trends were observed in monolayers exposed to mannitol (0.004 mM) solutions containing monocaprin (Fig. 2).

### 3.3. Reversibility of TEER and paracellular barrier properties in Caco-2 monolayers

The recovery of monolayer barrier properties following exposure to 1.3 and 2.0 mM concentrations of monocaprin has been assessed by mea-

surement of TEER and mannitol permeability. In Fig. 3, the mean TEER values measured across Caco-2 monolayers immediately at the end of a 60 min exposure to drug solutions containing 1.3 and 2.0 mM monocaprin are compared to the mean TEER values measured across the same monolayers following a recovery period in which the cells were incubated in DMEM for 30 h. A significant increase in mean TEER (from 43 to 80% of the initial value) was recorded across monolayers exposed to 1.3 mM monocaprin. No similar recovery of barrier properties was noted with monolayers exposed to 2.0 mM monocaprin; the mean TEER of these monolayers was unchanged at the end of the 30 h incubation period.

Mean apparent permeability coefficients ($P_{app}$) were calculated from

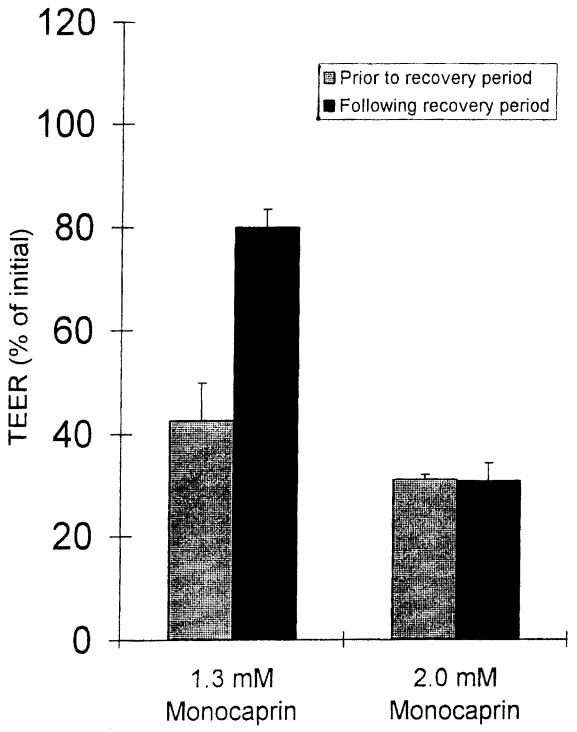



Fig. 3. TEER of Caco-2 monolayers exposed for 1 h to 2.0 mM solutions of BTA-243 containing 1.3 and 2.0 mM monocaprin, prior to and after a 30 h recovery period. Error bars represent SE, $n = 3$.

$$P_{app} = \frac{dQ}{dt}\frac{1}{AC_o},$$

where $dQ/dt$ is the permeability rate, $C_o$ is the initial concentration in the donor chamber and $A$ is the surface area of the monolayer. Permeability coefficients for mannitol transport across monolayers exposed to monocaprin and then subjected to a recovery period are presented in Table 1. The mean apparent permeability coefficient calculated for mannitol across fresh monolayers has been included for comparison. Statistical analysis of the $P_{app}$ values, performed using an ANOVA method, showed no statistically significant difference between the mean $P_{app}$ value calculated for mannitol transport across fresh Caco-2 monolayers and that across Caco-2 monolayers that had undergone a period of recovery following exposure to drug solutions containing 1.3 mM monocaprin. These findings are consistent with the TEER measurements and show that the barrier modification initiated by a 60 min exposure to drug solutions containing 1.3 mM monocaprin is reversible. Although monolayer TEER is not completely restored following the recovery period, the results suggest that sufficient recovery in TEER occurred to restore mannitol permeability to levels similar to those recorded across fresh monolayers that had not been exposed to monocaprin. In contrast, the high mannitol $P_{app}$ measured across monolayers that had been exposed to 2.0 mM monocaprin suggests that no significant recovery of barrier properties occurred in these monolayers during the recovery period, in agreement with the results from TEER measurements.

Table 1
Mean apparent permeability coefficient ($P_{app}$) of mannitol for Caco-2 monolayers ($n = 3$) measured after a 30 h period of recovery following a 60 min exposure to solutions containing monocaprin

| Concentration of monocaprin applied (mM) | $P_{app} \times 10^6$ [min–max] (cm $s^{-1}$) |
|---|---|
| 0 | 0.55 [0.43–0.64]* |
| 1.3 | 1.25 [0.31–2.08] |
| 2.0 | 23.8 [9.71–31.9] |

* $P_{app}$ of mannitol measured across fresh monolayers over 45 min.

### 3.4. The influence of monocaprin on the gross morphology of Caco-2 cell monolayers

Scanning electron micrographs showing the apical surfaces of Caco-2 monolayers incubated for 60 min in HBSS containing 2 mM BTA-243 and 0, 1.3 and 2.0 mM monocaprin are compared in Fig. 4. The surface features of monolayers exposed to a solution containing 1.3 mM monocaprin were similar to those of monolayers not exposed to monocaprin, both sets exhibiting a comprehensive, dense carpet of microvilli. These results suggest that a 60 min exposure to 1.3 mM monocaprin had not caused significant structural damage to the monolayer surface. Structural changes were, however, observed in monolayers following exposure to solutions containing 2.0 mM monocaprin. Some areas of the surface appeared to lack microvilli, although none of the cells appeared to be disrupted and no holes in the monolayers were observed.

The results from Caco-2 cell studies have shown the time dependence of BTA-243 and mannitol permeability enhancement in the presence of monocaprin to be similar. This observation suggests that a similar mechanism of enhancement may be responsible for the increased permeability of the drug and paracellular marker. The decrease in TEER that accompanied exposure to 1.3 mM monocaprin and its reversible nature upon removal is consistent with either permeation enhancement by modulation of paracellular transport or with the enhanced removal of cells from the monolayer. Examination of the monolayer using SEM showed no holes or disrupted cells in monolayers exposed to 1.3 mM monocaprin and thus it may be speculated that monocaprin at this concentration predominantly enhances the permeability of BTA-243 and mannitol by modulation of tight junction permeability. Both are hydrophilic molecules, the permeability of which would be expected to be increased by enhanced availability of the paracellular pathway. On the other hand, the structural changes observed in monolayers exposed to 2.0 mM monocaprin are consistent with the higher observed transport of both drug and mannitol (Figs. 1 and 2) and the inability of these monolayers to regain

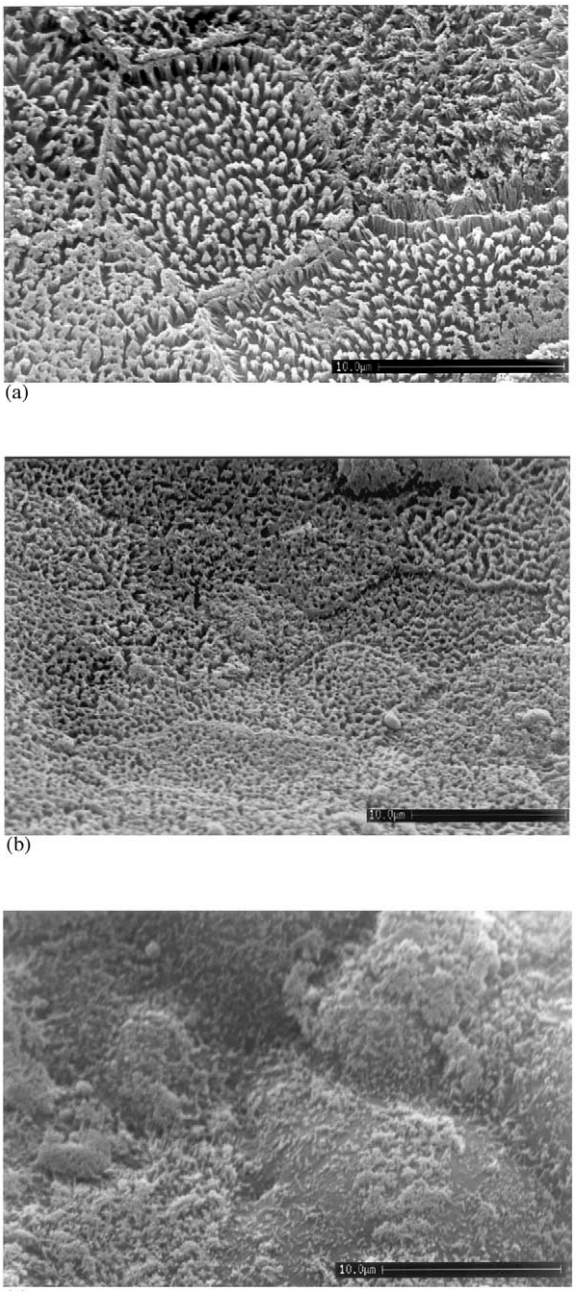



Fig. 4. Electron micrographs of the apical surface of Caco-2 cells exposed for 1 h to 2.0 mM solutions of BTA-243 containing: (a) no added monocaprin; (b) 1.3 mM monocaprin; and (c) 2.0 mM monocaprin.

their transport properties during the recovery period. Other workers (Schipper et al., 1997) noted absorption enhancement, by the charged molecule chitosan, of poorly absorbable drugs through Caco-2 cell monolayers. They suggested that enhanced transport occurred through the paracellular pathways due to interactions of the chitosan with the cell membrane resulting in structural reorganisation of tight junction-associated proteins. Monocaprin is an uncharged molecule and is unlikely to enhance permeability by the same mechanism as chitosan.

### 3.5. The influence of monocaprin on the transfer of BTA-243 across everted gut sacs

The transfer of BTA-243 across everted gut sacs in solutions containing 0, 1.3 and 2.0 mM monocaprin is shown in Fig. 5. A linear relationship between the amount of BTA-243 transferred into the serosal cavity and time was observed both in the presence and absence of monocaprin. Transfer rates from linear regression analysis were 0.86, 1.26 and 1.17 nmol $cm^{-2}$ $min^{-1}$ in the presence of 0, 1.3 and 2.0 mM monocaprin, respectively, indicating an apparent enhancement of BTA-243 transfer rate in the presence of monocaprin. Statistical analysis of the data using SPSS software showed that the increase in the BTA-243 transfer rate in the presence of 1.3 mM monocaprin was significant compared to transfer in its absence, while that in the presence of 2.0 mM monocaprin was not.

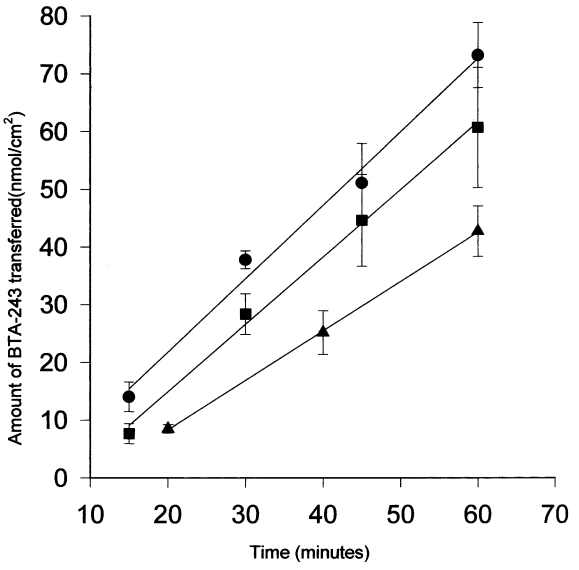



Fig. 5. Variation of BTA-243 transport across everted gut sacs with time for solutions containing: ▲, 0; ●, 1.3; and ■, 2.0 mM monocaprin. Error bars represent SE, $n = 8$.

The enhancement of BTA-243 transfer rates by monocaprin observed in the everted gut sac model, were smaller than those noted in the Caco-2 model. The greater enhancement observed in the Caco-2 model is likely to be a consequence of the greater paracellular permeability of the everted gut sacs prior to exposure to monocaprin. Everted gut sacs represent a model of leaky epithelia and may be more indicative of in vivo intestinal absorption than Caco-2 monolayers, which represent a model of tight epithelia.

### 3.6. The influence of monocaprin on the release of LDH from everted gut sacs

LDH is an intracellular enzyme, detected following damage to cell membranes, which has been used as a biochemical marker of intestinal wall damage (Schasteen et al., 1992; Swenson et al., 1994). The influence of BTA-243, monocaprin and the nonionic surfactant Triton X-100 on the release of LDH from everted gut sacs, as measured by the activity of LDH in the bathing solution following a 60 min exposure, is presented in Fig. 6.

The mean dehydrogenase activity detected in the bathing solutions containing 2.0 mM BTA-243 was similar to that detected in the control (no added drug) bathing solutions. Enzyme detected in these solutions is likely to have been released from the cut ends of the gut sac and from damage inflicted upon the tissue during the preparation stages. Although the difference between mean LDH activities detected in bathing solutions containing 1.3 and 2.0 mM monocaprin and that of the control was not significant at a probability level of 95%, a trend for a greater LDH activity in the presence of monocaprin was indicated, suggesting that some cellular damage may be associated with exposure to the monocaprin. However, Fig. 6 shows that any such cellular damage was significantly less than that caused by Triton X-100

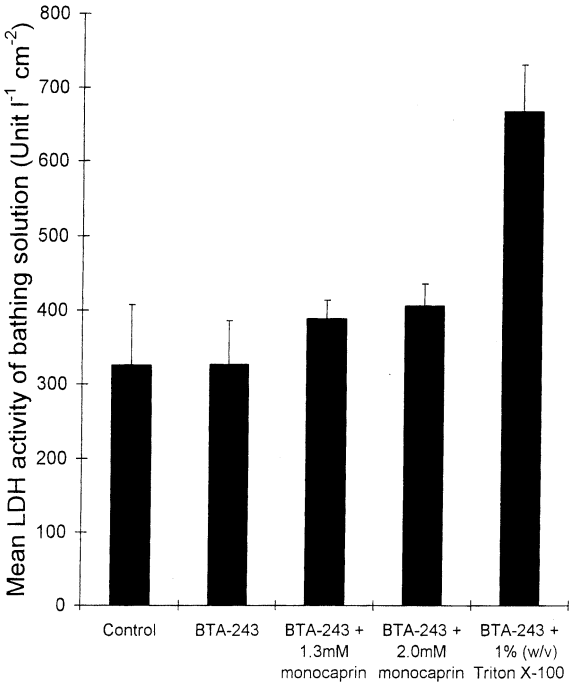



Fig. 6. The influence of BTA-243, monocaprin and Triton X-100 on LDH release from everted gut sacs incubated in TC199 medium for 1 h. Error bars represent SE, $n = 3$.

(1% w/v), a surfactant that is known to disrupt membranes (Gulik-Krzywicki, 1975).

## 4. Conclusions

Our investigations confirm that the permeability of the triple charged, surface active BTA-243 molecule in epithelial monolayer and everted gut sac models may be enhanced by monocaprin. Measurements of the TEER have shown a decrease to a threshold value after 30–40 min exposure of monolayers to 1.3 and 2.0 mM monocaprin, which is thought to be indicative of a modulation of tight junction permeability. The attainment of a threshold TEER value coincided with the onset of a sustained enhancement of transport of drug and mannitol across the Caco-2 monolayers, the extent of permeability enhancement being very much higher in solutions containing 2.0 mM monocaprin. Measurements of TEER and mannitol permeability across monolayers exposed to 1.3 mM monocaprin, after a recovery period of 30 h, indicated partial reversibility of the effects of the monocaprin on the monolayers. No structural changes in the apical surfaces of Caco-2 monolayers exposed to these solutions could be detected by SEM. Our results have shown that although a higher concentration of monocaprin (2.0 mM) increased permeability to a greater extent, the monolayers failed to recover their barrier properties after 30 h and changes in their gross morphology were observed.

Monocaprin enhanced transfer of the drug into the serosal cavity of the gut sac, although to a lesser extent than in the Caco-2 model. The greater release of intracellular LDH from gut sacs exposed to 1.3 and 2.0 mM monocaprin compared to control sacs shows that exposure to this monoglyceride is associated with some structural damage to epithelial cells in organised tissues as well as to cell monolayers.

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