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Comparison of different intestinal epithelia as models for absorption enhancement studies

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Abstract

In this study we compared the effect of two surfactants (laureth-6 and sodium docusate) on the permeability of a model hydrophilic drug across three different epithelia (Caco-2 cells, stripped porcine jejunum and rat ileo-jejunum). Among the tested epithelia Caco-2 cells are the tightest with the trans-epithelial electrical resistance of $372 \pm 4\ \Omega\ cm^2$ followed by porcine jejunum ($124 \pm 8\ \Omega\ cm^2$) and rat ileo-jejunum ($33 \pm 2\ \Omega\ cm^2$). Both surfactants decreased the trans-epithelial electrical resistance and increased the permeability of a model drug across Caco-2 cells at concentrations as low as 0.02 mg/ml, with more pronounced effect observed for laureth-6. On the other hand, ten times higher concentrations (0.2 mg/ml) did not affect the permeability of the model drug across the porcine jejunum. Similarly, laureth-6 at this high concentration had no effect on the trans-epithelial electrical resistance of the rat ileo-jejunum and did not increase the permeability of the model drug across this tissue. On the basis of these results we concluded that Caco-2 cells are much more sensitive to the investigated surfactants, that act as permeation enhancers, than the native intestinal tissues. Therefore, the results obtained in the experiments with Caco-2 cells might exaggerate the effects of the surfactants on the permeability compared to in vivo situation.

Keywords: Absorption enhancement; Caco-2 cells; Rat ileo-jejunum; Porcine jejunum; Trans-epithelial electrical resistance; Surfactants

1. Introduction

Many hydrophilic drugs such as bisphosphonate drugs, proteins, peptides and peptide-like drugs are poorly absorbed from the gastrointestinal tract, due to low permeability across the intestinal epithelium. One approach to improve the permeability of these drugs is co-administration of absorption enhancers, including surfactants, bile salts, calcium chelating agents, fatty acids, cyclodextrins, chitosans and other mucoadhesive polymers (Junginger and Verhoef, 1998; Aungst, 2000; Zadravec et al., 2000). These substances promote the permeability of poorly permeable drugs mainly by opening the tight junctions, leading to the increased

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paracellular permeability (Ward et al., 2000). Besides that, it was shown that several surfactants often used as pharmaceutical excipients could improve the permeability in the absorptive direction by the inhibition of secretory transporters including P-glycoprotein and several multidrug resistance associated proteins (MRPs) (Lo, 2003).

The main problem of absorption enhancers is the narrow concentration range between their ability to increase the intestinal permeability and cytotoxicity (Ward et al., 2000). In general, a positive correlation between the toxic and the enhancement effects is established (Quan et al., 1998). Different models of intestinal epithelia such as Caco-2 cells and rat native intestine are used for studying the effectiveness and toxicity of various absorption enhancers (Aungst, 2000). The concentrations of absorption enhancers that is needed to improve the permeability of polar drugs across the native intestine are usually very high (>10 mM) (Yamamoto et al., 1996; Sugiyama et al., 1997; Lo and Huang, 2000), while the absorption enhancers promote the permeability of drugs across Caco-2 cells at much lower concentration (Quan et al., 1998). These observations suggest that Caco-2 cells are much more sensitive to the absorption enhancers than the native intestinal tissue. Therefore, it is possible that the conclusions drawn out from the experiments with Caco-2 cells might not adequately reflect the activity of certain absorption enhancers in the native intestinal tissue.

The purpose of the present study was to compare the effects of two surfactants (laureth-6 and sodium docusate) on the permeability of a model hydrophilic drug across the different models of intestinal epithelia. For that purpose Caco-2 cell monolayers, stripped porcine jejunum and rat ileo-jejunum were used. Sensitivity of these models with different transepithelial resistance to the studied absorption enhancers was investigated.

2. Materials and methods

2.1. Materials

Stable model hydrophilic drug ($c\log P = -1.87$; $pK_a = 2.8$ (acid)), was obtained from Lek (Ljubljana, Slovenia). Fluorescein sodium was obtained from Fluka (Deisenhofen, Germany). Laureth-6 (dodecyl hexaoxyethylene monoether) and sodium docusate (dioctylsulfosuccinate, sodium salt) were supplied by Sigma (Deisenhofen, Germany).

2.1.1. Cell culture chemicals

Dulbecco's modified eagles medium (DMEM) with supplements was used for the Caco-2 culture. DMEM, non-essential amino acids and gentamicine sulphate were obtained from Biochron KG (Berlin, Germany). Trypsin–EDTA solution was obtained from Sigma (Deisenhofen, Germany). Fetal calf serum was supplied by Greiner Labortechnik (Frickenhause, Germany). For the transport experiments Krebs–Ringer bicarbonate buffer with pH 7.4, containing 25 mM of D-glucose (KRB) was used.

2.2. Experiments with Caco-2 cells

Caco-2 cells were obtained from the German Cell Culture Collection DSMZ, DSMZ-no. ACC 169. The cells were cultured at 37 °C, 90% humidity and 10% CO_2. For the transport studies 10^4 cells/cm^2 were sown on clear polyester transwell filters (Costar, Germany) with an area of 1.13 cm^2 and 0.4 μm pore size.

Before the transport experiments, the cells were washed twice with KRB to remove the cell culture medium. Fresh pre-warmed KRB and the transport solution were added to the acceptor compartment (1.5 ml) and donor compartment (0.5 ml), respectively. The transport solution was prepared by dissolving the model drug (350 μM, final concentration) with and without surfactants in the DMSO (1% in the final volume) and diluted with fresh KRB. DMSO at such low concentrations is known to not affect the integrity of Caco-2 cells (Yamashita et al., 2000) or the integrity and viability of excised rat jejunal segments (Watanabe et al., 2000).

The monolayers were pre-incubated for 35 min to saturate the transport sites in the test system with the model drug. Afterwards, the KRB was removed completely from the acceptor compartment and replaced by fresh KRB (start of transport). Twenty-five millimolars D-glucose was present in the incubation medium at the apical and basolateral side throughout the experiments. During the transport study samples were taken from the acceptor compartments at defined time points (60, 120, 180 and 240 min). Between the