Effects of Deoxycholate on the Transepithelial Transport of Sucrose and Horseradish Peroxidase in Filter-Grown Madin–Darby Canine Kidney (MDCK) Cells

Yi-Jing Lin¹ and Wei-Chiang Shen¹,²

Received June 7, 1990; accepted October 18, 1990

Madin–Darby canine kidney (MDCK) epithelial cells grown on microporous polycarbonate filters were used as a model system to investigate the mechanisms of enhancement by deoxycholate in the transepithelial transport of horseradish peroxidase (HRP) and ¹⁴C-sucrose. Deoxycholate at 0.025% had no effect on the transepithelial electrical resistance (TEER); a fivefold enhancement on the transepithelial transport of HRP, but not on that of ¹⁴C-sucrose, was observed. Deoxycholate at 0.05% induced a reversible decrease of TEER; a 2- and 50-fold enhancement on the transepithelial transport of ¹⁴C-sucrose and HRP, respectively, was observed. At 0.1%, deoxycholate induced an irreversible decrease in TEER and the epithelial barrier in the cell monolayer was completely eliminated. A 3.3-fold increase in cellular uptake in HRP, but not in ¹⁴C-sucrose, was also observed in the presence of 0.025% deoxycholate. The increase in cellular uptake was abolished when HRP was conjugated to polysine. These results suggest that deoxycholate can increase the transepithelial transport by at least two different mechanisms, i.e., a transcellular pathway, possibly due to the enhancement of cellular uptake of selective molecules, and a nonsselective paracellular pathway, due to the loosening of tight junctions by deoxycholate at higher concentrations.

KEY WORDS: deoxycholate; transepithelial transport enhancement; Madin–Darby canine kidney (MDCK) cells.

INTRODUCTION

Bile salts are a class of penetration enhancers which have been widely used to promote the absorption of drugs, particularly peptides or proteins, across nasal (1), buccal (2), or rectal (3) epithelia. Even though enhancement by bile salts has been demonstrated in the in vivo absorption of insulin (4), interferon (5), or enkaphlin (6), the exact mechanism of the action at the molecular and cellular level has not been explicitly elucidated. Effects of bile salts on epithelia are difficult to investigate in animal models, because of the complexity of the in situ measurement of various parameters, such as the integrity of the junction between the cells, and the rates of the cellular uptake and the transepithelial transport of macromolecules.

Recent developments of the epithelial cell cultures have provided us with a useful in vitro model system for the detection of the response of epithelial cells to various treatments. For example, chelators, such as citrate, have been shown to lower the transepithelial electrical resistance (TEER) on MDCK cells, possibly due to the removal of Ca²⁺ ions involved in the tight junction formation (7). Similarly, both dimethyl sulfoxide and ethanol can cause a reversible decrease in TEER which correlates with an increase in the transepithelial transport of both ¹⁴C-sucrose and horseradish peroxidase (HRP) (8). Most recently, a decrease in TEER has also been demonstrated in cultured kidney epithelial cells upon the treatment of tumor necrosis factor, a cytokine which has been shown to cause epithelial and endothelial leakage (9).

In this paper, we describe the effects of one of the most commonly used bile salts, deoxycholate, on the filter-grown Madin–Darby canine kidney (MDCK) epithelial cell monolayers. We also demonstrate that MDCK cell monolayers can be useful as an in vitro epithelial model system for the elucidation of the mechanism of the transepithelial transport, particularly in the presence of penetration enhancers.

MATERIALS AND METHODS

Eagle’s minimum essential medium (MEM), fetal bovine serum (FBS), and trypsin/EDTA were obtained from GIBCO laboratories (Grand Island, NY). Transwells with 24.5-mm diameter and 0.4-µm pore size were obtained from Costar (Cambridge, MA). Sodium deoxycholate and horseradish peroxidase (HRP) were purchased from Sigma Chemical Co (St. Louis, MO). ¹⁴C-Sucrose was purchased from ICN Radiochemicals (Irvine, CA). Polysine conjugates of HRP (HRP-PLL) were prepared by a thioether coupling reaction as described in our previous report (11).

Strain 1 MDCK cells (a gift from Dr. M. J. Cho, The Upjohn Co.) were routinely cultured at 37°C in a T-25 flask with 5% CO₂ and Eagle’s MEM supplemented with 10% FBS as previously described (10,11). Cells (2.2 × 10⁴/cm²) were seeded on each of the wells in Transwells. The amount of FBS in the medium was reduced to 2.5% when the transepithelial electrical resistance (TEER), measured with an Ethigraph Voltohmeter, EVOM (World Precision Instruments, West Haven, CT), reached 1000 Ω · cm².Generally, confluent monolayers were obtained in 5–6 days with a final TEER of approximately 2000 Ω · cm² and were maintained in medium containing 1% FBS. All experiments were carried out in the presence of 1% FBS in the medium.

Effects of Deoxycholate on the Transepithelial Electrical Resistance (TEER) of MDCK Cell Monolayers

Confluent MDCK cell monolayers in Transwells were incubated at first with medium containing 0.01, 0.05, 0.1, and 0.5% of deoxycholate in the apical compartments. After 1 hr at 37°C, deoxycholate-containing medium was replaced by fresh medium without deoxycholate and the incubation was continued for another 5 hr. TEER across the cell monolayers was measured with EVOM electrodes at various time intervals. Results are expressed as ohms per square centimeter.

Transepithelial Transport of HRP and ¹⁴C-Sucrose in MDCK Cell Monolayers

Confluent MDCK cell monolayers in the Transwells

¹ Drug Targeting Research, Division of Pharmaceutics, University of Southern California School of Pharmacy, Los Angeles, California 90033.
² To whom correspondence should be addressed.
were incubated with 0.025% deoxycholate in the apical medium. Two fluid phase markers, i.e., \(^{14}\)C-sucrose and HRP, were added together to the apical medium to give final concentrations of 1 \(\mu\)Ci/ml and 1 mg/ml, respectively. The cell monolayers were then incubated at 37°C, and at various time intervals, TEER values across the monolayers were measured. Aliquots of 0.1 ml of the medium were pipetted from the basal compartments and the amounts of transported \(^{14}\)C-sucrose and HRP were determined by measuring the radioactivity and by the colorimetric HRP assay method (12), respectively.

**Cellular Uptake of HRP, \(^{14}\)C-Sucrose, and HRP-Polylysine Conjugate in MDCK Cell Monolayers**

Confluent MDCK cell monolayers grown in six-well cluster plates (Costar) were exposed to the medium containing 0.025% deoxycholate together with HRP (3 mg/ml), \(^{14}\)C-sucrose (1 \(\mu\)Ci/ml), or HRP-polylysine conjugate (3 \(\mu\)g/ml). After incubation at 37°C for 60 min, cells were removed from the plates by trypsin/EDTA, suspended in 2 ml of cold PBS, and transferred to test tubes. After centrifugation, the final cell pellets were washed twice each with 2 ml of cold PBS, then dissolved in 1 ml of 0.1% Triton X-100 in water, and the amount of the intracellular \(^{14}\)C-sucrose or HRP was determined. Lowry’s protein assay (13) was used to normalize the cell number in each sample.

**RESULTS**

The effects of various concentrations of deoxycholate on TEER of MDCK-cell monolayers are presented in Fig. 1. In the presence of 0.01% deoxycholate, no change in the TEER of the cell monolayers was found. However, in the presence of 0.05% deoxycholate, a decrease in TEER of the monolayers was observed. The TEER across the cell monolayers fell during the first hour of the treatment to 60% of the control, i.e., from 1356 to 598 \(\Omega \cdot \text{cm}^2\). A rapid recovery of TEER to as high as 120% of the initial values was observed when the deoxycholate-containing medium was replaced by fresh medium without deoxycholate. When the cell monolayers were exposed to 0.1% or higher deoxycholate, a sharp decrease in TEER of the cell monolayers was observed. The decrease in TEER from 1366 to 71 \(\Omega \cdot \text{cm}^2\) in the 1-hr treatment of 0.1% deoxycholate was not recovered during the course of the 6-hr experiment.

To determine if the decrease in TEER in the cell monolayers is due, at least in part, to the alteration of the intercellular gap junctions, the transepithelial transports of \(^{14}\)C-sucrose and HRP were studied. As shown in Figs. 2A and B, 0.05% deoxycholate induced 2- and 50-fold increases in the transepithelial transport of \(^{14}\)C-sucrose and HRP, respectively, i.e., an increase from 1.7 to 3.6% of the initial \(^{14}\)C-sucrose and from 0.3 to 13.6% of the initial HRP transported in 5 hr. The fact that transepithelial transport of HRP in the presence of 0.05% deoxycholate was higher than that of \(^{14}\)C-sucrose, a fluid phase transport marker, suggests that the transport of HRP was not limited only to the paracellular pathway. Furthermore, when the cell monolayers were exposed to 0.025% deoxycholate, a fivefold increase in HRP transport, i.e., from 0.3 to 1.7% of HRP (Fig. 2A), but not in \(^{14}\)C-sucrose transport (Fig. 2B), was observed. At this concentration, there was no effect in TEER of the cell monolayers after 5 hr of incubation, i.e., 3075 \(\Omega \cdot \text{cm}^2\) compared to 3066 \(\Omega \cdot \text{cm}^2\) for the control (Fig. 2C).

**Fig. 1.** Effects of various concentrations of deoxycholate on the transepithelial electrical resistance (TEER) of MDCK cell monolayers. The apical surface of the MDCK cells were exposed to medium containing 0.01% (A), 0.05% (B), 0.1% (C), and 0.5% (D) of deoxycholate during the first hour and then changed to fresh medium for the continuous incubation. TEER in each Transwell was measured at time intervals. Monolayers exposed to medium without deoxycholate were used as controls (O).

**Fig. 2.** Effects of deoxycholate on the transport of HRP (A) and \(^{14}\)C-sucrose (B) and on the TEER (C) in MDCK cell monolayers. Cells were exposed on the apical surface for 5 hr to a medium containing both HRP (1 mg/ml) and \(^{14}\)C-sucrose (1 \(\mu\)Ci/ml) with 0.025% (■), 0.05% (♦), or no deoxycholate (○). At various time intervals, 100 \(\mu\)l of the basal medium was pipetted for the measurements of the \(^{14}\)C-radioactivity and HRP enzymatic activity. Fresh medium was added to the Transwells after each sampling in order to maintain a constant volume of medium in each well. Standard deviations are represented as bars or are smaller than the symbols.