Paracellular Porosity and Pore Size of the Human Intestinal Epithelium in Tissue and Cell Culture Models

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ABSTRACT: The paracellular space defines the passive permeation of hydrophilic compounds in epithelia. The goal of this study was to characterise the paracellular permeation pathway in the human intestinal wall and differentiated epithelial cell models (MDCKII, Caco-2 and 2/4/A1). The permeabilities of hydrophilic polyethylene glycols (PEG) were investigated in diffusion chambers, and mass spectrometry was used to obtain accurate concentrations for each PEG molecule. The paracellular porosity and the size of the pores in the membranes were estimated from the PEG permeability data using an effusion-based approach. The porosities were found to be low (fraction 10^{-7}–10^{-5} of the epithelial surface) in all investigated membranes. Two different pore sizes (radii 5–6 and >10 Å) were detected in the human intestinal epithelium and the Caco-2 and MDCKII cells, while only one (about 15 Å) in the 2/4/A1 monolayer. The paracellular porosities of the human small intestine and 2/4/A1 monolayers were larger ($>10^{-7}$) than that of the MDCKII and Caco-2 cells ($<10^{-7}$). We report for the first time the quantitative values describing both porosity and pore size of the paracellular space in the human intestine. The cell models deviate from the small intestine either with respect to porosity (Caco-2, MDCKII) or pore size distribution (2/4/A1). © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:2166–2175, 2010

Keywords: paracellular permeation; drug absorption; human intestine; Caco-2; MDCKII; 2/4/A1; epithelial permeability

INTRODUCTION

Passive drug diffusion across an epithelium takes place either through the hydrophilic pores between the cells or across the lipoidal cell membrane. The route, which a compound prefers, depends on its shape, size and charge.[1,2] For instance, hydrophobic compounds are considered

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to traverse the cell monolayer predominantly by the transcellular route, whereas small hydrophilic solutes prefer the paracellular route.[3,4] The negative characteristics of the paracellular space determine that cations permeate across the paracellular route easier than neutral compounds, which, in turn, are more permeable than anions.[3,5,6]

Paracellular permeation depends not only on the properties of the permeating molecule but naturally also on the morphology of the epithelium. The hydrophilic pores constituting the paracellular route are protein-based structures formed of occludins and claudins.[7,8] These structures, also called tight junctions, seal the space between the epithelial cells thereby forming a protective barrier allowing paracellular diffusion of small solutes, but excluding potentially toxic macromolecules and micro-organisms.

Epithelial cell monolayers grown on semiporous filters have gained considerable popularity in the study of epithelial drug transport. Previous studies have shown that the commonly used Caco-2 cell model yields even 100 times lower permeability coefficients for low permeability drugs than the small intestine.[9] This difference has been proposed to result, at least partly, from the tight junctions of the Caco-2 cells being less permeable than those of the small intestine.[9] Another commonly used cell line, the MDCKII cell model, also suffers from the drawback of not predicting accurately the absorption of low permeability drugs.[10]

Alternative cell lines that would better mimic the human small intestine are under development. The recently introduced 2/4/A1 cell line forms a leakier paracellular pathway than the Caco-2 monolayer and therefore is thought to mimic the human small intestinal epithelium better than the Caco-2 model.[11,12] Tavelin et al.[12] estimated the average pore radius of the 2/4/A1 cell line to be 9.0 Å. In the same study, the average pore radius of the Caco-2 cell line was found to be 3.7 Å. Fine et al.[13] estimated that the average pore radius of the human intestinal epithelium was 8–13 Å.

Several previous studies, such as the study of Tavelin et al.,[12] have estimated the pore radii of the intestinal cell models using an approach called the Renkin method.[14] Hämäläinen et al.[15] introduced an alternative method, an effusion-based approach, to analyse the paracellular space of epithelial membranes. According to the effusion theory, paracellular drug permeation takes place when randomly colliding drug molecules happen to hit the pores in the membrane. This occurs infrequently, but once a molecule finds a pore, permeation across the pore is assumed to take place rapidly. The effusion approach can only be applied to membranes with short diffusion lengths across the pores, small pore densities and small pore sizes. In addition to predicting the pore size of the investigated membrane, the effusion theory also predicts the porosity of the membrane (the fraction of the paracellular space of the membrane surface), which is not possible with other techniques. Such quantitative information on epithelial cell monolayers and human tissues could provide a basis for improved predictions of drug absorption from cell data.

The aim of this study was to characterise the paracellular permeation route of (1) the 2/4/A1, MDCKII and Caco-2 cell lines and (2) excised human intestinal segments. The pore sizes and porosities of these membranes were estimated based on permeabilities of neutral polyethylene glycol (PEG) oligomers and an effusion theory-based data analysis.

MATERIALS AND METHODS

Polyethylene Glycol Stock Solution

PEGs with mean molecular weights of 200, 400, 600 and 1000 were obtained from Chemical Pressure (Pittsburgh, PA). PEG 200 (final concentration 0.2 mg/mL), PEG 400 (final concentration 4.0 mg/mL), PEG 600 (final concentration 0.6 mg/mL) and PEG 1000 (final concentration 1.0 mg/mL) were dissolved in glutathione bicarbonated Ringer's (GBR) solution (Caco-2 and MDCKII)/NaCl (2/4/A1 and human jejunum). On the basis of mean molecular weight the concentrations were 0.001 M.

Human Jejunum

Human proximal jejunum was obtained from one female patient (age 62, weight 75 kg) undergoing gastro-intestinal bypass surgery (Sahlgrenska University Hospital, Gothenburg, Sweden). A 2 cm × 2 cm part of the jejunum was stapled and put in a beaker with cold Krebs-bicarbonate Ringer's solution (KBR) on ice, which was continuously bubbled with an O_2/CO_2 (95%/5%) gas mixture. The segment was directly transported to the laboratory under these conditions. The jejunal