Identification of Tight Junction Modulating Lipids

SHU-CHIH CHEN-QUAY, KRISTINE T. EITING, ANGELA W.-A. LI, NAJIB LAMHARZI, STEVEN C. QUAY

Nastech Pharmaceutical Company, Inc., 3830 Monte Villa Parkway, Bothell, Washington

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ABSTRACT: Tight junctions (TJs) play an important role in regulating paracellular drug transport. The aim of this study was to identify lipids that rapidly and reversibly alter transepithelial electrical resistance (TER) and/or TJ permeability in epithelial tissue. In this study, we developed a screen for identifying lipids that alter TJ properties. Measurement of TER was used to monitor TJ activity on bronchial/tracheal epithelial tissues using a microtiter format. Among seven groups of lipids tested, four classes were identified as TJ modulators (sphingosines, alkylglycosides, oxidized lipids and ether lipids). Individual lipids within these four classes showed up to 95% TER reduction at noncytotoxic concentrations. Alkylglycosides, however, showed high cytotoxicity and low viability at concentrations (0.2–0.4%) reported to enhance transmucosal absorption (Ahsan et al., 2003, Int J Pham 251: 195–203). Several active lipids also showed enhanced permeation of FITC-labeled dextran (m.w. 3000). Immunofluorescence staining of PGPC-treated cells with antibodies against ZO-1, occludin and claudin 4 showed no detectable changes in TJ structural morphology, indicating that a nondestructive, submicroscopic alteration in TJ function may be involved in TER reduction and permeation enhancement. This study demonstrates that three new classes of lipids, excluding alkylglycosides, show potential utility for transmucosal drug delivery. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:606–619, 2009

Keywords: tight junction; epithelial delivery/permeability; lipids/lipoproteins; mucosal delivery; nasal absorption; ether lipids; glycosylated sphingosine; oxidized lipid; alkylglycosides; ceramides

INTRODUCTION

Tight junctions (TJs) are intercellular permeability barriers located in the apical region of the lateral plasma membrane in epithelial and endothelial tissue layers. The three primary functions of TJs are: regulating the passage of ions, water, and molecules through the paracellular pathway (gate function); limiting the diffusion of proteins and lipids between the apical and basolateral domains of the plasma membrane (fence function);[1] and recruiting cytoskeletal and signaling molecules involved in cell polarity, growth, differentiation and apoptosis.[2] Freeze-fracture replica electron microscopic (EM) images show that TJs are composed of a network of continuous beaded strands interlacing on the

Abbreviations used: TJ, tight junction; TER, transepithelial electrical resistance; EM, electron microscopy; LDH, lactate dehydrogenase; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; POVPC, 1-palmitoyl-2-(5′-oxo-valeroyl)-*sn*-glycero-3-phosphocholine; PGPC, 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine; C16-09:0, 1-O-hexadecyl-2-azelaoyl-*sn*-glycero-3-phosphocholine; 16:0-09:0(COOH)PC, 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine; 16:0-09:0(ALDO)PC, 1-palmitoyl-2-(9′-oxo-nonanoyl)-*sn*-glycero-3-phosphocholine; PAF, platelet-activation factor; OMGPC, 1-O-octadecyl-2-O-methyl-glycerol-3-phosphocholine; DPGPC, 1,2-di-O-phytanyl-glycero-3-phosphocholine; DO-ethyl-PC, 1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine; HAG, 1-O-hexadecyl-2-acetoyl-*sn*-glycerol.

Correspondence to: Steven C. Quay (Telephone: 425-908-3687; Fax: 425-908-3655; E-mail: squay@nastech.com)

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protoplasmic face of the plasma membrane.[2] Ultra-thin section EM micrographs reveal TJs as a series of fusion points (also referred to as kissing points) between the outer leaflets of the membrane of the adjacent cells.

TJs are composed of transmembrane proteins such as claudins, occludin and junction adhesion proteins (JAMs), as well as cytoplasmic plaque proteins including ZO-1, ZO-2, ZO-3, cingulin and 7H6.[3] At least 24 claudin genes have been identified.[2] Both claudin and occludin are tetra-span proteins consisting of four transmembrane regions and two extracellular domains, with both the amino and carboxyl termini located intracellularly.[2] These tetra-span proteins are capable of interactions with complementary molecules on adjacent cells to form adhesion points and through lateral copolymerization to form continuous, anastomosing strands.[3] Cytoplasmic plaque proteins are involved in: (i) linking claudin and occludin molecules to the actin cytoskeleton through PDZ domains, (ii) cross-linking the transmembrane junction proteins, (iii) vesicular trafficking to the TJ and cell signaling through their associated kinases, and (iv) other functions that are not as well characterized, such as their role in gene expression due to their nuclear shuttling and specific binding to transcription factors.[3]

The existence of lateral asymmetric membrane lipid domains enriched with cholesterol, ceramides, glycosphingolipids, ether and oxidized lipids, and arachidonic acid precursors (including plasmenylethanolamine) comprise structures known as lipid rafts. These structures and specific associated proteins have been studied extensively.[4] Both ZO-1 and hyperphosphorylated occludin are found in detergent-insoluble glycolipid (DIG) rafts along with the DIG-abundant protein, caveolin-1, suggesting that TJ proteins may reside in cholesterol-enriched microdomains.[5] This is supported by the finding that a rapid reduction of membrane cholesterol by methyl-β-cyclodextrin reduces transepithelial electrical resistance (TER) and increases mannitol paracellular permeability,[6] indicators of a change in TJ integrity. Therefore, it is possible that TJ permeability can be modulated by perturbing the lipid environment in which TJ proteins integrate or associate. To that end, we have screened more than 50 lipids ranging from normal lipid raft associated lipids such as ceramides, glycosylated sphingosines, ether lipids, and oxidized lipids, to synthetic nonionic detergent lipids, such as alkylglycosides for potential TJ modulating properties. We used an *in vitro* differentiated bronchial–tracheal mucosal epithelial tissue model to identify candidates that are able to reduce TER and enhance permeability for fluorescein-labeled dextran (m.w. 3000) and also evaluated the apparent cytotoxicity of these lipids across a range of concentrations. The most active candidates, glycosylated sphingosine, oxidized lipids and ether lipids, offer new prospects as excipients for mucosal drug delivery via the paracellular pathway. The presence of these candidates in normal lipid rafts suggests that they may enable the development of novel excipients for drug delivery that have reduced toxicity during long-term administration compared to other, nonphysiological compounds.

MATERIALS AND METHODS

Lipids

All lipids other than the alkylglycosides were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). The alkylglycosides were from Calbiochem (EMD Biosciences, Inc., San Diego, CA). Most of the lipids were dissolved in chloroform, evaporated to form a thin film, and then resuspended in Dulbecco's phosphate buffered saline (DPBS) with magnesium (Mg) and calcium (Ca), or dissolved directly in DPBS with Mg and Ca. A few lipids were dissolved in 20% ethanol and the appropriate percentage of ethanol was used as a control in parallel to evaluate the effect of ethanol.

EpiAirway™ Tissue Model

Normal human tracheal/bronchial epithelial cells were grown in a 96-well high throughput format (AIR-196-HTS) or individual 24-well cell culture inserts (AIR-100) to form pseudostratified mucociliary airway tissue (EpiAirway™, MatTek Corporation, Ashland, MA). The AIR-196-HTS inserts were used for TER, lactate dehydrogenase (LDH), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), and permeability assays, while the AIR-100 inserts were used for immunofluorescent staining. Inserts were recovered following shipment according to the manufacturer's recommended procedure using the

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