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Cytoskeletal modulation and tyrosine phosphorylation of tight junction proteins are associated with mainstream cigarette smoke-induced permeability of airway epithelium ☆

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Abstract

Cigarette smoke increases the permeability of the lung epithelium. Consequences of increased permeability include increased access of toxins and pathogens from the air spaces to the interstitium and even the blood stream, and leakage of fluids into the air spaces. The mechanisms for permeability alterations have not been elucidated for airway epithelia. By analogy with other types of epithelia, we hypothesized that changes in the phosphorylation status and function of tight junction (TJ) or cytoskeletal proteins might mediate the smoke-induced permeability changes. We investigated the effects of exposure to mainstream cigarette smoke (MS) on cultures of Calu-3 cells, an airway epithelial cell line. Specifically, MS exposure caused increases in phosphorylation of the myosin-binding subunit (MBS) of myosin phosphatase and myosin light chain (MLC), proteins involved in the regulation of actin polymerization. These results implicate activation of Rho kinase (ROCK), consistent with previously reported data indicating that inhibition of ROCK activation suppressed MS-induced increases in permeability. MS exposure also increased polymerized (filamentous) actin (f-actin) content and caused redistribution of the TJ proteins from the normal apical circumferential band to a more basal location. The translocation of the TJ proteins was spatially associated with local increases in both f-actin and macromolecular permeability. Finally, MS exposure increased tyrosine phosphorylation of occludin but not ZO-1 and decreased association between the two TJ proteins. These results indicate that MS exposure causes alterations in cytoskeletal and TJ structure and function, resulting in increased macromolecular permeability that may contribute to the adverse health effects of MS.

Keywords: Cigarette smoke; Airway epithelium; Tight junction proteins; Cytoskeleton

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Introduction

Cigarette smoking causes a vast array of adverse effects, both in the lung itself and distal to this primary site of smoke exposure. Systemic effects include increased incidence of cardiovascular diseases (atherosclerosis, myocardial infarctions, and strokes) and increased susceptibility to infection. Cigarette smoke (CS) exposure is known to increase the permeability of the lung epithelial/endothelial barrier, potentially allowing access of atmospheric components and pathogens to the circulation and leakage of serum and its constituents to the lumen. The mechanisms by which CS disrupts epithelial integrity have not been fully defined but are likely to involve alterations in the function of the tight junctions (TJ), which normally maintain the polarity of the epithelial cells and limit flow of ions and macromolecules from one side of the monolayer to the other. We previously reported that cigarette smoke temporarily increases macromolecular permeability ($\approx$30 min) at focal sites within cultured epithelium by protein tyrosine kinase (PTK)- and Rho kinase (ROCK)-dependent mechanisms. Conversely, the role of myosin light chain kinase (MLCK) was shown to be protective in this system (Olivera et al., 2007). These results suggested that regulation of permeability in this tissue involves actomyosin and protein phosphorylation.

Tyrosine phosphorylation of the TJ proteins has been linked to increased permeability in a number of models. In epidermal carcinoma cells (Van Itallie et al., 1995), phosphorylation of ZO-1 resulted in its redistribution, and in the blood–brain barrier (BBB), tyrosine phosphorylation of occludin caused its redistribution and increased macromolecular permeability (Kago et al., 2006). In an intestinal model, oxidative stress increased tyrosine phosphorylation of occludin and ZO-1, which is associated with loss of ZO-1:occludin complex, loss of TJ:cytoskeleton association, and increased permeability to ions and macromolecules (Rao et al., 2002).

ROCK is an important modulator of the cytoskeleton, known to induce stress fibers (Amano et al., 1997; Fujita et al., 2000), phosphorylate myosin light chain, similar to MLCK (Amano et al., 1996; Kimura et al., 1996); phosphorylate and inactivate the myosin-binding subunit (MBS) of myosin phosphatase (Totsukawa et al., 2000); and phosphorylate Lin-11, Isl-1, and Mec-3 kinase (LIMK) (Ohashi et al., 2000), which inhibits cofilin, the actin-severing factor (Takaishi et al., 2000). The results of these phosphorylation events are the polymerization of filamentous actin at the cell periphery (Takaishi et al., 2000; Bruewer et al., 2004), cytoskeletal contraction through increased net phosphorylation of myosin light chain (Wojciak-Stothard et al., 2001), increased motor activity of myosin heavy chain (Totsukawa et al., 2000), TJ protein redistribution (Stamatovic et al., 2003; Bruewer et al., 2004), and increased permeability (Wojciak-Stothard et al., 2001; Stamatovic et al., 2003).

Our previous data indicated that inhibition of protein tyrosine kinases partially protects barrier function in lung epithelial cells exposed to mainstream smoke (MS) at an air–liquid interface (Olivera et al., 2007). We therefore investigated tyrosine phosphorylation of ZO-1 and occludin and ZO-1:occludin associations. Because inhibition of ROCK also partially protected barrier function, we also examined interactions of the cytoskeleton with the TJ proteins, densities of filamentous actin, and MBS and MLC phosphorylation status following exposure to cigarette smoke.

Materials and methods

Cell culture

Calu-3 adenocarcinoma cells were selected for these experiments due to their airway epithelial origin and ability to form monolayers that are highly impermeable to ionic and macromolecular flux (Wan et al., 2000; Olivera et al., 2007). Cells were obtained from the American Type Cell Collection (Rockville, MD) and used at passages 30–40 for all experiments. Cells to be exposed were seeded $1 \times 10^5/cm^2$ into Transwell® inserts, 0.4 μm pore size, 1 cm^2 surface area (Costar, Cambridge, MA) and grown in RPMI 1640 medium (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (FBS) (Hyclone, Logan, UT) and 100 units/ml penicillin/streptomycin (Invitrogen), with 500 μl in the upper compartment and 1000 μl in the lower compartment. The first day after cultures were seeded, they were washed with 37 °C phosphate-buffered saline (PBS) to remove nonadherent cells. Cultures were then maintained at 37 °C and 5% CO_2 until confluent, as assessed microscopically.

TER measurement

Tight junction ionic barrier integrity was measured by trans-epithelial electrical resistance (TER), using a Millicell electrical resistance system (ERS) meter with "chopstick" style AgCl electrodes (Millipore, Bedford, MA), as previously described (Olivera et al., 2007). Cultures with TERs between 1000 and 1300 Ωcm^2 were used for exposures. Cultures exceeded 1000 Ωcm^2 2–3 days after confluence was reached and were given fresh medium, incubated overnight, and exposed to mainstream cigarette smoke (MS) or filtered air (FA) the following day.