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NF-κB activation as a key mechanism in ethanol-induced disruption of the F-actin cytoskeleton and monolayer barrier integrity in intestinal epithelium

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Abstract

Intestinal barrier disruption has been implicated in several intestinal and systemic disorders including alcoholic liver disease (ALD). Using monolayers of intestinal (Caco-2) cells, we showed that ethanol (EtOH) disrupts the barrier integrity via *de*stabilization of the cytoskeleton. Because proinflammatory conditions are associated with activation of NF-kappa B (NF-κB), we hypothesized that EtOH induces disruption of cytoskeletal assembly and barrier integrity by activating NF-κB. Parental cells were pretreated with pharmacological modulators of NF-κB. Other cells were stably transfected with a dominant negative mutant for the NF-κB inhibitor, I-κBα. Monolayers of each cell type were exposed to EtOH and we then monitored monolayer barrier integrity (permeability); cytoskeletal stability and molecular dynamics (confocal microscopy and immunoblotting); intracellular levels of the I-κBα (immunoblotting); subcellular distribution and activity of NF-κB (immunoblotting and sensitive ELISA); and intracellular alterations in the 43 kDa protein of the actin cytoskeleton, polymerized F-actin, and monomeric G-actin (SDS-PAGE fractionation). EtOH caused *de*stabilizing alterations, including I-κBα degradation, NF-κB nuclear translocation, NF-κB subunit (p50 and p65) activation, actin disassembly (↑G-, ↓F-), actin cytoskeleton *in*stability, and barrier disruption. Inhibitors of NF-κB and stabilizers of I-κBα (e.g., MG-132, lactacystin, etc) prevented NF-κB activation while protecting against EtOH-induced injury. In transfected I-κBα mutant clones, stabilization of I-κBα to inactivate NF-κB protected against all measures of EtOH-induced injury. Our data support several novel mechanisms where NF-κB can affect the molecular dynamics of the F-actin cytoskeleton and intestinal barrier integrity under conditions of EtOH injury. (1) EtOH induces disruption of the F-actin cytoskeleton and of intestinal barrier integrity, in part, through I-κBα degradation and NF-κB activation; (2) The mechanism underlying this pathophysiological effect of the NF-κB appears to involve instability of the assembly of the subunit components of actin network.

Keywords: Cytoskeleton; Actin; Gut barrier integrity; Transfection and molecular biology; ALD; Caco-2 cells

Introduction

The intestinal epithelium is a selective barrier that normally prevents the passage of immunoreactive and toxic molecules into the mucosa and the systemic circulation (Hollander, 1992, 1998; Keshavarzian et al., 1999; Menconi et al., 1997; Unno et al., 1997). This barrier thus represents a crucial line of defense against pathogenic and noxious agents including proinflammatory antigens and luminal bacterial products like endotoxin, which could lead to local and systemic necroinflammatory reactions. Indeed, disruption of gut barrier integrity is thought to lead to the penetration of these luminal proinflammatory factors into the mucosa, and cause the initiation or continuation of inflammatory processes and mucosal and systemic damage. Not surprisingly, then, disruption of barrier integrity has been implicated in the pathogenesis of a wide variety of gastrointestinal and systemic disorders (Hermiston and Gordon, 1995; Hollander, 1992, 1998; Keshavarzian et al., 2003, 2001, 1994, 1999; Mathurin et al., 2000; Nanji et al., 1994; Yamada et al., 1993).

Abbreviations: Inflammatory bowel disease, IBD; Alcoholic liver disease, ALD.

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One of the systemic disorders associated with disruption of intestinal barrier integrity (leaky gut) and endotoxemia is alcoholic liver disease (ALD) (Bode et al., 1987; Keshavarzian et al., 2003, 2001, 1994, 1999; Mathurin et al., 2000; Nanji et al., 1994). It is now well established that only a minority of alcoholics develop ALD. Thus, excessive alcohol ingestion, although is required, is not sufficient to cause ALD. We have previously shown that alcoholics with no liver disease have a normal intestinal barrier while those with ALD have a leaky gut and thus suggested that the key and the required cofactor for development of ALD is gut leakiness (Keshavarzian et al., 2003, 2001, 1994, 1999). Accordingly, increased understanding of the mechanisms involved in alcohol-induced intestinal barrier dysfunction should lead to the development of more effective preventive and/or treatment strategies for ALD.

Ethanol (EtOH) intake injures the functional and structural integrity of the intestinal mucosa (Bjorkman and Jessop, 1994) and causes loss of intestinal epithelial barrier function (Bode et al., 1987; Keshavarzian et al., 1994, 1999). Less has been known, however, about the underlying mechanisms by which EtOH contributes to ALD. While investigating these mechanisms, we showed (Banan et al., 1999, 2000a, 2000b, 1998a, 2000d), using monolayers of intestinal epithelial cells as a model of the gut barrier function, that EtOH-induced disruption of barrier integrity requires oxidative stress and disassembly and instability of the cytoskeleton. We also showed that the instability of cytoskeletal cytoarchitecture is required for mucosal damage caused by a variety of noxious agents under both in vivo (Banan et al., 1998b, 1996) and in vitro conditions (Banan et al., 1999, 2000a, 2002a, 2002c, 2002b, 2001b, 2000b, 2001a, 2001c, 2001d, 1998a, 2000c, 2000d).

Among factors that might cause cytoskeletal and barrier disruption in the context of oxidative stress by EtOH, we focused on NF-kappa B (NF-κB) for several reasons. Several studies have shown that gut oxidation and inflammation is associated with the activation of the nuclear transcription factor NF-κB (Banan et al., 2003a, 2003b, 2003d; Barnes and Karin, 1997; Neurath et al., 1999; Rogler et al., 1998; Shreiber et al., 1998) and increases in oxidative stress, and that these changes appear to be key contributors to intestinal epithelial and mucosal tissue injury (Banan et al., 2000a, 2000c, 2000d; Dinda et al., 1996; Keshavarzian et al., 1992, 2003; McKenizie et al., 1996). Once activated, NF-κB upregulates several important cellular processes involved in oxidative stress responses such as inducible-nitric oxide synthase (iNOS) (Banan et al., 2002c, 2000b, 2001d; Barnes and Karin, 1997; Chen et al., 1995; Jobin et al., 1999; Neurath et al., 1999). We previously showed (Banan et al., 1999, 2000b) that EtOH increased several measures of oxidative stress and upregulated iNOS activity in intestinal Caco-2 monolayers. Because NF-κB is a known transcription factor for iNOS enzyme and because iNOS promoter in fact has consensus sequences for NF-κB binding, we investigate in the current study whether NF-κB by itself is key to EtOH-induced injurious effects on intestinal monolayers. Indeed, the precise molecular mechanisms through which NF-κB might contribute to the development of mucosal epithelial abnormalities, especially following EtOH exposure, are unclear. NF-κB is typically composed of p50 and p65 subunits and its activation is regulated by an endogenous inhibitor, I-Kappa Bα (I-κBα) (Moon et al., 1999). Accordingly, in the current study, we investigated whether damage to the actin cytoskeleton caused by NF-κB activation mediates EtOH-induced intestinal epithelial barrier disruption.

We hypothesized that EtOH induces disruption of the epithelial F-actin cytoskeleton and of barrier integrity through degradation of I-κBα and activation of NF-κB. To explore this possibility, we exposed intestinal epithelial cells to pharmacological or targeted molecular interventions, including using mutant clones that are unable to activate NF-κB. We now report that the ability of NF-κB to promote the EtOH injury and inhibit the molecular dynamics of F-actin cytoskeleton are novel mechanisms in EtOH effects on the GI tract, ones not previously attributed to NF-κB in cells.

Materials and methods

Cell culture

Caco-2 cells, which were obtained from American type culture collection (Rockville, MD) at passage 15, were chosen because they form monolayers that morphologically resemble small intestinal cells, with defined apical brush borders and a highly organized apical ring of actin (Banan et al., 2002a, 2000d; Meunier et al., 1995). Cells were maintained at 37°C in complete Dulbecco's minimum essential medium (DMEM) in an atmosphere of 5% CO_2 and 100% relative humidity. Parental cells or stably transfected cells (see below) were split at a ratio of 1:6 on reaching confluency, and set up in either 6-or 24-well plates for experiments, or T-75 flasks for propagation. Cells grown for barrier function experiments were split at a ratio of 1:2 and seeded at a density of 200,000 cells/cm^2 into 0.4 μM Biocoat Collagen I Cell Culture Inserts (0.3 cm^2 growth surface; Becton Dickinson Labware, Bedford, MA) and experiments were performed at least 3 weeks postconfluence. The media was changed every 2 days. The utility and characterization of this cell line has been previously reported (Banan et al., 1998a; Meunier et al., 1995).

Plasmids and stable transfection by selection

The dominant negative mutant (super-repressor) of I-κBα was constructed and used as described (Banan et al., 2003a). This mutant contains double point mutations substituting key serine residues—32 and 36—with alanine residues, which prevents the degradation of I-κBα. The construct was cloned into a CMV expression vector to