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Pregnane X receptor agonists enhance intestinal epithelial wound healing and repair of the intestinal barrier following the induction of experimental colitis

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ABSTRACT

The intestinal epithelial barrier plays a key role in the maintenance of homeostasis within the gastrointestinal tract. Barrier dysfunction leading to increased epithelial permeability is associated with a number of gastrointestinal disorders including the inflammatory bowel diseases (IBD) – Crohn's disease and ulcerative colitis. It is thought that the increased permeability in patients with IBD may be driven by alterations in the epithelial wound healing response. To this end considerable study has been undertaken to identify signaling pathways that may accelerate intestinal epithelial wound healing and normalize the barrier dysfunction observed in IBD.

In the current study we examined the role of the pregnane X receptor (PXR) in modulating the intestinal epithelial wound healing response. Mutations and reduced mucosal expression of the PXR are associated with IBD, and others have reported that PXR agonists can dampen intestinal inflammation. Furthermore, stimulation of the PXR has been associated with increased cell migration and proliferation, two of the key processes involved in wound healing. We hypothesized that PXR agonists would enhance intestinal epithelial repair.

Stimulation of Caco-2 intestinal epithelial cells with rifaximin, rifampicin and SR12813, all potent agonists of the PXR, significantly increased wound closure. This effect was driven by p38 MAP kinase-dependent cell migration, and occurred in the absence of cell proliferation. Treating mice with a rodent specific PXR agonist, pregnenolone 16α-carbonitrile (PCN), attenuated the intestinal barrier dysfunction observed in the dextran sulphate sodium (DSS) model of experimental colitis, an effect that occurred independent of the known anti-inflammatory effects of PCN.

Taken together our data indicate that the activation of the PXR can enhance intestinal epithelial repair and suggest that targeting the PXR may help to normalize intestinal barrier dysfunction observed in patients with IBD. Furthermore, our data provide additional insight into the potential mechanisms through which rifaximin elicits its clinical efficacy in the treatment of IBD.

1. Introduction

The inflammatory bowel diseases (IBD; Crohn's disease – CD; ulcerative colitis – UC) are complex diseases of the gastrointestinal tract thought to be caused by aberrant inflammatory responses to luminal antigens/microbes in genetically susceptible individuals (Xavier and Podolsky, 2007). Patients with IBD exhibit alterations in innate immune function, enhanced T cell-dependent inflammation and defects in intestinal epithelial barrier function, each thought to contribute to IBD pathogenesis in an interdependent fashion (Xavier and Podolsky, 2007). As such, therapies designed to inhibit the inflammatory responses in IBD are effective, however the clinical efficacy of these agents is associated with enhanced mucosal wound healing and ulcer restitution (Iacucci and Ghosh, 2011; Turner, 2009).

Maintenance of the intestinal epithelial barrier is key to optimal gastrointestinal health. Increased permeability is associated with IBD (Blair et al., 2006; Heller et al., 2005; Zeissig et al., 2007), thus studying the mechanisms that regulate intestinal epithelial growth/survival and epithelial barrier function may provide insight into the pathogenesis of IBD and provide new therapeutic targets for its treatment. The PXR, a ligand-activated nuclear receptor, is a key regulator of xenobiotic metabolism that is expressed in various regions of the gastrointestinal tract and has been implicated in the regulation of metabolic pathways in intestinal epithelial cells (IECs)

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(Cheng et al., 2012). The PXR can be activated by a number of diverse ligands including steroids, bile acids, antibiotics and other exogenous compounds (Staudinger et al., 2013). As such, the PXR is often coined a "xenobiotic sensor" and master regulator of detoxification pathways throughout the gastrointestinal tract, through its ability to upregulate the expression of key proteins involved in metabolism including specific cytochrome P450 enzymes and various transporters (Jana and Paliwal, 2007; Staudinger et al., 2013).

Several genome-wide association studies have reported that specific single nucleotide polymorphisms (SNPs) in coding and non-coding regions of the PXR gene are associated with increased risk for CD and UC (Dring et al., 2006; Glas et al., 2011; Martinez et al., 2007). Furthermore, aberrant PXR expression has been observed in the colonic mucosa of patients with IBD (Langmann et al., 2004). Interestingly, PXR-deficient mice exhibit spontaneous small intestinal inflammation (Zhou et al., 2006) and selective PXR agonists can dampen the inflammatory response in experimental models of colitis (Dou et al., 2013; Shah et al., 2007). Furthermore, rifaximin, an antibiotic used to treat IBD and a gut specific human PXR agonist (Ma et al., 2007), attenuates experimental colitis in humanized PXR mice (Cheng et al., 2010). In each case, the anti-inflammatory effects observed in experimental colitis were attributed to PXR-dependent inhibition of NFkappaB driven cytokine/chemokine production (Cheng et al., 2010; Dou et al., 2013; Shah et al., 2007).

In addition to regulating gene transcription and directly inhibiting NFkappaB signaling, activation of the PXR has been linked to intracellular signaling pathways associated with cytoskeletal rearrangement, cell motility and cell proliferation. Kodama and Negishi (2011) reported that the activation of the PXR triggered cytoskeletal rearrangement enhancing cell migration in a p38 MAP kinase-dependent fashion (Kodama and Negishi, 2011). Furthermore, rifampicin, a prototypical PXR agonist, enhanced the migration of colonic epithelial cell lines through the activation of FGF19-dependent pathways (Wang et al., 2011). Taken together these data suggest that the activation of the PXR may promote cell proliferation and migration, two of the key processes involved in epithelial wound healing.

Given that alterations in the intestinal epithelial barrier play a key role in the induction of inflammation in experimental colitis, and that PXR activation can drive the cellular responses associated with the wound healing process, we posit that activation of the PXR may provide its anti-colitic effects, in part, through its ability to enhance the intestinal epithelial barrier by accelerating the wound healing process.

In the current manuscript we report that PXR agonists enhance intestinal epithelial wound healing by triggering cell migration in a p38 MAP kinase-dependent fashion, an effect that occurs in the absence of increased cell proliferation. Furthermore, oral administration of a mouse specific PXR agonist attenuates the intestinal barrier defect observed in experimental colitis, an effect that occurs independent of the inflammatory status.

2. Methods

2.1. Reagents

Human PXR agonists: rifaximin, rifampicin and SR12813 (all from Sigma Aldrich) were dissolved in sterile DMSO to stock concentrations of 10 mM. Volumes of stock solutions were added to culture media to reach the appropriate experimental concentration (10 μM). Matching volumes of sterile DMSO were added to culture media for experimental vehicle controls. p38 MAP kinase inhibitor: SB202190 (Sigma Aldrich) was dissolved in sterile DMSO to stock concentrations of 10 mM. Volumes of stock solutions were added to culture media to reach the appropriate experimental concentration (10 μM). SB202190 was added 30 min. prior to addition of PXR agonists. Mouse PXR agonist: pregnenolone 16α-carbonitrile (PCN; Sigma Aldrich) was dissolved in sterile corn oil. Each mouse was administered PCN via oral gavage at a dose of 25 mg/kg. Control mice were administered matching volumes of sterile corn oil via oral gavage.

2.2. Cell culture

Caco-2 cells (adult human colonic epithelial cell line derived from a colorectal adenocarcinoma; ATCC) were propagated in Dulbecco's modified Eagle medium supplemented with 20% fetal bovine serum and penicillin–streptomycin (100 μg/mL, 1 nmol/L; Invitrogen, Carlsbad, CA). Cells were split 1:10 upon reaching 75% confluence. All experiments were performed on cell passages 15–25.

2.3. Wound healing

To examine the effects of PXR agonists on wound healing, cells were seeded at a density of 3×10^7 cells/mL in ibidi cell-culture inserts (ibidi) in standard 12 well plates (Corning). After 48 h, the culture insert was removed revealing a 500 μm gap/wound between cell fronts. Wells were then loaded with 5% FBS-OptiMEM (Invitrogen) containing SB202190 (10 μM) with either rifaximin, rifampicin or SR12813 (each at 10 μM). Wound closure was assessed at 0, 12 and 24 h following the addition of PXR agonists. The distance between the cell fronts was measured at 10 random positions per captured image using Nikon NIS Elements software (Nikon). Data are expressed as a percentage of wound closure compared to initial wound.

2.4. Western blots

For experiments assessing p38 MAP kinase activation, cells were seeded at a density of 5×10^7 cells/mL onto 12-well plates and allowed to grow for 7-days post-confluence. Cells were treated with PXR agonists or vehicle control for 0, 15, 30, 60 and 120 min. Following the treatment period the media was removed and the cells washed with ice-cold PBS. Cell lysis buffer was added (150 mM NaCl, 20 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, and protease inhibitor cocktail; phosphatase inhibitor cocktail – Complete Minitab; Complete PhoStop, Roche). Total protein was quantified using the Precision Red Advanced Protein Assay (Cytoskeleton) and sample protein concentration equalized. Samples were resolved and transferred to nitrocellulose membranes (0.2 μm pores; BioRad) and blotted with the following antibodies: Phospho-p38 MAPK (Thr180/Tyr182; D3F9; Cell Signaling) and total p38 MAPK (D13E1; Cell Signaling). Blots were imaged using a Biorad ChemiDoc XRS (Biorad) and band intensity quantified using ImageJ (NIH). Data are expressed as the percentage of phospho-p38 compared to total p38 in each sample.

2.5. Migration assay

To assess cell migration in response to treatment with PXR agonists, 1×10^4 Caco-2 cells were seeded onto the upper surface of a Transwell Boyden chamber (8 μm pore; Corning) in standard media (Dulbecco's modified Eagle medium supplemented with 20% fetal bovine serum and penicillin–streptomycin (100 μg/mL, 1 nmol/L; Invitrogen)). After 24 h, the upper chambers were loaded with 5% FBS-OptiMEM (Invitrogen) containing SB202190 (10 μM) and the bottom chambers were loaded with 5% FBS-OptiMEM (Invitrogen) containing SB202190 (10 μM) with either rifaximin, rifampicin or SR12813 (each at 10 μM). After 48 h, the membranes