Pregnane X receptor activation ameliorates DSS-induced inflammatory bowel disease via inhibition of NF-κB target gene expression

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Steroid and xenobiotic receptor (SXR) and its rodent ortholog pregnane X receptor (PXR) are members of the nuclear receptor (NR) superfamily (22, 23). The NR superfamily is characterized by the presence of a DNA-binding domain and a conserved ligand-binding domain (38). Upon activation, PXR heterodimerizes with the retinoid X receptor and binds DNA response elements arranged as direct and everted repeats (22). PXR is activated by several structurally diverse ligands that display species specificity. Rifampicin is an agonist for SXR (human PXR) and induces cytochrome P-450 (CYP)3A in humans but not in rodents, whereas pregnenolone-16α-carbonitrile (PCN), a rodent-specific PXR agonist, induces CYP3A in rodents but not in human hepatocytes (22). Activated PXR behaves as a xenosensor and regulates a battery of genes including hepatic metabolism enzymes encoding CYPs (4, 33), UDP-glucuronosyltransferases (41), and glutathione-S-transferases (GSTs) (9). PXR is a major regulator of CYP3A4, which metabolizes >50% of drugs clinically used in humans (13). In addition, PXR also regulates several drug efflux pumps and transporters including the ATP-binding cassette (ABC) multidrug drug resistance 1 (MDR1) (11), multidrug resistance protein 2 (MRP2) (20), MRP3 (37), and the organic anion transporter 2 (14, 15, 35).

More recently, evidence was presented in humans that PXR was involved in inflammatory bowel disease (IBD) (8, 26). IBD, which manifests as either ulcerative colitis (UC) or Crohn’s disease (CD), is associated with chronic inflammation of the intestinal tract. While the precise etiology of IBD is unclear, it is thought to be a combination of altered intestinal epithelial barrier function and dysregulation of the mucosal immune system (1). Gene expression profiling of inflamed colon tissues from UC and CD patients identified several downregulated detoxification genes and ABC transporters in the colon of patients with UC (26). In addition to the decrease in several phase II enzymes and xenobiotic transporters, there was a significant downregulation of PXR, suggesting a role for PXR in the pathogenesis of IBD (26). Furthermore, several single-nucleotide polymorphisms linked to a decrease in PXR activity or expression have been identified in patients with IBD (8). It is hypothesized that the detoxification properties of PXR and its target genes are necessary to maintain the integrity of the intestinal epithelial barrier. However, recently, cross-talk between PXR and NF-κB signaling pathways was demonstrated. NF-κB activation repressed PXR association with the CYP3A4 promoter, a well-characterized PXR target gene (12). In addition, PXR was shown to be a key regulator of inflammation in hepatocytes and the small intestine. PXR activation inhibited the activity of NF-κB and the expression of its target genes. This inhibition was shown to be PXR dependent and potentiated by PXR ligands (40).

For a more thorough understanding of the role of PXR in colon inflammation, wild-type and Pxr-null mice were subjected to dextran sulfate sodium (DSS)-induced colitis. The present study demonstrates that PXR activation ameliorates DSS-induced colonic injury as assessed by classical clinical parameters of IBD. The activation of PXR restricts NF-κB activity and target gene expression, which is demonstrated to be a critical mechanism by which PXR affords protection.

Materials and methods

Cell lines. HCT116 cells were grown at 37°C with 5% CO2 in DMEM (Invitrogen, Grand Island, NY) supplemented with 10% FBS (Gemini Bio-Products, Woodland, CA) and 1% penicillin-streptomycin (Invitrogen).

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Luciferase assay. HCT116 cells were plated in 24-well plates (5 x 10^4 cells/well) and cultured in DMEM containing 10% FBS. Cells were transfected with the expression vector as indicated in the figures using Fugene transfection reagent (Roche, Indianapolis, IN). After 24 h posttransfection, cells were incubated with vehicle and PCN (100 nM, 1 µM, and 10 µM; Sigma, St. Louis, MO) for 24 h. In experiments in which TNF-α (10 ng/ml, Sigma) was used, cells were incubated for 4 h with PCN followed by an incubation with TNF-α for 24 h. A standard dual luciferase assay was used and normalized to a cotransfected control reporter (Promega, Madison, WI).

Animals. Pxr-null mice on a C57Bl/6 background were kindly provided by GlaxoSmithKline and Dr. Steven A. Kliewer (Dallas, TX). Pxr-null mice (6–8 wk old) and their corresponding wild-type controls were used throughout the study. Animals were housed in temperature- and light-controlled rooms and were given water and pelleted chow ad libitum. All animal experiments were carried out in accordance with the Institutional Animal Research guidelines and approved by the National Cancer Institute Animal Care and Use Committee.

Induction and assessment of colitis. Pxr-null mice and their corresponding wild-type control mice (6–8 wk old) were administered 2.5% (wt/vol) DSS (mol wt 35,000–44,000, MP Biomedicals, Aurora, OH) in drinking water for 7 days. For PCN (10 mg/kg ip) and vehicle (corn oil ip) treatment, the drug was administered for 3 days prior to DSS treatment and continued to the end of the DSS treatment. Daily changes in body weight and clinical signs of colitis, such as rectal bleeding, diarrhea, and bloody stool, were assessed and reported as a score from 0 to 4. Hemoccult SENSA (Beckman Coulter, Fullerton, CA) was used for the examination of rectal bleeding. For macroscopic colonic damage, colons were opened longitudinally, flushed with PBS, and fixed in 10% buffered formalin. Colons were Swiss rolled to examine the entire length of the colon and processed in paraffin. Colitis was scored on a routine hematoxylin and eosin-stained section, according to the morphological criteria previously described (5).

RNA analysis. RNA was extracted using TRIzol reagent (Invitrogen). Quantitative real-time PCR (qPCR) was performed using cDNA generated from 1 µg total RNA with the SuperScript II Reverse Transcriptase kit (Invitrogen). Primers were designed for qPCR using Primer Express software (Applied Biosystems, Foster City, CA), and sequences are available upon request. qPCRs were carried out using SYBR green PCR master mix (Applied Biosystems) in an ABI Prism...
7900HT Sequence Detection System (Applied Biosystems). Values were quantified using the comparative threshold cycle method, and samples were normalized to 
/H9252-actin.

In vivo intestinal permeability assay. Intestinal permeability was examined in mice following 7-day DSS treatment. Mice were gavaged with 0.6 mg/g body wt of FITC-conjugated dextran (Sigma) for 4 h (n = 6 mice/condition). Retroorbital bleeding was performed, and serum concentrations of FITC were measured.

Coimmunoprecipitation. For coimmunoprecipitation experiments, whole cell extracts were immunoprecipitated with NF-κB antibody (Santa Cruz Biotechnology, Santa Cruz, CA) overnight and then for 2 h with 25 μl of protein A/G-magnetic beads (New England Biolabs, Ipswich, MA). Immunoprecipitated proteins were recovered by boiling the magnetic beads in 2× SDS sample buffer, and Western blot analysis was carried using either a NF-κB antibody (Santa Cruz Biotechnology) or a PXR antibody (Santa Cruz Biotechnology).

Data analysis. Results are expressed as means ± SD. P values were calculated using a multifactorial ANOVA test and an independent t-test. P < 0.05 was considered significant.

RESULTS

PXR is highly expressed in the mouse colon. PXR mRNA concentrations were measured in the lung, heart, liver, kidney,
stomach, duodenum, jejunum, ileum, and colon. PXR mRNA was expressed at low levels in the lung, heart, kidney, and stomach, with the highest mRNA levels in the liver and gastrointestinal tract (Fig. 1A). In contrast, Pxr-null mice did not express PXR in any tissue assessed. Pxr-null mice were shown to have an increase mononuclear cell inflammatory infiltrate in the mucosa of the small intestine and a spontaneous induction of NF-κB target genes in the small intestine (40). However, the colons from Pxr-null mice did not show any clinical signs of inflammation (Fig. 1B). In addition, there were no significant differences in colon TNF-α levels between wild-type and Pxr-null mice (Fig. 1C). Interestingly, PCN was able to decrease basal colonic TNF-α levels in wild-type mouse colons, whereas PCN had no effect in Pxr-null mouse colons. TNF-α was significantly higher in the small intestine of Pxr-null mice compared with wild-type mice (Fig. 1C). Similar to the colon, small intestine TNF-α basal levels were decreased upon PCN administration to wild-type mice and not in Pxr-null mice (Fig. 1C). These data demonstrate that there was no overt colon inflammation in the absence of PXR; however, these data suggest that PCN may have an anti-inflammatory role via a PXR-mediated mechanism.

**Activated PXR decreases the susceptibility of mice to DSS-induced IBD.** Wild-type and Pxr-null mice were administered 2.5% DSS in drinking water for 7 days. Mice were then killed, and their colons were analyzed. No significant differences were found in the severity of IBD between wild-type and Pxr-null mice as assessed by major clinical symptoms (body weight loss, rectal bleeding diarrhea, and shortening of the colon) and histology (Figs. 2 and 3). However, wild-type mice administered PCN demonstrated a decrease in the severity of DSS-induced IBD compared with vehicle-treated mice. The administration of 2.5% DSS in drinking water for 7 days induced a significant body weight loss in vehicle-treated mice compared...
with PCN-treated mice (Fig. 2A). PCN significantly improved diarrhea scores, bleeding scores, and colon lengths compared with vehicle-treated mice (Fig. 2, B–D). In addition, histological analysis showed that DSS-induced colonic injury was less severe in PCN-treated mice (Fig. 3, A and B). PCN did not improve the clinical symptoms of DSS-induced IBD in Pxr-null mice, therefore demonstrating that the protective effects of PCN in DSS-induced IBD were mediated via a receptor-dependent mechanism.

**PCN induces expression of phase II detoxification enzymes and xenobiotic transporters but does not increase epithelial barrier function in DSS-induced IBD.** Phase II enzymes and cellular efflux transporters are critical components in intestinal barrier function (31). Therefore, their gene expression profiles were compared in whole colons of vehicle- and PCN-treated mice following 7-day DSS treatment. PCN treatment increased the expression of GSTa1, GSTm1, GSTt1, MDR1a, and MRP2 mRNA in the colons of wild-type mice compared with vehicle-treated mice (Fig. 4A). GSTa1, GSTm1, GSTt1, and MDR1a expression remained significantly increased in PCN-treated wild-type mice following 7-day DSS treatment (Fig. 4A). Intestinal epithelial permeability is a direct in vivo measurement of epithelial barrier function. Vehicle- and PCN-treated wild-type mice following 7-day DSS treatment were either gavaged with PBS or FITC-dextran solution, serum was collected 4 h

![Fig. 4. Phase II enzyme and xenobiotic transporter expression and intestinal permeability assay from colonic tissue following 7-day DSS or control treatment from vehicle- or PCN-treated WT mice.](http://ajpgi.physiology.org/)
later, and permeability was measured. Seven-day DSS treatment significantly increased intestinal permeability to the same magnitude in both vehicle- and PCN-treated wild-type mice (Fig. 4B). Together, these data suggest that the major mechanism by which PXR affords protection in DSS-induced IBD is not by increasing intestinal epithelial barrier.

**Activated PXR inhibits NF-κB target gene expression during DSS-induced IBD.** To gain further insight into the mechanism of protection by PXR, NF-κB gene expression profiles were compared in whole colons of vehicle- and PCN-treated wild-type and Pxr-null mice following 7-day DSS treatment. Chemokine (C-C) motif receptor 2, ICAM-1, IL-1β, IL-6, IL-10, inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein 1 (MCP-1), and TNF-α mRNA expression was assessed by qPCR in colon RNA isolated from WT or Pxr-null mice treated with vehicle or PCN. Expression was normalized to β-actin, and each bar represents the mean value ± SD. *P < 0.05 compared with vehicle + DSS-treated WT or Pxr-null mice; **P < 0.05 compared with vehicle + DSS-treated WT mice.

![Graphs showing cytokine expression from colonic tissue following 7-day DSS or control treatment of vehicle- or PCN-treated WT or Pxr-null mice.](http://ajpgi.physiology.org)

Fig. 5. Cytokine expression from colonic tissue following 7-day DSS or control treatment of vehicle- or PCN-treated WT or Pxr-null mice. Chemokine (C-C) motif receptor (CCR)2, CD68, ICAM, IL-1β, IL-6, IL-10, inducible nitric oxide synthase (iNOS), MCP-1, and TNF-α mRNA expression was assessed by qPCR in colon RNA isolated from WT or Pxr-null mice treated with vehicle or PCN. Expression was normalized to β-actin, and each bar represents the mean value ± SD. *P < 0.05 compared with vehicle + DSS-treated WT or Pxr-null mice; **P < 0.05 compared with vehicle + DSS-treated WT mice.
model using HCT116 colon cancer cells was assessed. HCT116 cells were transfected with a DR3-luciferase reporter alone or cotransfected with a PXR expression plasmid and incubated with increasing concentrations of either PCN or vehicle. In cells transfected with the DR3-luciferase reporter alone, there were no increases in luciferase expression following PCN incubation (Fig. 6A). In cells cotransfected with the DR3-luciferase reporter and PXR, there were increases of basal luciferase expression, indicating ligand-independent activation of PXR, and PCN incubation further enhanced luciferase expression (Fig. 6A). Next, HCT116 cells were transfected with a NF-κB-luciferase reporter or cotransfected with a NF-κB-luciferase reporter and PXR and incubated with either recombinant TNF-α or PCN or coincubated with TNF-α and PCN. In cells transfected with the DR3-luciferase reporter alone, TNF-α increased luciferase expression, PCN had no effect on basal luciferase expression; however, coincubation with PCN and TNF-α repressed TNF-α-induced luciferase expression (Fig. 6C). These data indicate that ligand-activated PXR directly inhibits the activity of the proinflammatory transcription factor NF-κB in colon cells. When protein-protein interactions were assessed between PXR and the p65 subunit of NF-κB, no PCN-induced interactions were observed (data not shown).

DISCUSSION

IBD refers to two chronic diseases that cause inflammation of the intestine: UC and CD. Although the diseases have some features in common, there are critical differences. UC is an inflammatory disease of the colon, whereas CD affects the last part of the small intestine, the ileum. However, CD is not restricted to these areas and can affect anywhere from the anus to the esophagus. UC generally only affects the mucosal lining of the intestinal wall, whereas CD tends to involve the entire bowel wall (1). The present study used an acute model of IBD by administration of DSS in drinking water. The inflammation was mainly localized to the colon, resembling UC; however, the inflammation affected deep into the intestinal wall, similar to CD.

In the present study, PXR was found to be expressed in the colon. In a DSS-induced IBD model, treatment with the PXR ligand PCN decreased the severity of colitis as revealed by body weight, colon length, diarrhea, rectal bleeding, and macroscopic and histological analysis compared with vehicle-treated mice. It should be noted that PCN is also a glucocorticoid receptor (GR) ligand and thus may activate GR-mediated anti-inflammatory effects (3, 39). However, the protective effect of PCN was not recapitulated in Pxr-null mice, demonstrating a PXR-dependent mechanism. In addition, TNF-α-induced NF-κB-luciferase expression was repressed only when cells were cotransfected with PXR.

PXR expression and several of its target genes were shown to be decreased in CD and UC patients (26), suggesting that PXR may be critical in the pathogenesis of IBD. However, in the mouse model, the absence of PXR did not increase the susceptibility of mice to DSS-induced IBD. The molecular basis for this discrepancy is unclear; however, compensatory mechanisms could be activated in the absence of PXR. In Pxr-null mice, other xenobiotic NRs or tissue-specific transcription factors may genetically compensate, whereas in colitis patients a temporal decrease in PXR may restrict the formation of compensatory networks. Interestingly, no overt phenotypic changes were observed in any Pxr-null tissues with the exception of the small intestine (40). This may suggest a ubiquitous compensation mechanism that is not found in the small intestine.

Intestinal epithelial cells are critical in maintaining the intestinal epithelial barrier (31), and several key junctional proteins were shown to be dysregulated upon colonic inflammation (18, 21, 32). Indeed, disruption of the epithelial barrier in mice and humans can cause IBD (17, 18). In addition, xenobiotic detoxification enzymes and transporters were shown to be critical in maintaining intestinal barrier integrity (31). For example, mice with a genetic deficiency in MDR1a develop spontaneous colitis (27), and humans with polymorphisms in the MDR1a gene are more susceptible to IBD (34).
However, the present study demonstrates that PXR-induced phase II enzymes and transporters do not have a major role in increasing the barrier function in colonocytes. The present study suggests that PXR-mediated suppression of NF-κB target genes (IL-1β, IL-10, iNOS, and TNF-α) may be the mechanism by which PXR provides protection to DSS-induced IBD. However, other mechanism cannot be excluded. Several studies (6, 10, 16) have demonstrated a protective role for NF-κB inhibition in animal models of colitis. Therefore, the present study further validates the importance of NF-κB activity in IBD. Interestingly, specific inhibition of NF-κB activity in animal models of IBD repressed a similar subset of proinflammatory mediators (TNF-α, IL-6, IL-1β, and MCP-1) (6) as shown in the present study.

Several NRs were reported to inhibit inflammatory responses. GR’s anti-inflammatory activity is the most extensively studied among NRs (3, 39). However, liver X receptor (LXR)-α, LXR-β (19), estrogen receptor-α (36), peroxisome proliferator-activated receptor (PPAR)-α, PPAR-β, and PPAR-γ (24) have all been shown to influence inflammatory activity. The GR-mediated anti-inflammatory response was shown to occur through several diverse mechanisms. Reciprocal inhibition between GR and proinflammatory transcriptions factors activator protein-1 and NF-κB is mediated by protein-protein interactions (29, 30). Also, GR and PPAR-α increase the synthesis of IkB-α, which attenuates NF-κB signaling (2, 7). Recently, PPAR-γ was shown to inhibit NF-κB target genes by directly binding to NF-κB and recruiting a corepressor complex (28).

The exact mode by which PXR mediates the repression of NF-κB is still unclear, but the present study provides further in vivo data demonstrating the anti-inflammatory role of PXR. Since most IBD patients become refractory to primary therapy for UC and CD, new therapeutic modalities are needed. The exact mode by which PXR mediates the repression of NF-κB is still unclear, but the present study provides further in vivo data demonstrating the anti-inflammatory role of PXR. Since most IBD patients become refractory to primary therapy for UC and CD, new therapeutic modalities are needed. The present study provides rationale to explore PXR agonists as a therapeutic strategy for IBD. Furthermore, NF-κB inhibitors are being intensely investigated as a therapeutic modality for IBD (25). However, their ubiquitous expression may lead to deleterious side effects, thus reducing their clinical impact. On the other hand, PXR may be an attractive target due to its tissue-specific expression pattern.

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REFERENCES


