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Pregnane-X-receptor mediates the anti-inflammatory activities of rifaximin on detoxification pathways in intestinal epithelial cells

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ABSTRACT

The pregnane-X-receptor (PXR) is master gene overseeing detoxification of wide number of xenobiotics and is critical for maintenance of intestinal integrity. The intestinal expression of genes involved in cellular detoxification is down-regulated in patients with inflammatory bowel diseases (IBD). Rifaximin is a non-absorbable antibiotic endowed with a PXR agonistic activity. In the present study we have investigated whether rifaximin activates PXR in primary human colon epithelial cells and human colon biopsies and assessed whether this antibiotic antagonizes the effect of tumor necrosis factor (TNF)-α on expression of PXR and PXR-related genes. Present results demonstrate that primary colon epithelial cells express PXR and that their exposure to rifaximin induces the expression of genes involved in cellular detoxification. Exposure to TNFα reduces the expression of PXR mRNA as well as expression of its target genes. This inhibitory effect was prevented by that co-treatment with rifaximin. Knocking down the expression of PXR in colon epithelial cells by an anti-PXR siRNA, abrogated the counter-regulatory effects exerted by rifaximin on cell exposed to TNFα. Finally, *ex vivo* exposure of colon biopsies obtained from ulcerative colitis patients to rifaximin increased the expression of genes involved in xenobiotics metabolism. In aggregate, these data illustrate that rifaximin increases the expression of PXR and PXR-regulated genes involved in the metabolism and excretion of xenobiotics and antagonizes the effects of TNFα in intestinal epithelial cells and colon biopsies. These non-antibiotic effects of rifaximin could contribute to the maintenance of the intestinal barrier integrity against xenobiotics and products generated by luminal bacteria.

1. Introduction

The pathophysiology of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC) is not yet completely understood [1–5]. In recent years, it has become clear that genetic, immunological, environmental and microbial factors contribute to the aetiology of IBD [1–5]. The epithelial cells, the first line of defence against potentially harmful luminal antigens, are remarkably similar to hepatocytes in their ability to carry out detoxification and biotransformation of luminal agents of dietary, bacterial or fermentative origin. In the last decade results from animal models as well as studies of IBD patients have shown that colonic epithelial cells might become unable to detoxify toxic metabolites in inflammation [6–11]. Despite the pathogenesis of this epithelial dysfunction is multifactorial and somewhat elusive, the concept of a multilevel alteration of the intestinal detoxification system is recognized as pathogenetic mechanism in IBD [6,12].

The steroid and xenobiotic receptor (SXR), also known as pregnane-X-receptor (PXR), is a nuclear hormone receptor activated by a diverse array of endogenous hormones, dietary steroids, pharmaceutical agents and xenobiotics [13–15]. PXR has a highly flexible, hydrophobic, ligand binding domain (LBD) which accommodates a wide array of ligands conferring to the receptor a remarkably divergent array of activities across mammalian species. PXR is master gene overseeing detoxification of wide number of xenobiotics and is critical for maintenance of intestinal integrity. Gene expression studies have shown that a down-regulation of xenobiotic metabolism and a dysregulation of PXR transcriptional activity in the gut are strongly associated with the development of IBD [16]. In the dextran sulfate sodium (DSS)-induced colitis, a widely used mouse model for IBD, administration of pregnenolone 16-carbonitrile (PCN), a rodent-specific PXR ligand, attenuates development of colitis and protects against immune dysfunction [17].

A major regulator of inflammation is the transcription factor NF-κB which regulates the expression of a diverse array of genes

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associated with both innate and adaptive immunity (including many cytokines, chemokines, adhesion proteins and stress response genes) [18]. Recently, it has been shown that the p65 sub-unit of NF-κB interacts with the PXR partner RXRα, and this interaction prevents the binding of PXR to the promoters of target genes [19]. This interaction may account for the inhibition of liver drug metabolism observed in inflammatory states [20]. Conversely, interaction of NF-κB with PXR leads to inhibition of NF-κB activity [20]. Confirming the reciprocal regulation, an increased expression of NF-κB target genes occurs in PXR null mice [20].

ATP binding cassette (ABC) transporters are ATP-dependent membrane proteins predominantly expressed in excretory organs, such as the liver, intestine and kidney [21]. ABC transporters have an important role in tissue defence through the excretion of toxic compounds and their metabolites. The expression of these transporters is tightly regulated, emphasizing their importance in organ protection [21]. Many of these genes are specific targets for PXR. A growing body of data demonstrates that a reciprocal regulation exists between genes that mediate detoxification and inflammation [21]. The role of these genes in regulating the inflammatory signalling is made evident from studies on MDR-1α [22]. Thus, mice deficient in the *mdr1a* gene which encodes for P-glycoprotein, a membrane efflux pump, expressed, among others, by intestinal epithelial cells, develop a spontaneous colitis presumably due to an intestinal epithelial "barrier" defect [22]. Further, MDR1a polymorphisms increase the susceptibility to IBD [23,24]. These data highlight the fact that the intestinal detoxification system serves a dual role during inflammation, both sending out inflammatory signals as well as protecting the intestinal epithelium. A previous study has shown that rifaximin, a non-absorbable antibiotic, is a gut-specific human PXR agonist [25].

In the present study we have investigated whether rifaximin regulates the expression of genes involved in detoxification in human intestinal epithelial cells and colon biopsies from IBD patients. The results of this study support the notion that rifaximin is a ligand for human PXR and that, under rifaximin binding, PXR robustly counter-regulates pro-inflammatory effects of TNFα in colon epithelial cells.

2. Material and methods

2.1. Material

Rifaximin, polymorph-α, named rifaximin-α (Alfa Wassermann, Bologna, Italy), batch number 2008.8001312, was dissolved in DMSO at the final concentration of 10 mM and subsequently serial dilutions were made daily in complete F12 medium. The final concentration of DMSO was 0.5%. TNFα was from Invitrogen (Milan, Italy) while all other reagents were from Sigma–Aldrich (Milan, Italy).

2.2. Cells treatment

CRL-1790 cells, a human epithelial cell line, (ATCC, Manassas, VA, USA) were used for this study. CRL-1790 cells were grown in F-12 medium enriched with the following components: 0.02 mg/ml insulin, 0.01 mg/ml transferrin, 25 nM sodium selenite, 50 nM hydrocortisone, 1 ng/ml epidermal growth factor, 0.01 mM ethanolamine, 0.01 mM phosphorylethanolamine, 100 pM triiodothyronine, 0.5% (w/v) bovine serum albumin, 10 mM HEPES, 0.5 mM sodium pyruvate, extra 2 mM L-glutamine (final concentration 4.5 mM). Cell density was maintained at approximately 100,000 cells per milliliter of medium. Cells were then plated in fibronectin- and collagen type I- coated T-25 flasks. The cells were incubated at 37 °C in 5% CO_2 and 95% O_2 atmosphere and underwent cell passage at ≈70% confluence. Rifaximin and TNFα were added 20 h before mRNA extraction. In co-treatment experiments rifaximin was added 3 h before TNFα and cells incubated for 20 h.

2.3. Cellular modulation of PXR expression by small interfering (si) RNA

CRL-1790 cells were suspended to a final concentration of 100,000 cells per milliliter in complete F12 medium and incubated at 37 °C. The lipid-based transfection agent was used for transfection with PXR siRNA (Origene, Rockville; MD, USA) in accordance to the manufacturer's indication. The cells were incubated for at least 72 h at 37 °C in 5% CO_2 after transfection with the anti-PXR siRNA before their use.

2.4. RNA extraction, reverse transcriptase and polymerase chain reaction

Quantification of the expression of PXR and genes involved in cellular detoxification was performed by quantitative real-time polymerase chain reaction (RT-PCR) using sense and antisense primers as indicated in Table 1. All PCR primers were designed using PRIMER3-OUTPUT software using published sequence data from the NCBI database. Total RNA was isolated from biopsies specimens by TRIzol reagent (Invitrogen srl, Milan, Italy). 1 μg of

Table 1
List of primers used for real-time PCR and the PXR responsive elements (PXR-RE) expressed in the promoter of each gene.

Gene and relative PXR-RE	Primer sense	Primer antisense
Phase I		
CYP-3A4 (ER6)	CAAGACCCCTTTGTGGAAAA	CGAGGCGACTTTCTTTCATC
CYP-2C9 (DR4)	AATTTTGGGATGGGGAAGAG	AAGTGGGATCACAGGGTGAG
CYP-3A7 (ER6)	CAAGACCCCTTTGTGGAAAA	TGTCTCTTTGAGGCGACCTT
CES-2 (DR3)	CTGGGGAGTCTTGTCCATGT	CCCTCACACCACTCCAAGAT
GXP-1 (DR3)	CCAAGCTCACCTGGTCT	TCGATGTCAATGGTCTGGAA
Phase II		
GST-A1 (DR3)	ATCGCTACTTCCCTGCCTTT	CTTCTTCACTGTGGGCAGGT
GST-M4 (ER6)	TTTCCTCGCCTATGATGTCC	CAGACAGCCACCCTTGTGTA
GST-T1 (DR3)	GCCAAGAAGAACGACATTCC	CCTTATATTTGCGCGTCAGG
SULT-1A1 (DR3)	CATGGTCGGAGAAGTGTCCT	GAATCTCCCTTTTCGGGTTC
SULT-1A3 (DR3)	CACGTCGTTCAAGGAGATGA	GCCATCTTCTCCGCATAGTC
SULT-2A1 (DR4)	GATCCAATCTGTGCCCATCT	TAAATCACCTTGGCCTTGGA
UGT-1A3 (DR3)	TAAGTGGCTACCCCAAAACG	GCTTTGCATTGTCCATCTGA
Phase III		
ABC-B1 (DR4)	CTTATGCTCTGGCCTTCTGG	GGAGATGCCTGTCCAACACT
ABC-C2 (DR3)	AGAGCTGGCCCTTGTACTCA	AGGGACAGGAACCAGGAGTT
ABC-C3 (DR4)	TTCTGGGACTCCAACCTGTC	ACACCCAGGACCATCTTGAG
PXR	CTGGAGGTGAGACCCAAAGA	CACATACACGGCAGATTTGG

ER = Everted repeat; DR = Direct repeat.

purified RNA was treated with Dnase I for 15 min at room temperature, followed by incubation at 95 °C for 5 min in the presence of 2.5 mmol/l EDTA. The RNAs were reverse transcribed with Superscript II (Invitrogen srl, Milan, Italy) in a 20 μl reaction volume using random primers. For quantitative RT-PCR, 50–100 ng of template was dissolved in a 25 μl solution containing 0.2 μmol/l of each primer and 12.5 μl of SYBR GreenER qPCR SuperMix for iCycler (Invitrogen srl, Milan, Italy). All reactions were performed in triplicate, and the thermal cycling conditions were as follows: 2 min at 50 °C, 10 min at 95 °C, followed by 50 cycles of 95 °C for 15 s and 60 °C for 60 s in an iCycler iQ instrument (Bio-Rad, Hercules, CA, USA). The mean value of the replicates for each sample was calculated and expressed as the cycle threshold (CT; cycle number at which each PCR reaction reaches a predetermined fluorescent threshold, set within the linear range of all reactions). The amount of expression of each gene was then calculated as the difference (dCT) between the CT value of the sample for the target gene and the mean CT value of that sample for the endogenous control (GAPDH). Relative expression was calculated as the difference (ddCT) between dCT values of the test control sample for each target gene. The relative expression level was expressed as 2-ddCT.

2.5. Culture of colon biopsies

Colon biopsies were obtained from 6 patients (5 men, mean age 36.3 ± 4.5 years) undergoing colonscopy for clinical staging of their disease. All patients were affected by Crohn's disease with a colonic localization. Two of them had also ileal involvement. All subjects were taking active drugs: budesonide (4 patients) and azathioprine (2 patients). None of the patients had previous surgery. Each patient had an active disease at the macroscopic examination. Samples were taken from inflamed mucosa for histo-pathology analysis, as a part of standard diagnostic assessment. Written consent was obtained from each patient. Biopsies were maintained in cold (4 °C) culture medium, gently washed, 3 times in RPMI with 3% of penicillin/streptomycin and cultured on 40 μM mesh filters over a culture dish in 24 well tissue culture plates in complete RPMI medium. They were cultured in the presence or absence of rifaximin (100 μM) and incubated at 37 °C with 5% CO_2. After 18 h, culture supernatants were removed, while biopsies were processed for RNA extraction. For each subject 4–6 biopsies were obtained. Each colon biopsy was plated individually. Half of the biopsies were used as a control (i.e. not treated with rifaximin) and half were exposed to rifaximin. The effect of rifaximin was compared in each subject with control (i.e. untreated) biopsies.

2.6. Data analysis

All values are expressed as mean ±SE of "*n*" experiments. The statistical analysis was done by GraphPad Prism software. The variation between data sets was tested by Student *t* test for unpaired samples, when we compared two groups. Comparisons of more than 2 groups were made with a 1-way analysis of variance with post hoc Tukey tests. Differences were considered statistically significant if p was <0.05.

3. Results

3.1. Rifaximin modulates the expression of PXR and PXR-regulated genes in intestinal epithelial cells

To investigate whether PXR directly regulates the expression of genes involved in the gut detoxification, we have first evaluated the expression of PXR in CRL-1790 cells, a normal human colon epithelial cell line, and compared it to HepG2 cells, a hepatocarcinoma cell line that express high levels of PXR. Results shown in Fig. 1, demonstrates that colon epithelial cells express PXR mRNA even if the expression was lower than that of HepG2 cells ($p < 0.05$ versus HepG-2 $n = 3$).

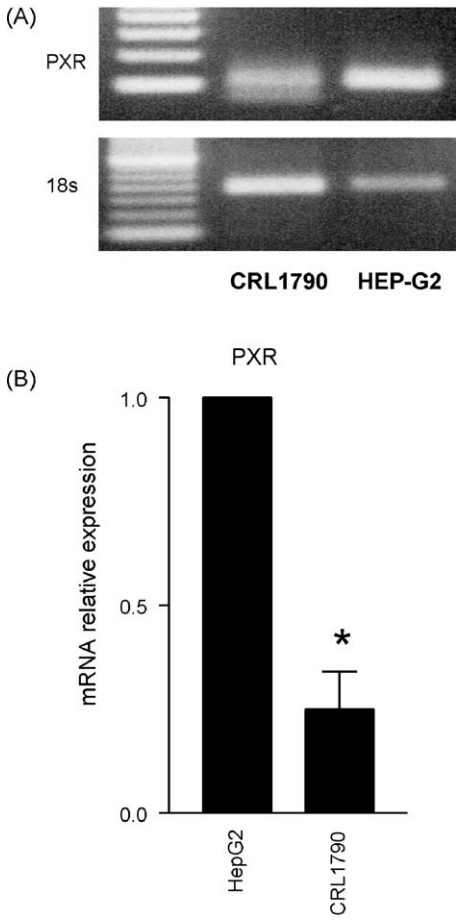

Fig. 1. The nuclear receptor PXR is expressed by normal human colon epithelial cells. (A and B) Qualitative and quantitative PCR showing expression of PXR mRNA by normal human colon cells (CRL1790). Hep-G2 cells, a human hepatic carcinoma cell line, was used as a positive control ($^*p < 0.05$ versus HepG2; $n = 3$).

We have then examined whether rifaximin (50 μM) modulates the expression of genes involved in intestinal detoxification. Cytochrome P450 (*CYP*) genes encode for phase I monooxygenases which catalyze essential reactions in drug metabolism and synthesis of cholesterol, steroids and other lipids [26]. Exposure of CRL-1790 cells to rifaximin induced a 2–3-fold increase in the expression of CYP-3A4, CYP-2C9 and CYP-3A7 mRNAs (Fig. 2A; $p < 0.05$ versus control cells; $n = 5$).

The glutathione S-transferase is a phase II enzyme that functions in the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress, by conjugation with glutathione [27]. Eight distinct classes of the soluble cytoplasmic mammalian glutathione S-transferases have been identified: alpha, kappa, mu, omega, pi, sigma, theta and zeta [27]. Exposure of CRL-1790 to rifaximin induced GST-A1 mRNA by 2–3 folds (Fig. 2B; $p < 0.05$ versus control cells; $n = 5$) but failed to change the expression of GST-M4 and GST-T1 mRNA.

The sulfotransferases are phase II enzymes that transfer a sulfonyl moiety from the cofactor 3′-phosphoadenosine-5′-phosphosulfate to hydroxyl, amino, sulfhydryl and N-oxide groups of their substrates [28]. Exposure of CRL-1790 cells to rifaximin induced SULT1A1 mRNA expression and SULT2A1 by 10 folds

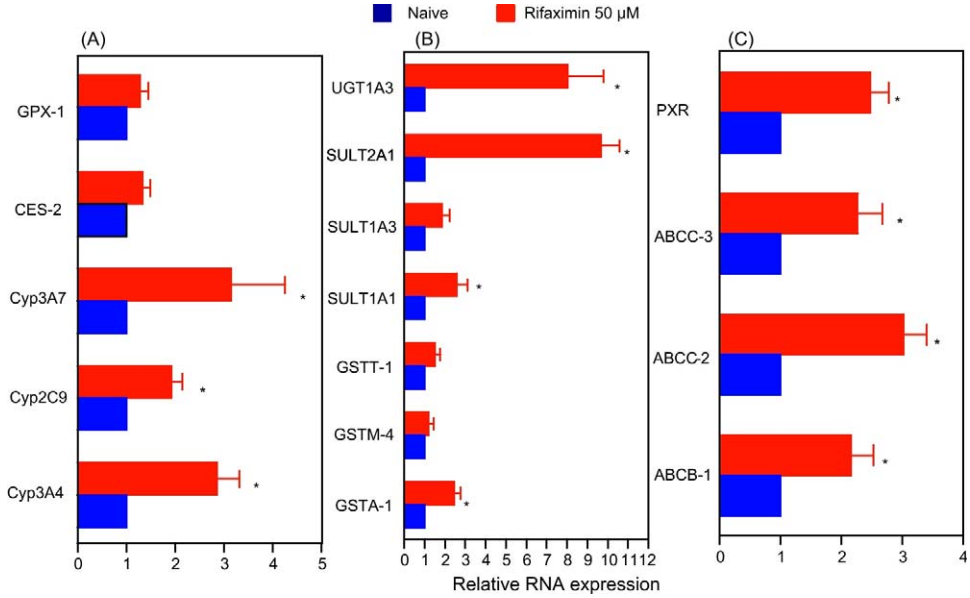

Fig. 2. Rifaximin modulates the expression of genes involved in xenobiotic detoxification in normal colon epithelial cells. Exposure of human colon epithelial cells to rifaximin (50 μM) for 20 h increases the expression of genes involved in cellular detoxification. Panel (A) shows the expression of phase I genes, panel B shows phase II genes and panel C shows phase III genes and PXR mRNA ($^{*}p < 0.05$ versus untreated cells; $n = 5$).

(Fig. 2B; $p < 0.05$ versus control cells; $n = 5$). Further on, rifaximin, was a potent inducer of UGT-1A3 an enzyme involved in glucuronidation pathway that transforms small lipophilic molecules, such as steroids, bilirubin, hormones, and drugs, into water-soluble, excretable metabolites (Fig. 2B; $p < 0.05$ versus control cells $n = 5$).

The superfamily of ABC transporters are phase III proteins that translocate a wide variety of substrates across extra- and intracellular membranes, including metabolic products, lipids and sterols, drugs and environmental agents [21]. Exposure of CRL-1790 cells to rifaximin induced ABC-B1, ABC-C2 and ABC-C3 by ≈2 folds (Fig. 2C; $p < 0.05$ versus control cells; $n = 5$). Finally, exposure to rifaximin increased PXR mRNA level by ≈2 folds (Fig. 2C; $p < 0.05$ versus control cells; $n = 5$).

3.2. Rifaximin reverts the repression of PXR and PXR-regulated genes caused by TNFα

Previous studies have shown that inflammation-driven NF-κB activation antagonizes PXR signalling in liver cells of human and mouse origin causing a robust reduction of CYP genes [21,19]. Because it is unknown whether a similar effect occurs in normal intestinal epithelial cells we investigated whether TNFα regulates the expression of PXR and PXR-regulated genes in intestinal epithelial cells. As shown in Fig. 3, exposure of CRL-1790 cells to TNFα (100 ng/ml) for 20 h caused a robust reduction in the expression of PXR and all but GPX-1, CES-2 and GST-T1, PXR-regulated genes (panels A–C; $p < 0.05$ versus control cells; $n = 5$). These effects were prevented by treating the cells with rifaximin. Thus, rifaximin effectively enhanced

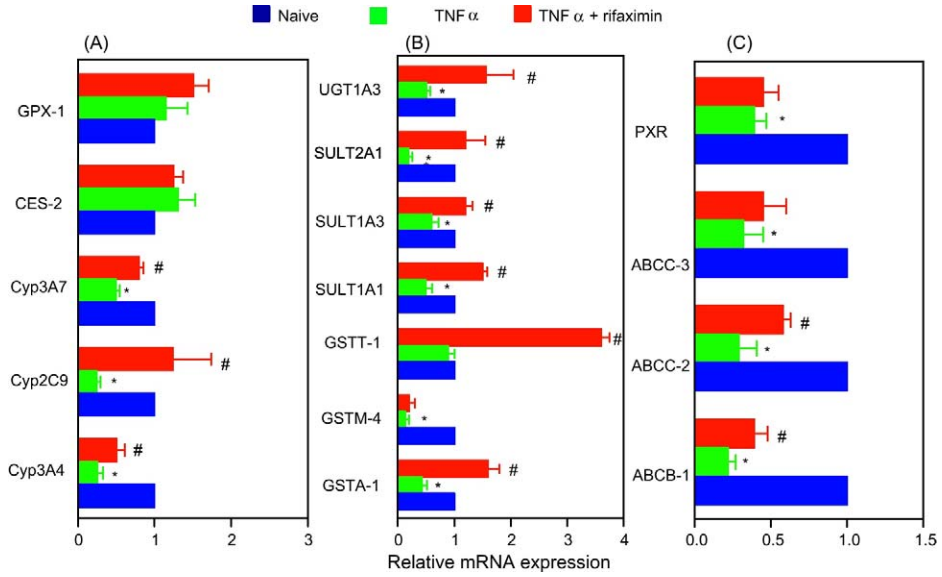

Fig. 3. TNFα is a negative modulator of genes involved in xenobiotic detoxification in normal colon epithelial cells. Exposure to TNFα (100 ng/ml) decreases the expression of genes involved in cellular detoxification. Pre-treating CRL1790 cells with rifaximin (50 μM) antagonized the effects of TNFα. Panel A shows the expression of phase I genes, panel B shows phase II genes, and panel C shows phase III genes and PXR mRNA ($^{*}p < 0.05$ versus naïve cells and $^{\#}p < 0.05$ versus TNFα treated cells; $n = 3$–5).

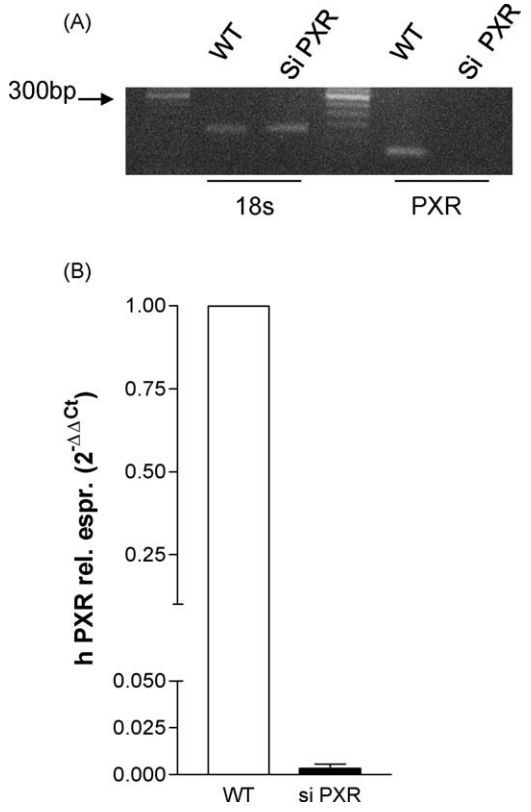

Fig. 4. PXR silencing in normal colon epithelial cells. Assessment of PXR mRNA expression in cells transfected with anti-PXR siRNA. (A) Qualitative RT-PCR of PXR mRNA; (B) quantitative RT-PCR of PXR mRNA in CRL1790 cells.

the expression of PXR and PXR-regulated genes in CRL-1790 cells exposed to TNF α ($p < 0.05$ versus TNFα alone).

3.3. Effects of rifaximin of detoxification genes is abrogated by PXR silencing

To explore the mechanistic involvement of PXR in the effect exerted by rifaximin, PXR gene expression was silenced by anti-PXR siRNA. As illustrated in Fig. 4, the treatment of CRL-1790 cells with an anti-PXR siRNA almost completely abrogated the expression of PXR ($p < 0.05$ versus non-transfected cells). With few exceptions, we found that exposure of CRL-1790 cells that were made deficient in PXR expression by anti-PXR siRNA completely abrogated the ability of rifaximin to counteract the effects of TNFα (Fig. 5A–C; $p < 0.05$ versus TNFα treated cells; $n = 3$).

3.4. Rifaximin regulates the expression of detoxification genes in human colon biopsies

To investigate whether the effect of rifaximin is maintained in a complex cellular system, colon biopsies obtained from macroscopically inflamed areas of colons of patients with ulcerative colitis were cultured *ex-vivo* with rifaximin. Results from these experiments (Fig. 6A; $p < 0.05$ versus untreated biopsies; $n = 16$ and $n = 17$, respectively) demonstrated that PXR is expressed in colon human biopsies and that rifaximin (100 μM) effectively increased the expression of CYP-3A7 and CYP-2C9 by 2 folds, sulfotransferases and glucuronosyltransferases, with the exception of SUL-1A3, by 2 folds (Fig. 6B; $p < 0.05$ versus untreated biopsies; $n = 10$), and ABC-B1 and ABC-C3 by 2–3 folds (Fig. 6C; $p < 0.05$ versus untreated biopsies; $n = 12$–15).

4. Discussion

A single layer of intestinal epithelial cells makes up the barrier between the host and the luminal content of the intestine. The cells of the mucosal immune system are protected against the luminal antigen load by this layer of epithelial cells. Disturbance of the integrity of the epithelial cell barrier contributes to the development of mucosal inflammation exposing the intestinal immune system to bacteria products and toxins [12]. Protective mechanisms that maintain intestinal barrier function include detoxification and biotransformation of luminal substances. The ability of intestinal epithelial cells to handle luminal antigens and xenobiotics is dependent on the activity of a large group of genes. Phase I reactions are largely dependent on the activity of proteins encoded by the cytochrome P450 (*CYP*) superfamily [7,8]. The phase I metabolites can either be eliminated directly from the body

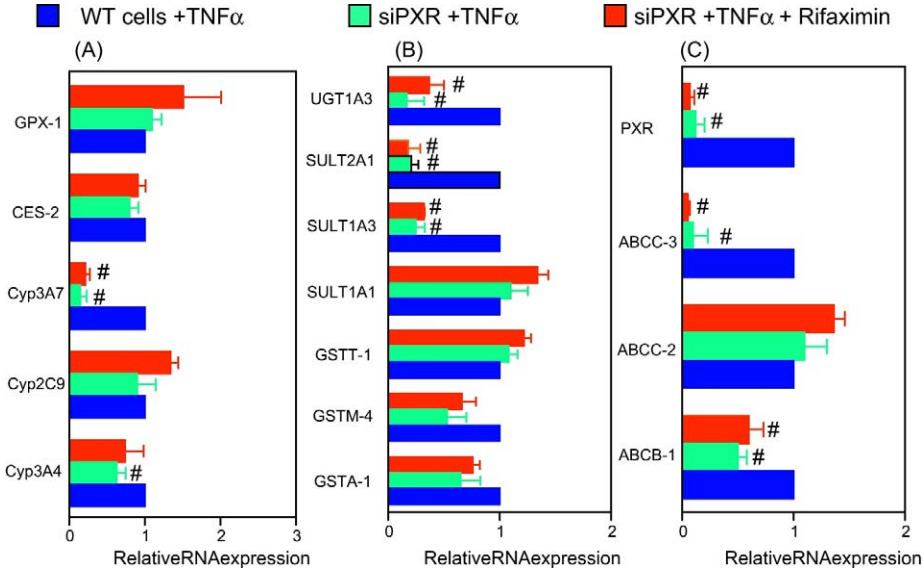

Fig. 5. Counter-regulation of TNFα activities exerted by rifaximin in normal intestinal epithelial cells requires PXR. Human colon epithelial cells (CRL1790 cells) transfected with anti-PXR siRNAs were treated with TNFα (100 ng/ml) alone or in combination with rifaximin (50 μM). Expression of tested genes was compared with their expression in wild-type cells treated with TNFα alone. Panel (A): expression of phase I genes; panel (B): phase II genes and panel (C): phase III gene and PXR ($^{\#}p < 0.05$ versus wild-type TNFα treated cells; $n = 3$).

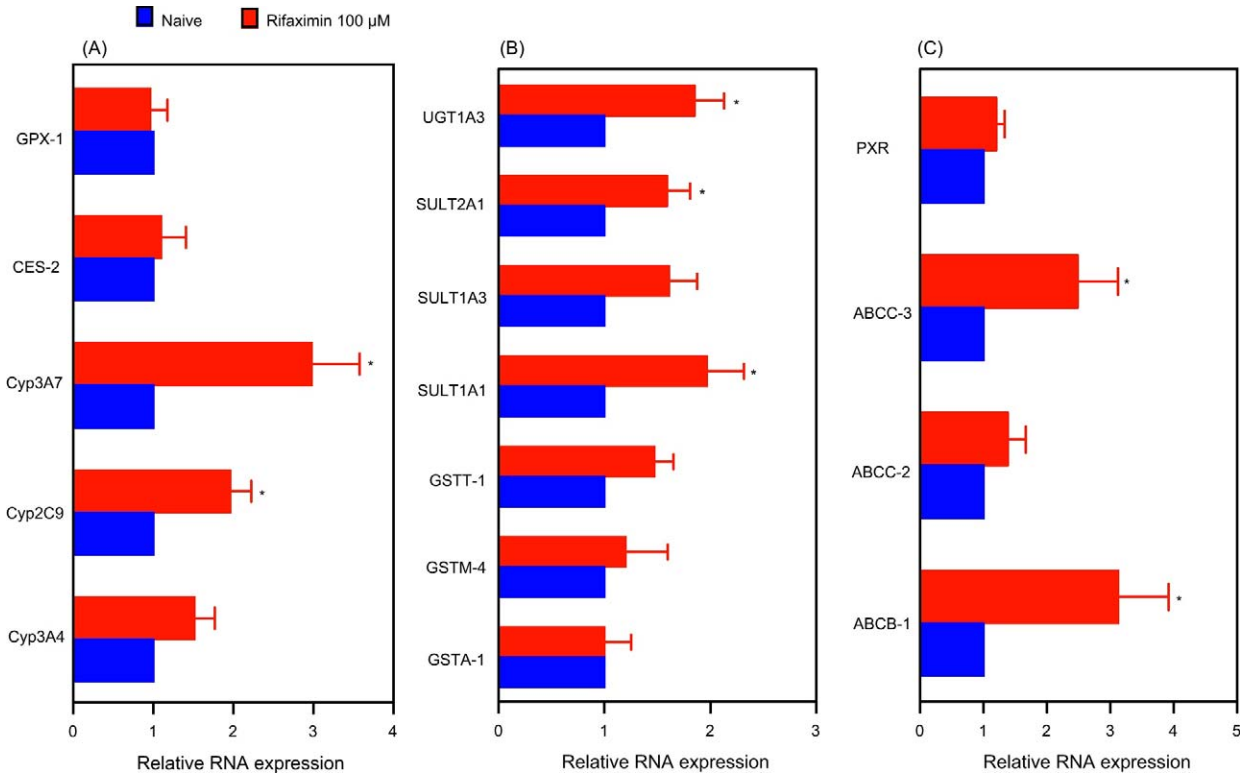

Fig. 6. Rifaximin modulates the expression of genes involved in epithelial detoxification in colon biopsies from IBD patients. Rifaximin (100 μM) increased the expression of genes involved in cellular detoxification in *ex vivo* experiments. Results of culturing colon biopsies obtained from areas of macroscopically inflamed colon of IBD patients with rifaximin are shown. Panel (A): expression of phase I genes; panel (B): phase II genes and panel (C): phase III genes and PXR mRNA ($^{*}p < 0.05$ versus untreated biopsies. $n = 10–15$).

or could subject to further biotransformation by phase II enzymes. The primary organ of drug metabolism is the liver, but the intestine is involved, as reflected by the expression of CYP enzymes in this tissue [9,10]. In addition, transporters, sometimes called phase III proteins, can determine foreign compound bioavailability, distribution, and elimination. The genes involved in drug metabolism provide an adaptive response to environmental challenge, and as a consequence, their expression is tightly regulated by foreign compounds themselves [11]. The ABCB1 (MDR1) and additional ABC transporters with a high expression in the gut such as ABCC1–3 (MRP1–3) are critically involved in the maintenance of the intestinal barrier by excluding drugs, nutrients, or bacterial compounds back into the gut lumen [22,29,30]. Furthermore, the association of MDR1 gene polymorphisms with susceptibility for IBD [23,24] implies that genetic variations or dysregulation of ABC transporters contributes to the development of IBD. Moreover it has been found that a down-regulation of detoxification genes, specifically sulfotransferases and glucuronosyltransferase and ABC transporters, occurs in the colon of patients with IBD [16]. This dysregulation is accompanied by a nearly complete loss of the transcriptional regulator PXR. This finding might have a pathogenetic relevance for IBD either because PXR is essential for regulation of detoxification pathways and for its anti-inflammatory activity and NF-κB modulating activity [16,19].

Rifaximin is a poorly absorbed oral antimicrobial agent that is concentrated in the gastrointestinal tract [31–33]. Rifaximin has a broad-spectrum of activity against Gram-positive and Gram-negative aerobic and anaerobic enteric bacteria [34–36]. In addition to its anti-bacterial activity rifaximin exerts anti-inflammatory and immunomodulatory effects [36] in human tissues and *in vivo*. Despite the compound is poorly absorbed there is evidence that it penetrates intestinal cells. This was, among others, shown recently by the fact that administration of rifaximin to mice harbouring a human PXR gene results in induction of PXR and PXR-regulated genes in the intestine [25].

In the present study we have demonstrated that exposure of primary epithelial colon cells to rifaximin modulates the expression of genes involved in cellular detoxification and that this effect requires PXR. Thus, exposure to rifaximin effectively increased the expression of CYP3-A4, CYP-3A7, CYP-2C9, GST-A1, SULT-1A1, SULT-1A3, SULT-2A1, UGT-1A3 and phase III transporters in naïve cells. In addition, rifaximin effectively counter-regulated the inhibitory effects caused by exposure of human epithelial cells to TNFα. Indeed, while TNFα reduced the expression of PXR and its target genes, with the exception of GPX-1 and CES-2 and GST-T, this effect was antagonized by incubating the cells with rifaximin. The effects exerted by rifaximin on the expression of genes involved in intestinal epithelial detoxification were mediated by PXR. This contention is supported by the following observations. First, all genes that are modified by exposure to rifaximin were modulated by PXR and specific PXR binding motifs, either as direct repeat (DR) or everted repeat (ER), ER6, DR4 and DR3 [21], were reported to be expressed in their promoter regions (Table 1). In addition, results from experiments, where the expression of PXR was knocked down by specific anti-PXR siRNA, demonstrate that the regulatory effect of rifaximin was lost in cells lacking the nuclear receptor. More specifically we have found that PXR is required by rifaximin in order to modulate the expression of CYP-3A4, CYP-3A7, GST-A1, SULT-1A1, SULT-1A3, SULT-2A1, UGT-1A3 and ABC-B1 (Table 2).

Besides its effect on human cultured epithelial cells, rifaximin modulates detoxification pathways in colon biopsies obtained from IBD patients. In these *ex vivo* studies we found that exposure to rifaximin effectively increased the expression of CYP-3A7, CYP-2C9, SULT-1A1, SULT-2A1, UGT-1A3, ABC-B1 and ABC-C3 in colon biopsies obtained from a macroscopically inflamed tissue. This

Table 2
Summary of the effects exerted by rifaximin and TNFα on the expression of detoxification genes.

Gene	Effect of rifaximin in naïve cells	Effect of TNF α in naïve cells	Effects of Rifaximin mediated by PXR (abrogated by anti-PXR siRNA)	Effect of rifaximin in IBD biopsies
Phase I				
CYP-3A4	Increased	Decreased	YES	No effect
CYP-2C9	Increased	Decreased	NO	Increased
CYP-3A7	Increased	Decreased	YES	Increased
CES-2	No effect	No effect	NO	No effect
GXP-1	No effect	No effect	NO	No effect
Phase II				
GST-A1	Increased	Decreased	YES	No effect
GST-M4	No effect	Decreased	NO	No effect
GST-T1	No effect	No effect	YES	No effect
SULT-1A1	Increased	Decreased	YES	Increased
SULT-1A3	Increased	Decreased	YES	No effect
SULT-2A1	Increased	Decreased	YES	Increased
UGT-1A3	Increased	Decreased	YES	Increased
Phase III				
ABC-B1	Increased	Decreased	YES	Increased
ABC-C2	Increased	Decreased	YES	No effect
ABC-C3	Increased	Decreased	NO	Increased
PXR	Increased	Decreased		No effect

Primary human colon epithelial cells were exposed to TNFα alone or to a combination of TNFα plus rifaximin (50 μM) and the expression of genes encoding for enzymes involved in detoxification assessed by RT-PCR. The list of genes that were induced by rifaximin, in a PXR-dependent manner is shown in green and includes: phase I: cytochrome P450 family CYP3-A4 and CYP-3A7; phase II: GST-A1, Sulfotransferases family SULT-1A1, SULT-1A3, SULT-2A1 and UDP glucuronosyltransferase UGT-1A3; and phase III: ATP-binding cassette ABC-B1. Genes whose expression was induced by exposure of either primary epithelial cells or human colon biopsies to rifaximin are shown in orange.

observation might have a clinical readout. Indeed, since TNFα negatively regulates the expression of genes involved in intestinal detoxification *in vitro* and the activities of detoxification pathways is highly compromised in IBD patients, as a part of the intestinal inflammatory syndrome, it appears that counter-regulation of inhibitory activities of TNFα on detoxification genes might contribute to the beneficial effects of rifaximin in IBDs.

In contrast to normal intestinal epithelial cells rifaximin was unable to induce PXR expression in CRL1790 cells exposed to TNFα (Fig. 3, panel C). Similar results were obtained in colon biopsies (Fig. 6). Thus it appears that during inflammation rifaximin induces PXR activity but not its expression. Support to this notion comes from the observation that the mutual regulation of NF-κB and PXR did not involve modulation of their expression but is rather mediated by changes in transcriptional activity [20,19].

In conclusion, present results demonstrate that rifaximin a non-absorbable antibiotic has a direct effect on intestinal epithelial cells. Rifaximin increases the expression of PXR in intestinal epithelial cells and modulates the expression of genes involved in intestinal detoxification in a PXR-dependent manner [37,38]. A PXR-mediated effect is required by rifaximin to counter-regulate the inhibitory effects of TNFα on genes involved in metabolism and excretion of xenobiotics in intestinal cells. Collectively, these data establish that, in addition to its antibiotic activities, rifaximin regulates the expression of genes essential to the intestinal detoxification of xenobiotic and luminal antigens in a PXR-dependent manner.

Contributors

AM participated in the design of the study, data analysis and writing of the manuscript. MM contributed RT-PCR data. MB contributed to interpretation of data and drafting of the manuscript. SC contributed to cell culture experiments. GP contributed to RT-PCR data. ED and BR contributed to data analysis and manuscript writing. SF designed the study, contributed to interpretation of data and wrote the manuscript. All authors have read and approved the final version of manuscript.

References

[1] Scholmerich J. New developments in aetiological mechanisms of inflammatory bowel disease. Eur J Gastroenterol Hepatol 2003;15:585–6.
[2] Rogler G. Update in inflammatory bowel disease pathogenesis. Curr Opin Gastroenterol 2004;20:311–7.
[3] Schmidt C, Stallmach A. Etiology and pathogenesis of inflammatory bowel disease. Minerva Gastroenterol Dietol 2005;51:127–45.

[4] Bamias G, Nyce MR, De La Rue SA, Cominelli F. New concepts in the pathophysiology of inflammatory bowel disease. Ann Intern Med 2005;143:895–904.
[5] Ahmed FE. Role of genes, the environment and their interactions in the etiology of inflammatory bowel diseases. Expert Rev Mol Diagn 2006;6:345–363.
[6] Langmann T, Schmitz G. Loss of detoxification in inflammatory bowel disease. Nat Clin Pract Gastroenterol Hepatol 2006;3:358–9.
[7] Wolf CR, Seilman S, Oesch F, Mayer RT, Burke MD. Multiple forms of cytochrome P-450 related to forms induced marginally by phenobarbital. Differences in structure and in the metabolism of alkoxyresorufins. Biochem J 1986;240:27–33.
[8] Gonzalez FJ, Gelboin HV. Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins. Drug Metab Rev 1994;26:165–83.
[9] Bergheim I, Bode C, Parlesak A. Distribution of cytochrome P450 2C, 2E1, 3A4, and 3A5 in human colon mucosa. BMC Clin Pharmacol 2005;5:4.
[10] Sarikaya D, Bilgen C, Kamataki T, Topcu Z. Comparative cytochrome P450-1A1, -2A6, -2B6, -2C, -2D6, -2E1, -3A5 and -4B1 expressions in human larynx tissue analysed at mRNA level. Biopharm Drug Dispos 2006;27:353–9.
[11] Xie W, Uppal H, Saini SP, Mu Y, Little JM, Radominska-Pandya A, et al. Orphan nuclear receptor-mediated xenobiotic regulation in drug metabolism. Drug Discov Today 2004;9:442–9.
[12] Roediger WE, Babidge W. Human colonocyte detoxification. Gut 1997;41:731–734.
[13] Kliewer SA, Goodwin B, Willson TM. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. Endocr Rev 2002;23:687–702.
[14] Rosenfeld JM, Vargas Jr R, Xie W, Evans RM. Genetic profiling defines the xenobiotic gene network controlled by the nuclear receptor pregnane X receptor. Mol Endocrinol 2003;17:1268–82.
[15] Sonoda J, Chong LW, Downes M, Barish GD, Coulter S, Liddle C, et al. Pregnane X receptor prevents hepatorenal toxicity from cholesterol metabolites. Proc Natl Acad Sci U S A 2005;102:2198–203.
[16] Langmann T, Moehle C, Mauerer R, Scharl M, Liebisch G, Zahn A, et al. Loss of detoxification in inflammatory bowel disease: dysregulation of pregnane X receptor target genes. Gastroenterology 2004;127:26–40.
[17] Shah YM, Ma X, Morimura K, Kim I, Gonzalez FJ. Pregnane X receptor activation ameliorates DSS-induced inflammatory bowel disease via inhibition of NF-κB target gene expression. Am J Physiol 2007;292:G1114–22.
[18] Ghosh S, Karin M. Missing pieces in the NF-κB puzzle. Cell 2002;109:S81–96.
[19] Gu X, Ke S, Liu D, Sheng T, Thomas PE, Rabson AB, et al. Role of NF-κB in regulation of PXR-mediated gene expression, a mechanism for the suppression of cytochrome P-4503A4 by pro-inflammatory agents. J Biol Chem 2006;281:17882–9.
[20] Morgan ET. Regulation of cytochromes P450 during inflammation and infection. Drug Metab Rev 1997;29:1129–88.
[21] Klaassen CD, Aleksunes LM. Xenobiotic, bile acid, and cholesterol transporters: function and regulation. Pharmacol Rev 2010;62:1–96.
[22] Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, *mdr1a*, spontaneously develop colitis. J Immunol 1998;161:5733–44.
[23] Schwab M, Schaeffeler E, Marx C, Fromm MF, Kaskas B, Metzler J, et al. Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. Gastroenterology 2003;124:26–33.
[24] Brant SR, Panhuysen CI, Nicolae D, Reddy DM, Bonen DK, Karaliukas R, et al. MDR1 Ala893 polymorphism is associated with inflammatory bowel disease. Am J Hum Genet 2003;73:1282–92.
[25] Ma X, Shah YM, Guo GL, Wang T, Krausz KW, Idle JR, et al. Rifaximin is a gut-specific human pregnane X receptor activator. J Pharmacol Exp Ther 2007;322:391–398.
[26] Danielson P. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab 2002;3:561–97.
[27] Bogaards JJ, van Ommen B, van Bladeren PJ. Purification and characterization of eight glutathione S-transferase isoenzymes of hamster. Comparison of subunit composition of enzymes from liver, kidney, testis, pancreas and trachea. Biochem J 1992;286:383–8.
[28] Lilla C, Risch A, Verla-Tebit E, Hoffmeister M, Brenner H, Chang-Claude J. SULT1A1 genotype and susceptibility to colorectal cancer. Int J Cancer 2007;120:201–6.
[29] Ho GT, Moodie FM, Satsangi J. Multidrug resistance 1 gene (P-glycoprotein 170): an important determinant in gastrointestinal disease? Gut 2003;52:759–66.
[30] Langmann T, Mauerer R, Zahn A, Moehle C, Probst M, Stremmel W, et al. Real-time reverse transcription-PCR expression profiling of the complete human ATP-binding cassette transporter superfamily in various tissues. Clin Chem 2003;49:230–8.
[31] Gerard L, Garey KW, DuPont HL. Rifaximin: a nonabsorbable rifamycin antibiotic for use in nonsystemic gastrointestinal infections. Expert Rev Anti Infect Ther 2005;3:201–11.
[32] Jiang ZD, DuPont HL. Rifaximin: in vitro and in vivo antibacterial activity. Chemotherapy 2005;51:67–72.
[33] Debbia EA, Maioli E, Roveta S, Marchese A. Effects of rifaximin on bacterial virulence mechanisms at supra- and sub-inhibitory concentrations. J Chemother 2008;20:186–94.
[34] Fiorucci S, Distrutti E, Mencarelli A, Barbanti M, Palazzini E, Morelli A. Inhibition of intestinal bacterial translocation with rifaximin modulates lamina propria monocytic cells reactivity and protects against inflammation in a rodent model of colitis. Digestion 2002;66:246–56.
[35] Bass NM, Mullen KD, Sanyal A, Poordad F, Neff G, Leevy CB, et al. Rifaximin treatment in hepatic encephalopathy. N Engl J Med 2010;362:1071–81.
[36] Scarpignato C, Pelosini I. Rifaximin, a poorly absorbed antibiotic: pharmacology and clinical potential. Chemotherapy 2005;51:36–66.
[37] Arrese M, Karpen SJ. Nuclear receptors, inflammation, and liver disease: insights for cholestatic and fatty liver diseases. Clin Pharmacol Ther 2010;87:473–8.
[38] McKay LI, Cidlowski JA. Molecular control of immune/inflammatory responses: interactions between nuclear factor-κB and steroid receptor-signaling pathways. Endocr Rev 1999;20:435–59.