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Alimentary Tract

Constitutive androstane receptor and pregnane X receptor cooperatively ameliorate DSS-induced colitis

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ABSTRACT

Background: Nuclear receptor pregnane X receptor (PXR) was shown to be protective in case of dextran sulfate sodium (DSS)-induced colitis. Constitutive androstane receptor (CAR) belongs to the same nuclear receptor subfamily with PXR. The roles of both receptors in DSS-induced colitis were evaluated.
Methods: Wild-type, *Car*-null, *Pxr*-null, and *Car*/*Pxr*-null mice were treated with a CAR/PXR agonist or vehicle and administered 2.5% DSS in the drinking water. The typical clinical symptoms, histological scoring, proinflammatory cytokine, and apoptosis were analyzed.
Results: Mice treated with the PXR agonist pregnenolone-16α-carbonitrile (PCN) were protected from DSS-induced colitis, as in a previous study. Mice treated with the CAR agonist, 4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) were also protected from DSS-induced colitis. Interestingly, the protective effects of PCN in the *Car*-null mice and those of TCPOBOP in the *Pxr*-null mice both decreased. PCN or TCPOBOP pretreatment significantly decreased the macrophage and monocyte infiltration in DSS-induced colitis. PXR and CAR agonists reduced the mRNA expression of several proinflammatory cytokines in a PXR- and CAR-dependent manner, respectively. CAR inhibited apoptosis by inducing Gadd45b. PXR inhibited TNF-α and IL-1b and CAR induced Gadd45b in *in vitro* cell analyses.
Conclusions: We showed that CAR and PXR cooperatively ameliorate DSS-induced colitis. PXR and CAR protected against DSS-induced colitis by inhibiting proinflammatory cytokines and apoptosis, respectively.

1. Introduction

The pathophysiology of inflammatory bowel disease (IBD), Crohn's disease (CD), and ulcerative colitis (UC) is poorly understood [1,2]. However, considerable evidence indicates that IBD results from an interaction between genetic, immune, and environmental factors [3]. The aberrant interaction between environmental factors and intestinal microbiota in a genetically susceptible host is considered one of the pathogeneses of IBD [4,5]. Over the past three decades, there has been an exponential increase in the incidence and prevalence of both CD and UC [6]. As such, the clarification of the mechanism and establishment of new therapeutic options are urgently needed.

Xenobiotic nuclear receptors, including aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR), are key regulators in the response to chemicals or environmental factors. PXR and CAR belong to the same NR1I nuclear receptor subfamily [7] and regulate hepatic drug-metabolizing enzymes, including phase I–III enzymes, such as cytochrome P450 (CYP), UDP-glucuronosyltransferase (UGT), and multidrug resistance protein (MRP), in response to chemicals and environmental factors [8]. These receptors are expressed not only in the liver but also in the intestines, which play important roles in drug absorption and act as barriers against xenobiotics. It is therefore not strange that these receptors are related to the immune system, environmental factors, and intestinal microbiota in the intestinal tract. Many associations between IBD and these receptors have been reported [9–12].

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AhR and PXR have been suggested to play protective roles in the pathogenesis of IBD [9,10]. AhR deficiency reportedly results in increased susceptibility to colitis in mice, whereas the activation of AhR may ameliorate dextran sulfate sodium (DSS)-induced colitis [13,14]. Indigo Naturalis, which contains ligands for the AhR, has been used as a traditional herbal medicine for the treatment of UC and promotes the regeneration of the mucosa through AhR signaling activation [15].

PXR activation was also reported to ameliorate DSS-induced colitis [12]. Cheng et al. demonstrated the protective function of PXR activation by rifaximin in DSS-induced and trinitrobenzene sulfonic acid (TNBS)-induced IBD models [11]. They showed that nuclear factor κB (NF-κB) target genes were markedly down-regulated by rifaximin treatment [11]. The preventive and therapeutic roles of rifaximin on IBD were suspected to be through the PXR-mediated inhibition of the NF-κB signaling cascade [11]. Rifaximin, a human PXR activator, is currently undergoing clinical trials for the treatment of IBD and has demonstrated efficacy against CD and UC [16–18].

CAR and PXR share some common ligands and overlapping sets of target genes [7,19–22]. Therefore, these two receptors are closely associated and compensate for each other's functions. CAR and PXR play diverse physiological, pharmaceutical, and pathological roles in growth, apoptosis, tumorigenesis, energy metabolism, and the induction of drug-metabolizing enzymes [23,24].

Because AhR and PXR have been implicated in the pathogenesis of IBD, CAR may also be related to the pathogenesis of IBD. However, the precise roles of CAR in the pathogenesis of IBD are unknown. Hudson et al. [25] recently reported that the CAR expression was reduced in CD and UC samples and that CAR activation accelerated intestinal epithelial wound healing by enhancing cell migration in DSS-induced colitis. We have also previously evaluated the roles of CAR in the pathogenesis of IBD [26]. Because CAR and PXR are closely related, DSS-induced colitis should be evaluated from the viewpoint of both CAR and PXR.

In this study, we investigated the roles of CAR and PXR in the pathogenesis of DSS-induced colitis using CAR/PXR single- and double-knockout mice.

2. Materials and methods

2.1. Chemicals

1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) and pregnenolone 16α-carbonitrile (PCN) were purchased from Sigma (St. Louis, MO, USA). Dextran sulfate sodium (DSS) was purchased from MP Biomedicals, LLC (Aurora, OH, USA). All other chemicals were obtained from commercial sources at the highest grade of purity available.

2.2. Animals and experimental design

All experiments were approved by the Gunma University Animal Care and Experimentation Committee (#16-006) and carried out in accordance with the approved guidelines. *Car*-null, *Pxr*-null, and *Car/Pxr*-null mice were generated and backcrossed to a C57BL/6 genetic background [27,28]. *Car*-null and *Pxr*-null mice were crossed to generate double-null mice (*Car/Pxr*-null mice) [29]. The wild-type, single-knockout, and double-knockout mice used in these studies were all maintained in a C57BL/6 background. Mice were maintained in the Institute of Experimental Animal Research of Gunma University under specific-pathogen-free conditions. Mice were handled in accordance with the animal care guidelines of Gunma University.

Six- to eight-week old wild-type, *Car*-null, *Pxr*-null, and *Car/Pxr*-null male mice were fashioned into DSS-induced IBD models. Mice were placed into 3 groups ($n \geq 6$ per group) in the DSS-induced IBD study: control, TCPOBOP pretreatment, and PCN pretreatment. TCPOBOP was used as a CAR-ligand in a 6 mg/ml dimethylsulfoxide solution at a dose of 3 mg/kg of body weight. PCN was used as a PXR-ligand in a 25 mg/ml ethanol solution at a dose of 20 mg/kg of body weight. Control mice received the same dose of dimethylsulfoxide solution or ethanol per body weight. Mice in the PCN and DSS or TCPOBOP and DSS groups received the compounds for 10 days, and on the 4th day of treatment, the mice were administered 2.5% DSS in drinking water (wt/vol) or control drinking water.

All mice were sacrificed 10 days after treatment with PCN, TCPOBOP, or vehicle (Supplemental Fig. 1), and tissue samples were harvested and stored at −80 °C before the analysis. Total liver RNA was prepared from each mouse ($n = 5$ per group) using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and then it was further purified using an RNeasy kit (Qiagen, Valencia, CA, USA).

2.3. The colitis evaluation

Daily changes in the body weight, diarrhea, rectal bleeding, and bloody stool were assessed and reported as a score from 0 to 4. After sacrifice, the length of the colon from the colo-cecal junction to the anal verge was measured. For the assessment of macroscopic colon damage, the colon was opened longitudinally, flushed with phosphate-buffered saline, and fixed in 10% buffered formalin. Colitis was measured by a blinded analysis using routine hematoxylin and eosin staining according to the morphological criteria described previously (Supplemental Fig. 2) [30].

To analyze the infiltrated cells, macrophages and monocytes were stained by F4/80 antibody (Funakoshi Co., Ltd., Tokyo, Japan). For immunohistochemistry, F4/80 antibody was applied overnight at 4 °C, and biotinylated secondary antibody was applied for 1 h. Bound peroxidase was visualized after a 3,3-diaminobenzidine reaction for 2–5 min and after light counterstaining with hematoxylin, dehydration, and mounting.

2.4. In situ *analyses of apoptosis by a terminal deoxynucleotidyl transferase dUTP nick end labeling assay*

To examine the apoptotic cells, a terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP)-biotin nick end-labeling (TUNEL) assay was performed according to the manufacturer's protocol (*In situ* Apoptosis Detection kit; Apoptag Direct, Oncor, Gaithersburg, Germany). The sections were viewed and photographed using standard fluorescence microscopy techniques. The number of TUNEL-positive cells per 100 cells was counted in 10 randomly selected fields in each group. The mean number for the 10 fields was then calculated.

2.5. *Cell lines and* in vitro *analyses*

Ym17 cells, a stable cell line expressing CAR, were established from HepG2 cells that were transfected with a mouse pcDNA3.1-CAR-V5-His expression vector and selected for neomycin resistance [24]. The cells were cultured in minimal essential medium supplemented with 10% fetal bovine serum and antibiotics (100 U/ml of penicillin and 100 μg/ml of streptomycin). To determine the effect of CAR/PXR stimulation on the inflammatory pathway, the tumor necrosis factor-alpha (TNF-α)-induced cell signaling was evaluated. The mRNA expression of the cytokines or CAR/PXR-targeted genes were analyzed by real-time polymerase chain reaction (PCR). In brief, Ym17 cells were cultured for 24 h, after which 10 mM of Rifampicin or 250 nM of TCPOBOP was added to the wells. At 48 h, 10 ng/ml of TNF-α was additionally added to each well, and the

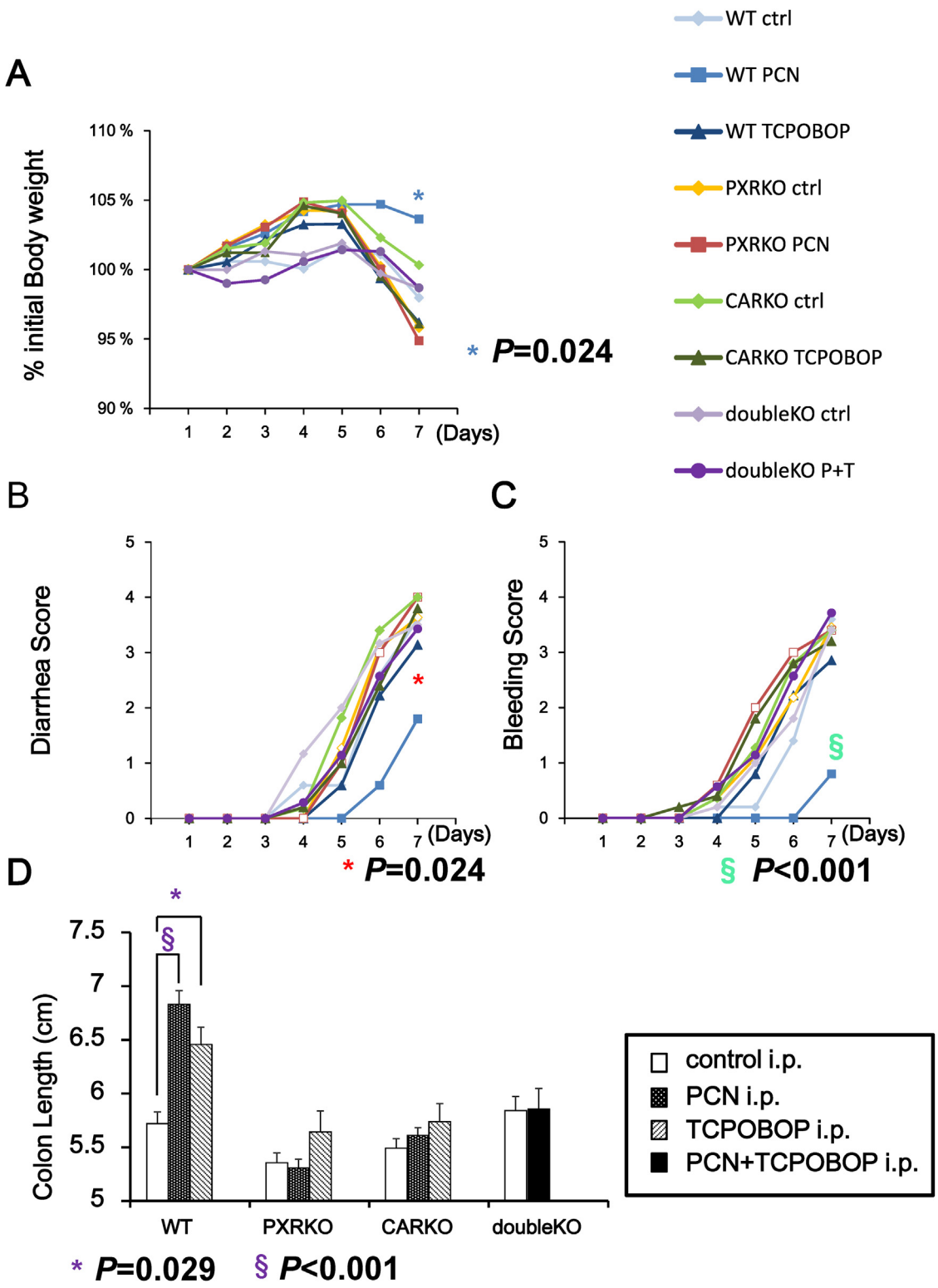

Fig. 1. The assessment of body weight changes and colitis in mice.
The body weight (A), diarrhea score (B), and bleeding score (C) in the DSS-induced colitis model were shown. DSS treatment decreased the body weight and caused diarrhea and rectal bleeding. Treatment with the PXR ligand PCN significantly improved the body weight loss ($P = 0.024$), diarrhea score ($P = 0.024$), and bleeding score ($P < 0.001$) in the wild-type mice compared with the wild-type control mice. (D) The colon lengths of each DSS-induced colitis model were evaluated. PCN significantly improved the colon length in the wild-type mice ($P < 0.001$). The preventive effects of PCN were not observed in the *Pxr*-null or *Car*/*Pxr*-*null* mice. TCPOBOP also significantly improved the colon length in the wild-type mice ($P = 0.029$). The effects of TCPOBOP were not observed in the *Car*-null mice. The preventive effects of TCPOBOP disappeared in the *Pxr*-null mice. A–D: ANOVA $P < 0.001$.

cells were cultured for 6 h at 37 °C. Total RNA was prepared from each well using TRIzol reagent (Invitrogen), and then real-time PCR was performed. cDNAs were amplified using the following sets of primers: TNF-α mRNA, 5′-AGCCCACGTCGTAGCAAACCACCAA-3′ and 5′-ACACCCATTCCCTTCACAGAGCAAT-3′; IL-1b mRNA, 5′-GAGGCCTAATAGGCTCATCTG-3′ and

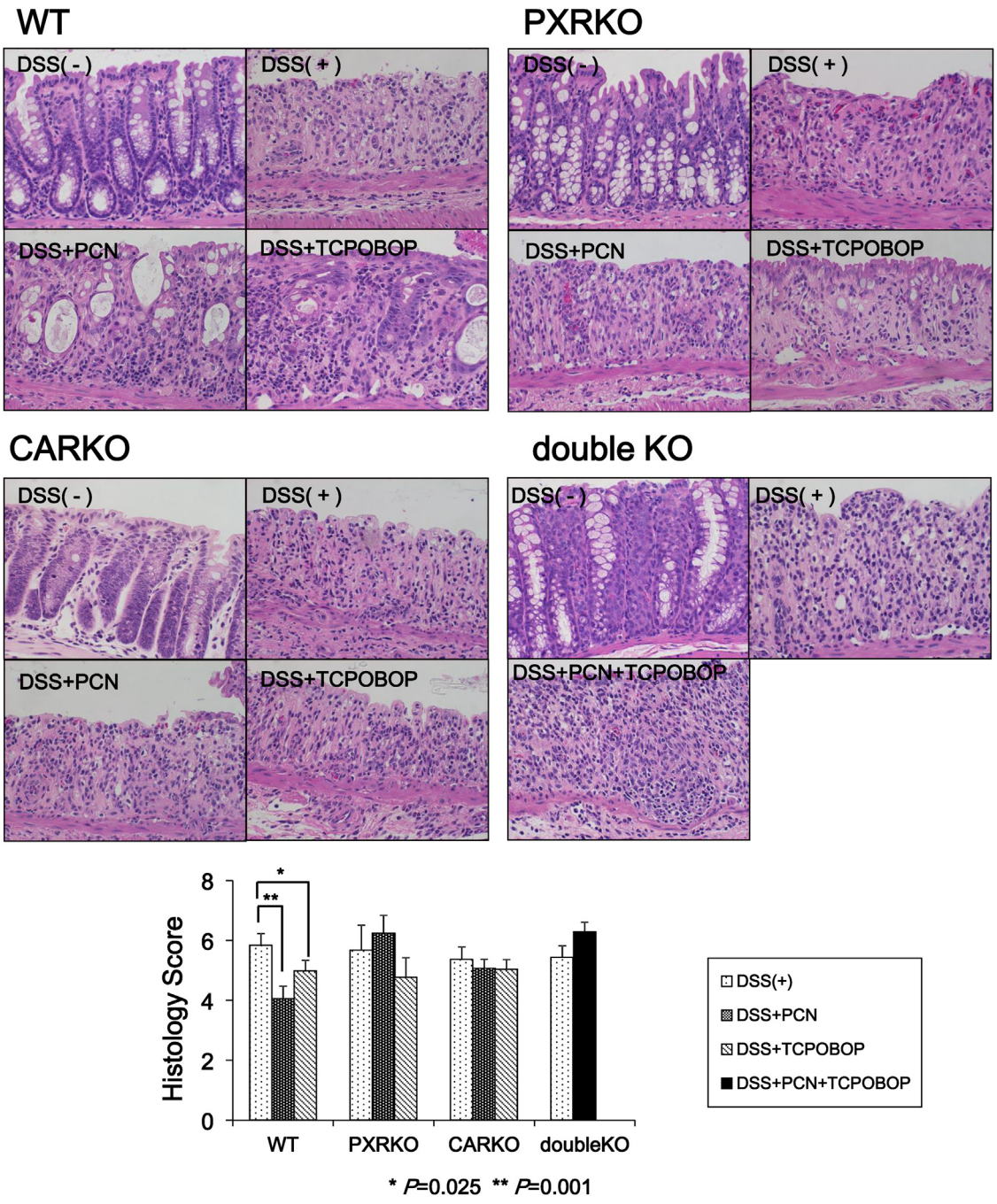

Fig. 2. Histological analyses in DSS-induced colitis models.
Representative hematoxylin and eosin-stained colon sections of wild-type, *Pxr*-null, *Car*-null, and *Car*/*Pxr*-null mice treated with DSS with and without PCN or TCPOBOP. Colitis was measured with routine hematoxylin and eosin staining according to the morphological criteria. DSS treatment caused epithelial cell damage and increased infiltration of granulocytes and mononuclear immune cells. PCN pretreatment significantly attenuated DSS-induced colon damage as assessed by the colon histology score in wild-type mice compared with DSS treatment alone ($P < 0.001$). TCPOBOP pretreatment also significantly attenuated DSS-induced colon damage in wild-type mice compared with DSS treatment alone ($P = 0.025$). ANOVA $P = 0.010$.

5′-GGGTCCGTCAACTTCAAAG-3′; MCP-1 mRNA, 5′-TCACTGAAGCCAGCTCTC-3′ and 5′-GGATCATCTTGCTGGTGAAT-3′; Gadd45b mRNA, 5′-TGCGACAATGACATTGACATC-3′ and 5′-GGAATCTGTATGACAGTTCGTGA-3′.

2.6. Real-time PCR

The mRNA expression of genes was analyzed by real-time PCR. Total RNA was extracted from the colon, and the subsequent syntheses of first strand cDNA were performed using the TRIzol reagent and the SuperScriptTM preamplification system (Invitrogen). cDNAs were amplified using the following sets of primers: IL-1b mRNA, 5′-GAGGCCTAATAGGCTCATCTG-3′ and 5′-GGGTCCGTCAACTTCAAAG-3′; CCR2 mRNA, 5′-CATTACACCTGTGGCCC-3′ and 5′-CTTCTCATTCCTACAGCGA-3′; TNF-α mRNA, 5′-AGCCCACGTCGTAGCAAACCACCAA-3′ and 5′-ACACCCATTCCCTTCACAGAGCAAT-3′; Gadd45b mRNA, 5′-TGCGACAATGACATTGACATC-3′ and 5′-GGAATCTGTATGACAGTTCGTGA-3′. One-twentieth of each cDNA

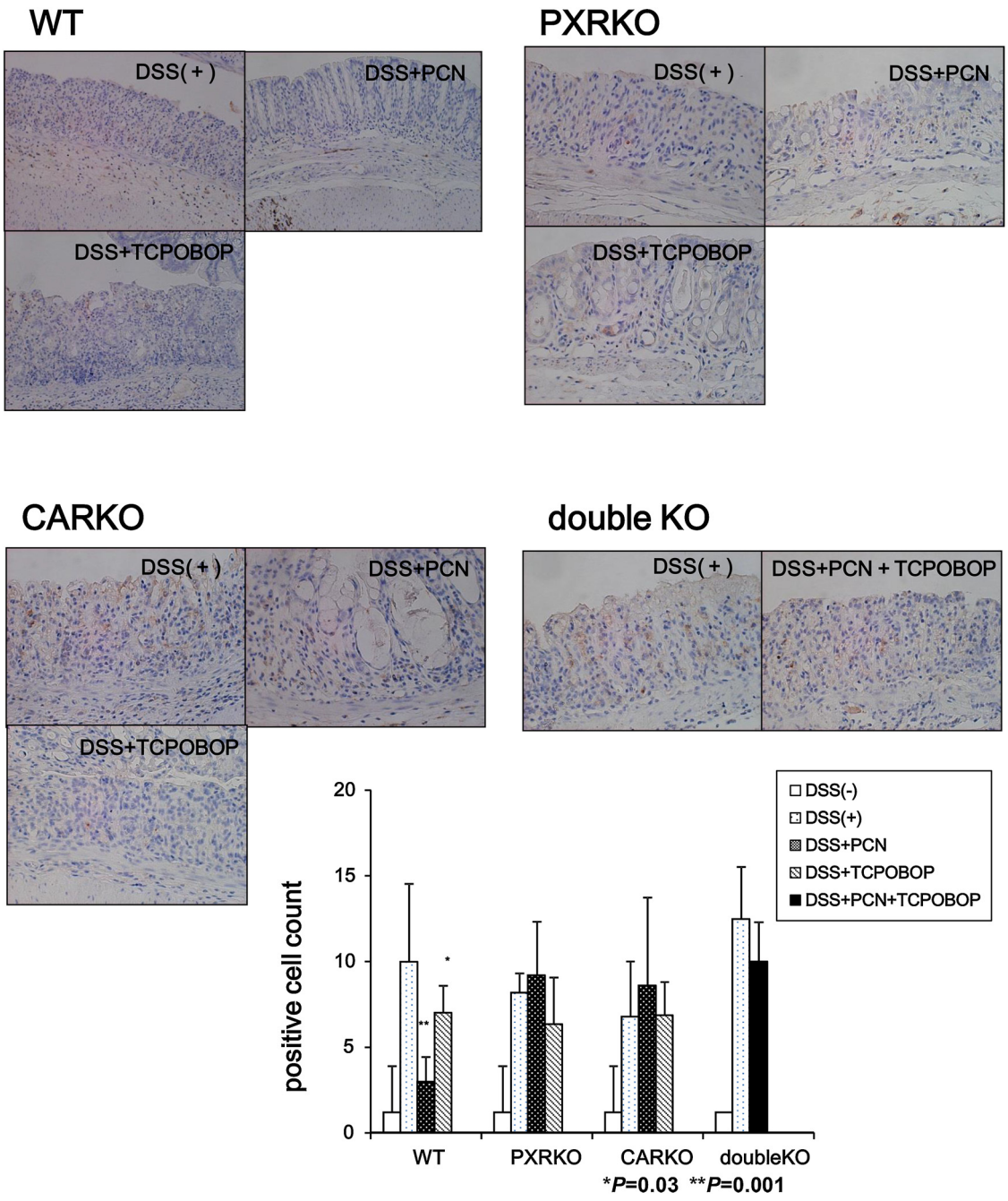

Fig. 3. F4/80 staining in DSS-induced colitis models.
To analyze the infiltrated cells, macrophages and monocytes were stained by F4/80 antibody. Representative F4/80-stained colon sections of wild-type, *Pxr*-null, *Car*-null, and *Car*/*Pxr*-null mice treated with DSS with and without PCN or TCPOBOP are shown. PCN ($P = 0.001$) and TCPOBOP ($P = 0.03$) pretreatment significantly decreased the macrophage and monocyte infiltration compared with DSS-treated control mice. PCN did not protect against DSS-induced colitis in *Pxr*-null mice, and TCPOBOP did not protect against DSS-induced colitis in *Car*-null mice. ANOVA $P = 0.0032$.

synthesized from 5 μg of RNA was subjected to real-time PCR using SYBR green dye (PE Applied Biosystems, Foster City, CA, USA).

2.7. Data analyses

All experimental data are shown as the mean ± standard deviation (SD). Multiple comparisons of parametric data were assessed using either a one or two-way factorial analysis of variance (ANOVA). A repeated-measures ANOVA was used to assess daily changes in the body weight, diarrhea, rectal bleeding, and bloody stool. Bonferroni's post hoc test was performed when the respective F values achieved $P < 0.05$ and no significant variance inhomogeneity was detected. Multiple comparisons of data that were not deemed normally distributed were assessed using the Kruskal–Wallis test followed by Dunn's post hoc test. The IBM SPSS software program, ver. 25, and the Statistics and Microsoft Excel2013 software programs were used for all analyses and to plot data points graphically.

3. Results

3.1. Body weight changes and colitis assessment of mice

The preventive roles of PCN or TCPOBOP were assessed in the DSS-induced colitis model. DSS treatment decreased the body

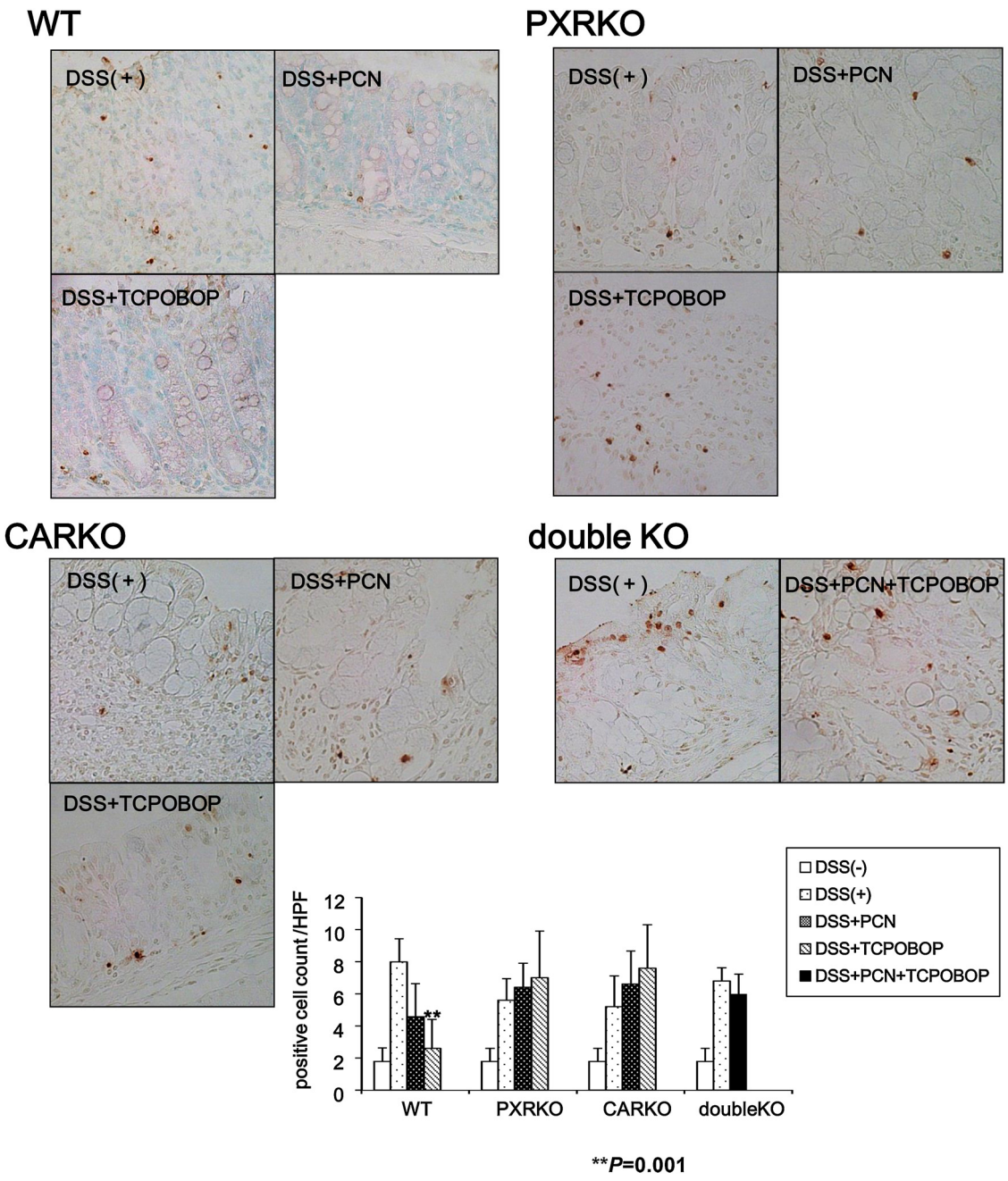

Fig. 4. TUNEL staining in DSS-induced colitis models.
To examine the apoptotic cells, a terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP)-biotin nick end-labeling (TUNEL) assay was performed. The number of TUNEL-positive cells per 100 cells was counted in 10 randomly selected fields in each group. The mean number for the 10 fields was then calculated. TUNEL staining showed a significant reduction in the number of apoptotic cells in the TCPOBOP-treated wild-type mice compared with the DSS-treated control mice ($P = 0.001$). TCPOBOP did not decrease the number of apoptotic cells in the *Car*-null mice. ANOVA $P = 0.005$.

weight (Fig. 1A) and caused diarrhea (Fig. 1B) and rectal bleeding (Fig. 1C). Treatment with the PXR ligand PCN significantly improved the body weight loss ($P < 0.01$), diarrhea score ($P < 0.01$), and bleeding score ($P < 0.01$) in the wild-type mice. Furthermore, PCN significantly improved the colon length in the DSS-induced colitis model ($P < 0.01$, Fig. 1D). The preventive effects of PCN were not observed in the *Pxr*-null or *Car*/*Pxr*-null mice. Interestingly, the preventive effects of PCN disappeared in the *Car*-null mice.

Treatment with the CAR ligand TCPOBOP tended to improve the diarrhea score and bleeding score, although it did not reach statistical significance. However, TCPOBOP significantly improved the colon length in this model ($P < 0.05$, Fig. 1D). No effects of TCPOBOP were observed in the *Car*-null mice. The preventive effects of TCPOBOP also disappeared in the *Pxr*-null mice.

3.2. Histological analyses

Histologically, DSS treatment caused epithelial cell damage and increased infiltration of granulocytes and mononuclear immune cells. Colitis was measured according to the morphological criteria by the colon histology score. PCN pretreatment significantly attenuated the DSS-induced colon damage based on the colon histology score in wild-type mice compared with DSS treatment alone ($P < 0.01$, Fig. 2). TCPOBOP pretreatment also significantly attenu-

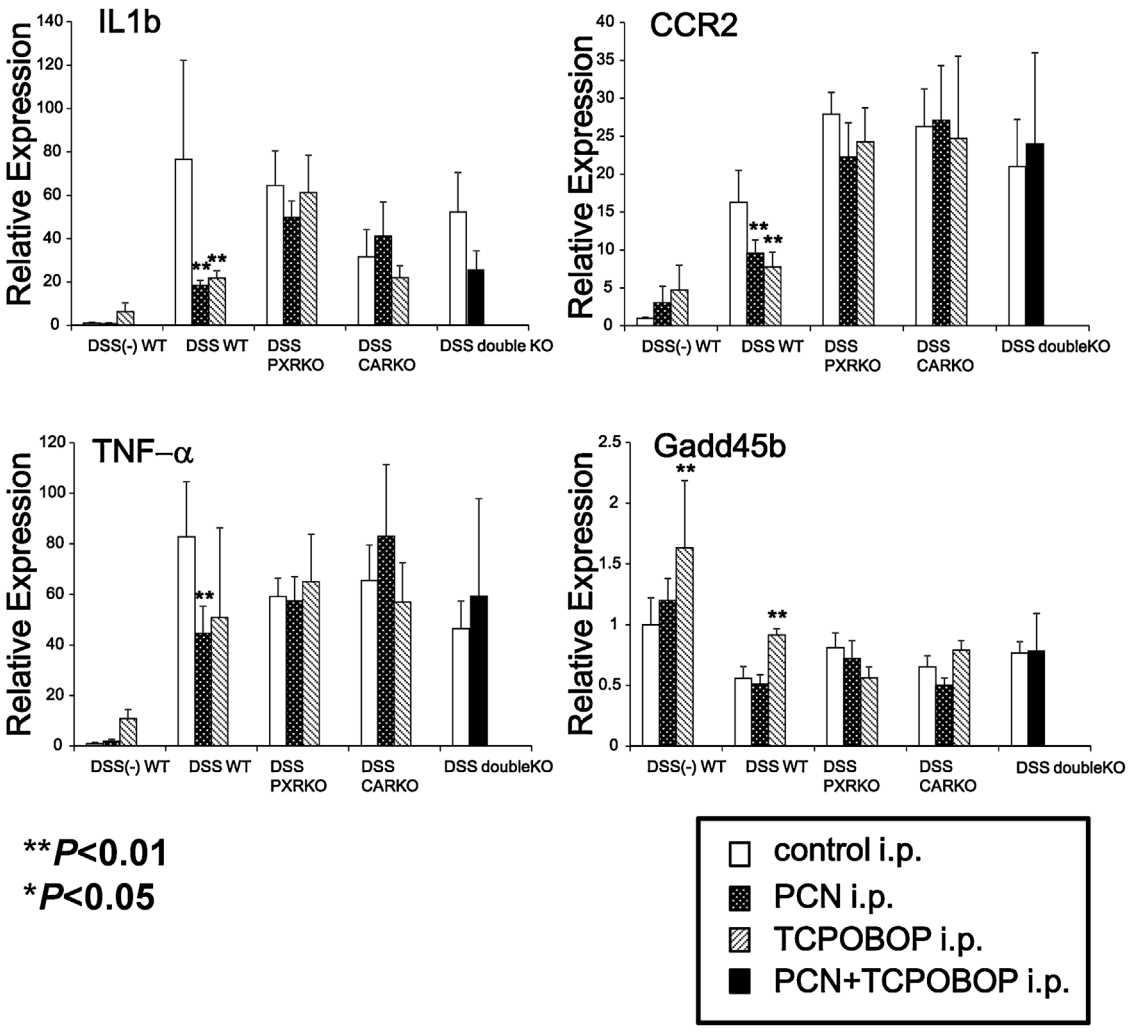

Fig. 5. The mRNA expression of NF-κB-targeted genes.
The mRNA expression of some NF-κB-targeted genes was analyzed by real-time PCR. Total RNA was isolated from the colon of the *Pxr*-null, *Car*-null, and *Car*/*Pxr*-null mice. The cDNA was then subjected to quantitative real time PCR. $^{**}P < 0.01$, in comparison to the control. Data represent the mean ± SD for five animals per group.

ated DSS-induced colon damage in wild-type mice compared with DSS treatment alone ($P < 0.05$, Fig. 2). The protective effects of PCN on colon damage were stronger than those of TCPOBOP in wild-type mice.

In *Pxr*-null mice, PCN did not protect against DSS-induced colitis as assessed by the colon histology score. These results suggest that the beneficial effects of PCN were attributed to its specific activation of PXR. TCPOBOP also did not protect against DSS-induced colitis in *Car*-null mice. These results suggest that the beneficial effects of TCPOBOP were attributed to its specific activation of CAR. In Car/*Pxr*-null mice, PCN and TCPOBOP did not protect against DSS-induced colitis as assessed by the colon histology score. Interestingly, the preventive effects of PCN disappeared in the Car-null mice, and the preventive effects of TCPOBOP also disappeared in the *Pxr*-null mice.

To analyze the infiltrated cells, macrophages and monocytes were stained by F4/80 antibody (Fig. 3). PCN ($P < 0.01$) or TCPOBOP ($P < 0.05$) pretreatment significantly decreased the macrophage and monocyte infiltration in the DSS-induced colon model. The anti-inflammatory effects of PCN were stronger than those of TCPOBOP. PCN did not protect against DSS-induced colitis in *Pxr*-null mice, and TCPOBOP did not protect against DSS-induced colitis in *Car*-null mice. In *Car*/*Pxr*-null mice, PCN and TCPOBOP did not decrease the macrophage and monocyte infiltration in the DSS-induced colon model. Interestingly, the preventive effects of PCN disappeared in the *Car*-null mice, and the preventive effects of TCPOBOP also disappeared in the *Pxr*-null mice.

TUNEL staining showed a significant reduction in the number of apoptotic cells in the TCPOBOP-treated wild-type mice compared with the control mice (Fig. 4, $P < 0.01$). TCPOBOP did not decrease the number of apoptotic cells in *Car*-null mice. These results suggest that the beneficial effects of TCPOBOP were attributed to its specific activation of CAR. Similarly, PCN treatment also did not decrease the number of apoptotic cells, although an inhibitory tendency was noted.

3.3. *mRNA expression of NF-κB target genes and apoptotic signals*

Because a previous study reported that PXR protected against DSS-induced colitis via the inhibition of NF-κB target gene expression [12], the mRNA expression of some NF-κB target genes was analyzed by real-time PCR (Fig. 5). IL-1b and CCR2 were significantly inhibited by PCN and TCPOBOP treatment in wild-type mice. However, these effects were not observed in *Pxr*-, *Car*-, or *Car*/*Pxr*-null mice. The TNF-α expression was significantly inhibited by PCN treatment in wild-type mice, as in a previous report [12]. In contrast, TCPOBOP treatment did not inhibit the TNF-α expression in

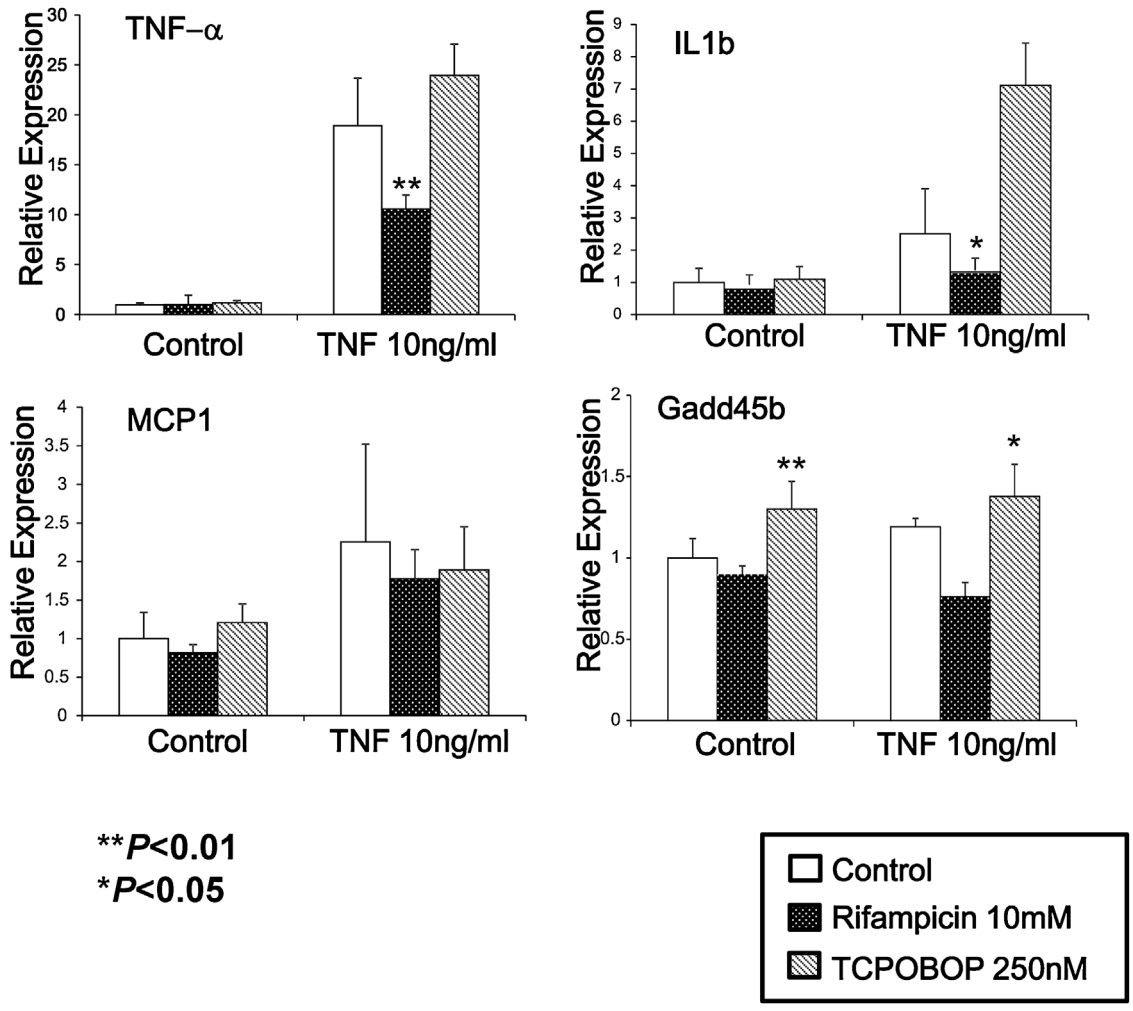

Fig. 6. CAR/PXR stimulation on inflammatory pathway *in vitro*.
To determine the effect of CAR/PXR stimulation on the inflammatory pathway, TNF-α-induced cell signaling was analyzed in Ym17 cells by real-time PCR. Ym17 cells were cultured for 24 h, after which 10 mM of Rifampicin, or 250 nM of TCPOBOP was added to the wells. At 48 h, 10 ng/ml of TNF-α was additionally added to each well, and the cells were cultured for 6 h. Total RNA was prepared from each well and then analyzed by real-time PCR. TNF-α and IL-1b were significantly inhibited by Rifampicin treatment. TCPOBOP treatment significantly increased the Gadd45b expression. $^{**}P < 0.01$, $^{*}P < 0.05$.

DSS-treated wild-type mice in this study, although it showed a tendency to inhibit the TNF-α expression.

Since TUNEL staining showed that CAR inhibited apoptosis in our DSS-induced colitis model, CAR target genes in apoptotic signals were evaluated. TCPOBOP treatment significantly increased the Gadd45b expression in DSS-treated wild-type mice ($P < 0.01$). The Gadd45b expression was not increased in *Car*-null mice. These findings, along with the results of TUNEL staining, showed that CAR inhibited apoptosis by inducing Gadd45b. PCN treatment did not increase the Gadd45b expression in DSS-treated wild-type mice.

3.4. *Effect of CAR/PXR stimulation on the inflammatory pathway* in vitro

To determine the effect of CAR/PXR stimulation on the inflammatory pathway, TNF-α-induced cell signaling were analyzed in Ym17 cells by real-time PCR (Fig. 6). TNF-α and IL-1b were significantly inhibited by Rifampicin treatment. These inhibitions were also observed *in vivo*. In contrast, TCPOBOP treatment did not inhibit the TNF-α and IL-1b expression.

Since CAR inhibited apoptosis *in vivo*, the involvement of CAR target genes in apoptotic signals was evaluated in Ym 17 cells. TCPOBOP treatment significantly increased the Gadd45b expression under both control and TNF-α-induced signaling conditions. In contrast, Rifampicin treatment did not affect the Gadd45b expression.

4. Discussion

In this study, we showed that CAR and PXR cooperatively ameliorate DSS-induced colitis. PXR protected DSS-induced colitis via the inhibition of NF-κB target gene expression, and CAR protected via the inhibition of apoptosis. CAR and PXR both protected against DSS-induced colitis but via different mechanisms. The protective effects of the PXR ligand PCN were not observed in *Car*-null mice, and the protective effects of the CAR ligand TCPOBOP were not observed in *Pxr*-null mice. This indicates that both PXR and CAR were required in order to protect against DSS-induced colitis. The precise mechanism underlying this phenomenon is not clear at this time. However, it is an interesting phenomenon, and further investigations are needed.

Hudson et al. [25] recently reported that the CAR expression was reduced in CD and UC samples and that CAR activation accelerated intestinal epithelial wound healing by enhancing cell migration. They suggested that CAR does not exert direct anti-inflammatory effects that dampen the response to DSS exposure [25], unlike PXR

and AhR. Although our protocol differed from theirs in that we evaluated the effects of pretreatment, the anti-inflammatory effects of the PXR ligand were also stronger than those of CAR ligand. Hudson et al. also showed that treating mice with TCPOBOP during recovery from colitis enhanced mucosal healing and reduced tissue inflammation. Both the previous [25] and present study showed that CAR exerted a protective effect against DSS-induced colitis and that CAR and PXR worked via different mechanisms to protect against DSS-induced colitis.

Our use of *Car*/*Pxr*-null mice was a novel aspect of this study. PXR and CAR belong to the same NR1I nuclear receptor subfamily [7] and share target genes or response elements. As such, they sometimes compensate for one another. Using *Car*/*Pxr*-null mice was therefore useful for evaluating the function of these two receptors.

Histologically, the protective effects of PCN on colon damage were stronger than those of TCPOBOP. The infiltrated cells stained by F4/80 antibody were significantly decreased by PCN or TCPOBOP treatment. However, the anti-inflammatory effects of PCN were stronger than those of TCPOBOP. Hudson et al. [25] suggested that CAR does not exert direct anti-inflammatory effects that dampen the response to DSS exposure [25]. Our results showing a stronger anti-inflammatory effect of PXR than CAR were similar to those previous findings. Treatment with a PXR agonist decreased the mRNA expression of several NF-κB target genes in a PXR-dependent manner, as described previously. Treatment with a CAR agonist also decreased the mRNA expression of several NF-κB target genes in a CAR-dependent manner. However, CAR treatment did not inhibit the TNF-α expression, unlike PXR treatment. In contrast, the Gadd45b expression was increased by CAR but not PXR. TUNEL staining showed that CAR but not PXR inhibited apoptosis in our model of DSS-induced colitis. We also showed that PXR agonist inhibited TNF-α and IL-1b and that CAR agonist induced Gadd45b in *in vitro* cell analyses. CAR and PXR both exerted protective effects against DSS-induced colitis via similar–but not the same–mechanisms.

Interestingly, the protective effects of PCN were decreased in the *Car*-null mice. While it is reasonable for the protective effects of PCN to disappear in *Pxr*-null mice, these effects were also decreased in the *Car*-null mice, indicating that CAR is needed to enjoy the full protective effect of PCN against DSS-induced colitis. Similarly, the protective effects of TCPOBOP were decreased in the *Pxr*-null mice. The precise mechanism underlying this phenomenon is unclear, and further investigations will be needed. CAR and PXR cooperatively ameliorate DSS-induced colitis.

PXR and CAR are closely related receptors that play important roles in the gastrointestinal tract, regulating the barrier function, inflammatory signaling, and mucosal repair following injury [31,32]. Xenobiotic receptors, including AhR, CAR, and PXR, play key roles in the maintenance of intestinal mucosal homeostasis, and their dysfunction may contribute to the pathogenesis of IBD. In this study, we showed that CAR and PXR cooperatively ameliorate DSS-induced colitis. Therapeutic agents targeting xenobiotic receptors may be useful for treating IBD.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2018.10.008.

References

[1] Torres J, Danese S, Colombel JF. New therapeutic avenues in ulcerative colitis: thinking out of the box. Gut 2013;62:1642–52.
[2] Bandzar S, Gupta S, Platt MO. Crohn's disease: a review of treatment options and current research. Cell Immunol 2013;286:45–52.
[3] Bamias G, Martin III C, Mishina M, Ross WG, Rivera-Nieves J, Marini M, et al. Proinflammatory effects of TH2 cytokines in a murine model of chronic small intestinal inflammation. Gastroenterology 2005;128:654–66.
[4] Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427–34.
[5] Matsuoka K, Kanai T. The gut microbiota and inflammatory bowel disease. Semin Immunopathol 2015;37:47–55.
[6] Hibi T, Ogata H. Novel pathophysiological concepts of inflammatory bowel disease. J Gastroenterol 2006;41:10–6.
[7] Raucy JL, Lasker JM. Cell-based systems to assess nuclear receptor activation and their use in drug development. Drug Metab Rev 2013;45:101–9.
[8] Xu C, Li CY, Kong AN. Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch Pharm Res 2005;28:249–68.
[9] 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.
[10] Dring MM, Goulding CA, Trimble VI, Keegan D, Ryan AW, Brophy KM, et al. The pregnane X receptor locus is associated with susceptibility to inflammatory bowel disease. Gastroenterology 2006;130:341–8.
[11] Cheng J, Shah YM, Ma X, Pang X, Tanaka T, Kodama T, et al. Therapeutic role of rifaximin in inflammatory bowel disease: clinical implication of human pregnane X receptor activation. J Pharmacol Exp Ther 2010;335:32–41.
[12] Shah YM, Ma X, Morimura K, Kim I, Gonzalez FJ. Pregnane X receptor activation ameliorates DSS-induced inflammatory bowel disease via inhibition of NF-kappaB target gene expression. Am J Physiol Gastrointest Liver Physiol 2007;292:G1114–22.
[13] Takamura T, Harama D, Matsuoka S, Shimokawa N, Nakamura Y, Okumura K, et al. Activation of the aryl hydrocarbon receptor pathway may ameliorate dextran sodium sulfate-induced colitis in mice. Immunol Cell Biol 2010;88:685–9.
[14] Islam J, Sato S, Watanabe K, Watanabe T, Ardiansyah, Hirahara K, et al. Dietary tryptophan alleviates dextran sodium sulfate-induced colitis through aryl hydrocarbon receptor in mice. J Nutr Biochem 2017;42:43–50.
[15] Kawai S, Iijima H, Shinzaki S, Hiyama S, Yamaguchi T, Araki M, et al. Indigo Naturalis ameliorates murine dextran sodium sulfate-induced colitis via aryl hydrocarbon receptor activation. J Gastroenterol 2017;52:904–19.
[16] Gionchetti P, Rizzello F, Ferrieri A, Venturi A, Brignola C, Ferretti M, et al. Rifaximin in patients with moderate or severe ulcerative colitis refractory to steroid-treatment: a double-blind, placebo-controlled trial. Dig Dis Sci 1999;44:1220–1.
[17] Guslandi M, Petrone MC, Testoni PA. Rifaximin for active ulcerative colitis. Inflamm Bowel Dis 2006;12:335.
[18] Mencarelli A, Migliorati M, Barbanti M, Cipriani S, Palladino G, Distrutti E, et al. Pregnane-X-receptor mediates the anti-inflammatory activities of rifaximin on detoxification pathways in intestinal epithelial cells. Biochem Pharmacol 2010;80:1700–7.
[19] Goodwin B, Moore JT. CAR: detailing new models. Trends Pharmacol Sci 2004;25:437–41.
[20] Ueda A, Hamadeh HK, Webb HK, Yamamoto Y, Sueyoshi T, Afshari CA, et al. Diverse roles of the nuclear orphan receptor CAR in regulating hepatic genes in response to phenobarbital. Mol Pharmacol 2002;61:1–6.
[21] Squires EJ, Sueyoshi T, Negishi M. Cytoplasmic localization of pregnane X receptor and ligand-dependent nuclear translocation in mouse liver. J Biol Chem 2004;279:49307–14.
[22] Tzameli I, Pissios P, Schuetz EG, Moore DD. The xenobiotic compound 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene is an agonist ligand for the nuclear receptor CAR. Mol Cell Biol 2000;20:2951–8.
[23] Kakizaki S, Yamazaki Y, Takizawa D, Negishi M. New insights on the xenobiotic-sensing nuclear receptors in liver diseases–CAR and PXR–. Curr Drug Metab 2008;9:614–21.
[24] Kakizaki S, Takizawa D, Tojima H, Yamazaki Y, Mori M. Xenobiotic-sensing nuclear receptors CAR and PXR as drug targets in cholestatic liver disease. Curr Drug Targets 2009;10:1156–63.
[25] Hudson GM, Flannigan KL, Erickson SL, Vicentini FA, Zamponi A, Hirota CL, et al. Constitutive androstane receptor regulates the intestinal mucosal response to injury. Br J Pharmacol 2017;174:1857–71.
[26] Tojima H, Kakizaki S, Yamazaki Y, Takizawa D, Horiguchi N, Mori M. Constitutive androstane receptor ameliorates the dextran sulfate sodium-induced colitis. Gut 2012;61:A273.
[27] Yamamoto Y, Moore R, Goldsworthy TL, Negishi M, Maronpot RR. The orphan nuclear receptor constitutive active/androstane receptor is essential for liver tumor promotion by phenobarbital in mice. Cancer Res 2004;64:7197–200.

[28] Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, et al. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc Natl Acad Sci U S A 2001;98:3369–74.
[29] Tojima H, Kakizaki S, Yamazaki Y, Takizawa D, Horiguchi N, Sato K, et al. Ligand dependent hepatic gene expression profiles of nuclear receptors CAR and PXR. Toxicol Lett 2012;212:288–97.
[30] Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest 1993;69:238–49.
[31] Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, et al. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. Immunity 2014;41:296–310.
[32] Garg A, Zhao A, Erickson SL, Mukherjee S, Lau AJ, Alston L, et al. Pregnane X receptor activation attenuates inflammation-associated intestinal epithelial barrier dysfunction by inhibiting cytokine-induced myosin light-chain kinase expression and c-Jun N-Terminal kinase 1/2 activation. J Pharmacol Exp Ther 2016;359:91–101.