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Rosiglitazone, an agonist of peroxisome proliferator-activated receptor gamma, reduces chronic colonic inflammation in rats

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Abstract

Recent studies have shown the implication of the peroxisome proliferator-activated receptor gamma (PPARγ) in control of inflammation, immune and apoptotic responses during early experimental colitis. However, there is little information about the effects of these agents on colonic mucosa under chronic inflammatory conditions. In this study, we have evaluated the effects of rosiglitazone, a PPAR-γ agonist, on the chronic injury caused by intra-colonic administration of trinitrobenzensulfonic acid (TNBS) in rats. Rosiglitazone (1 and 5 mg/kg p.o.) was administered by oral gavage, 24 h after TNBS instillation and daily during 2 weeks before killing the rats. Colons were removed for histological and biochemical analysis. Administration of rosiglitazone corrected the disorders in morphology associated to lesions, significantly reduced the ulceration index, the rise of myeloperoxidase (MPO) and the levels of tumour necrosis factor alpha (TNF-α). In addition, rosiglitazone treatment increased prostaglandin (PG)E_2 production and returned PGD_2 to basal levels. Also, reduced cyclooxygenase (COX)-2 and nuclear transcription factor NF-kappa B (NF-κB) p65 proteins expression. Furthermore, treatment of rats with rosiglitazone caused a significant increase of TNBS-induced apoptosis. In summary, rosiglitazone exerts protective effects in chronic experimental colitis. The anti-inflammatory effects seem to be related to impairment of neutrophil function, absence of up-regulation of TNF-α and decrease of nuclear NF-κB p65 expression. Our results also suggest that the activation of the PPARγ pathway reduces COX-2 overexpression, returns the increased PGD_2 values to basal levels and induces a significant increase of TNBS-induced apoptosis. We conclude that rosiglitazone represents a novel approach to the treatment of ulcerative colitis.

Keywords: PPAR-γ; Colitis; COX-2; Prostaglandins; NF-κB; Apoptosis

1. Introduction

One of the earliest factors involved in the pathogenesis of inflammatory bowel disease (IBD) includes the development of an abnormal immune and inflammatory response which is mediated predominantly by activated neutrophils, monocytes and macrophages and characterised by an enhanced formation of reactive oxygen and nitrogen species [1,2].

On the other hand, the role of prostanoids in the intestinal inflammatory process is not completely understood [3,4]. We have recently reported that the increased PGE_2 production during acute colitis is dependent upon the activity of COX-2 [5,6]. Previous reports have suggested that decrease of local PGs is correlated with colonic mucosal inflammation [7–9]. Moreover, recent studies have shown that inhibition of COX-2 activity during the healing process is detrimental, as it has been proven that this isoenzyme also exerts antiinflammatory effects [10,11]. Thus, both COX-1 and -2 are necessary in order to yield an amelioration of the damage [12].

Recently, the peroxisome proliferator-activated receptor PPARγ has been described as a regulator of cellular proliferation, apoptosis and anti-inflammatory response possibly through several signalling pathways [13–19]. Recent studies have shown that TZDs may participate in control of inflammation, specially in regulating the production of immunomodulatory and inflammatory mediators [13,17,20]. PPARγ is highly expressed in the colon, in which epithelial cells and macrophages are thought to be the main cellular sources [21], however its levels are

Abbreviations: COX, cyclooxigenase; IBD, inflammatory bowel disease; UC, ulcerative colitis; MPO, myeloperoxidase; NF-κB, nuclear transcription factor NF-kappa B; PG, prostaglandin; PPARγ, peroxisome proliferator-activated receptor gamma; ROS, rosiglitazone; TNBS, trinitrobenzensulfonic acid; TNF-α, tumour necrosis factor alpha

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decreased during chronic inflammation in humans and animals [22,23]. There are recent reports which document that PPARγ ligands decrease the degree of inflammation associated with acute experimental colitis [20,24–26]. Based upon these data, there is no doubt that PPARγ may play a central role in acute anti-inflammatory responses in colon [23,27]. However, there is no report related to the ligand agonist PPARγ activity, rosiglitazone, on chronic experimental colitis. Thus, the present study was designed to analyze the effects of the TZD, rosiglitazone, on chronic TNBS-induced colitis in rats. The inflammatory response was assessed by histology and MPO activity, as an index of quantitative inflammation and neutrophil infiltration in the mucosa. TNF-α production, histological and histochemical analyses of the lesions were also carried out. In order to gain a better insight into the action mechanism(s) of the observed protective effects of rosiglitazone, the expression of the nuclear transcription factor NF-κB p65, PGE_2 and PGD_2 generation and the expression of COX-1 and -2 by western blotting and immunohistochemistry have been investigated. Finally, since PPARγ agonists have been found to modulate apoptosis in colitis-related colon carcinogenesis [28] our aim was to study its effects in colonic mucosa under chronic inflammatory conditions.

2. Material and methods

2.1. Experimental animals

Male and female Wistar rats supplied by Animal Services, Faculty of Medicine, University of Seville, Spain, and weighing 180–220 g, were placed singly in cages with wire-mesh floors at a controlled room temperature 24–25 °C, humidity 70–75%, lighting regimen of 12L/12D and were fed a normal laboratory diet (Panlab, Barcelona, Spain). Rats were deprived of food for 24 h prior to the induction of colitis, but were allowed free access to tap water throughout. They were randomly assigned to groups of 8–14 animals. The experiments followed a protocol approved by the local animal Ethics Committee and the Local Government. All experiments were in accordance with the recommendations of the European Union regarding animal experimentation (Directive of the European Counsel 86/609/EC).

2.2. Induction of colitis

Colitis was induced according to the procedure described by Morris et al. [29]. Briefly, rats were lightly anesthetized with ether following a 24 h fast, and then a medical-grade polyurethane cannula for enteral feeding (external diameter 2 mm) was inserted into the anus and the tip was advanced to 8 cm proximal to the anus verge. TNBS (Sigma Aldrich-Company Ltd., Spain) dissolved in 50% (v/v) ethanol was instilled into the colon through the cannula 30 mg in a volume of 0.25 ml to induce chronic colitis. Following the instillation of the hapten, the animals were maintained in a head-down position for a few minutes to prevent leakage of the intracolonic instillate. Control groups were separated for comparison with TNBS/ethanol instillation: rats in the sham group received physiological saline instead of the TNBS solution, and the ethanol group received an enema of 0.25 ml 50% (v/v) ethanol. Rosiglitazone (1–5 mg/kg; Glaxo Smithkline, Company Ltd., Spain) was suspended in 0.9% saline solution and administered (p.o.) 24 h after TNBS instillation and daily during the 2 weeks before killing the rats. The animals were sacrificed, using an overdose of anesthetic. The rats were checked daily for behaviour, body weight, and stool consistency.

2.3. Assessment of colitis

Severity of colitis was evaluated by an independent observer who was blinded to the treatment. For each animal, the distal 10 cm portion of the colon was removed and cut longitudinally, slightly cleaned in physiological saline to remove fecal residues and weighed. Macroscopic inflammation scores were assigned based on clinical features of the colon (score 0–10: 0 (no damage), 1 (focal hyperaemia), 2 (ulceration without hyperaemia or bowel wall thickening), 3 (ulceration with inflammation at 1 site), 4 ($\geq$2 sites of ulceration and inflammation), 5 (mayor sites of inflammation $>$1 cm along the organ), 6–10 (mayor sites of inflammation $>$2 cm along the organ)). The presence of adhesions (score 0–2), and/or stool consistency (score 0–1) were evaluated according to the criteria of Bobin-Dubigeon et al. [30]. Pieces of inflammed colon were collected and frozen in liquid nitrogen for measurement of biochemical parameters.

2.4. Histological studies

For examination with the light microscope we used tissue samples from the distal colon of each animal fixed in 4% buffered paraformaldehyde, dehydrated increasing concentrations of ethanol, and embedded in paraffin. Thereafter, sections of tissue were cut at 5 μm on a rotary microtome (Leica Ultracut), mounted on clean glass slides and dried overnight at 37 °C. The sections were cleared, hydrated, and stained with haematoxylin and eosin, Giemsa and Alcian blue for histological evaluation of colonic damage and mucus content, respectively, according to standard protocols, and the slides were coded to prevent observer bias during evaluation. All tissue sections were examined in an Olympus BH-2 microscope for characterization of histopathological changes.

Photographs taken from colon samples were digitized using a Kodak D290 Zoom camera Eastman Kodak Co., U.S.A. and Motic®Images 2000 release 1.1 (MicroOptic Industrial Group CO., Ltd.; B1 Series System Micro-

scopes). Analysis of the figures was carried out by Adobe® Photoshop® Version 6.0 (Adobe Systems) image analysis program.

2.5. Immunohistochemical study

Colonic tissues were fixed in 4% buffered paraformaldehyde, dehydrated through graded concentrations of ethanol, embedded in paraffin, and sectioned. Sections (5 μm thick) were mounted on slides, cleaned, and hydrated. The sections were treated with a buffered blocking solution (3% bovine serum albumin in phosphate-buffered saline (PBS)) for 15 min. Then, the sections were co-incubated with primary antibodies for COX-1 and -2 (Santa Cruz Biotechnology) at a dilution of 1:400 in PBS v/v, at room temperature for 1 and 24 h respectively, followed by washing with PBS and co-incubated with secondary antibody (1:500 in PBS v/v, Santa Cruz Biotechnology), at room temperature for 1 h. Thereafter, sections were washed as before and with Tris–HCl 0.05 M, pH 7.66, and then co-incubated with a 3,3′-diaminobencidine solution in darkness, at room temperature for 10 min. The sections were washed with Tris–HCl, stained with haematoxylin according to standard protocols, mounted with glycerin and observed in an Olympus BH-2 microscope.

2.6. Assessment of leukocyte involvement

Myeloperoxidase (MPO) activity was assessed as an index of neutrophil infiltration according to the methods of Grisham et al. [31]. One sample from the distal colon was obtained from all animals. Samples were excised from each animal and rapidly rinsed with ice-cold saline, blotted dry, and frozen at −70 °C. The tissue was thawed, weighed and homogenized in 10 volumes 50 mM phosphate-buffered saline (PBS), pH = 7.4. The homogenate was centrifuged at 20.000 × *g*, 20 min, 4 °C. The pellet was again homogenized in 10 volumes 50 mM PBS, pH = 6.0, containing 0.5% hexadecyl-trimethylammonium bromide (HETAB) and 10 mM EDTA. This homogenate was subjected to one cycle of freezing/thawing and a brief period of sonication. A sample of homogenate (0.5 μl) was added to a 0.5 ml reaction volume containing 80 mM PBS, pH 5.4, 0.5% HETAB and 1.6 mM 3,3′,5,5′-tetramethylbenzidine (TMB). The mixture was incubated at 37 °C for 5 min and the reaction was started by adding 0.3 mM H_2O_2.

Each tube containing the complete reaction mixture was incubated for exactly 3 min at 37 °C. The reaction was terminated by the sequential addition of catalase (20 μg/ml) and 2 ml 0.2 M sodium acetate, pH = 3.0. The changes in absorbance at 655 nm were measured with a spectrophotometer. One unit myeloperoxidase activity was defined as the amount of enzyme present that produced a change in absorbance of 1.0 U/min at 37 °C in the final reaction volume containing the acetate. Results were quantified as U/mg tissue.

2.7. Production of PGE_2

PGE_2 was determined in colon tissue samples obtained from each group following the protocol established by Martíin et al. [5,6]. Briefly, colonic mucosa was excised and rapidly rinsed with ice-cold saline. The tissue was weighed and homogenized in 6 ml TEAP buffer (pH 3.24) which contained a COX inhibitor, lysine acetyl salicylate (Sigma Aldrich-Company Ltd. Spain). The homogenate was centrifuged (3000 rpm, 10 min, 4 °C) and the supernatant was removed and passed through a reverse-phase octadecylsilica C18 Sep Pak cartridge which was washed with 10 ml distilled water, 10 ml 15% (v/v) ethanol, 10 ml hexane and 10 ml ethylacetate, and the eluate collected. Each fraction was evaporated with ethylacetate, and the dry residue redisolved in ethanol. PGE_2 was determined by a competitive enzyme immunoassay kit (Assay Designs, Inc.). PGE_2 levels were quantified as PGE_2/mg tissue.

2.8. Production of PGD_2

PGD_2 was determined in colon tissue samples obtained from each group following the protocol established by Martín et al. [5,6]. Briefly, colonic mucosa was excised and rapidly rinsed with ice-cold saline. The tissue was weighed and homogenized in TEAP buffer (pH 3.24) which contained a COX inhibitor, lysine acetyl salicylate (Sigma-Aldrich Company Ltd. Spain). The homogenate was centrifuged (3000 rpm, 10 min, 4 °C) and an aliquot of supernatant had to be methoximated due to its chemical instability and rapid degradation. Later the supernatant was removed and passed through a reverse-phase octadecylsilica C18 Sep Pak cartridge which was washed with 10 ml distilled water, 10 ml 15% ethanol, 10 ml hexane and 10 ml ethylacetate, and the eluate collected. Each ethylacetate fraction was evaporated, and the dry residue re-dissolved in buffer. PGD_2 was determined by a competitive enzyme immunoassay kit (Cayman Chemical). Results are expressed as PGD_2/mg tissue.

2.9. TNF-α levels

Distal colon samples were weighed and homogenized, after thawing, in 0.3 ml phosphate buffer saline solution (PBS pH 7.2) at 4 °C. They were centrifuged at 12,000 rpm for 10 min. Mucosal TNF-α level was assayed with a quantitative TNF-α enzyme immunoassay kit (Quantikine® M, R&D Systems). The TNF-α values were expressed as pg/mg tissue.

2.10. Isolation of cytoplasmic and nuclear proteins and western blot assay

Nuclear proteins were isolated by the method of Helenius et al. [32]. Frozen colonic tissues were weighed and homogenized in ice-cold hypotonic buffer (1.5 mM $MgCl_2$,

10 mM KCl, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 1.0 mM dithiothreitol (DTT) and 10 mM Hepes, pH 7.9). Homogenates were incubated for 10 min on ice and centrifuged (25,000 × *g*, 15 min, 4 °C). Cytoplasmic proteins were collected from the supernatants and nuclear proteins from the pellets. These were washed once and centrifuged (10,000 × *g*, 15 min, 4 °C) after which they were suspended in ice-cold low-salt buffer (25% v/v glycerol, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.2 mM PMSF, 1.0 mM DTT, KCl, Hepes, pH 7.9). Nuclear proteins were released by adding a high-salt buffer (25% glycerol, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.2 mM PMSF, 1.0 mM DTT, 1.2 M KCl, 20 mM Hepes, pH 7.9) drop by drop to a final concentration of 0.4 M KCl. Samples were incubated on ice for 30 min, with smooth shaking. Soluble nuclear proteins were recovered by centrifugation (25,000 × *g*, 30 min, 4 °C) and proteins were stored at −80 °C.

Protein concentration of the homogenate was determined following Bradford's colorimetric method. Aliquots of supernatants containing equal amounts of protein (30 μg) were separated on 10% acrilamide gel by sodium dodecyl sulfate-polyacryamide gel electrophoresis. In the next step, the proteins were electrophoretically transferred onto a nitrocellulose membrane and incubated with specific primary antibodies (Santa Cruz Biotechnology, CA) for COX-1 (M-20) at a dilution of 1:2000, COX-2 (M-19) at a dilution of 1:400 and NF-κB p65, at a dilution of 1:200, respectively. Each filter was washed three times for 15 min and incubated with the secondary horseradish peroxidase-linked anti-goat (for COX-1 and -2) or anti-rabbit immunoglobulin G (for NF-κB p65) antibodies (Santa Cruz Biotechnology, CA). To prove equal loading, the blots were analysed for β-actin expression using an anti-β-actin antibody (Santa Cruz Biotechnology, CA). Immunodetection was performed using enhanced chemiluminiscence light-detecting kit (Amersham, Arlinghton Heights, IL). Densitometric data were studied following normalisation to the control (house-keeping gene). The signals were analyzed and quantified by a Scientific Imaging Systems (KODAK 1D Image Analysis Software).

2.11. Apoptosis

Citoplasmic DNA fragments, which are indicators of apoptosis, were measured with a DNA cell death detection ELISA PLUS KIT (Roche Diagnostics) according to the manufacturer's instructions. Results were expressed as absorbance × 10^3/mg protein.

2.12. Statistical evaluation

All values in the figures and text are expressed as arithmetic mean ± standard error of the mean (S.E.M.). The data were evaluated with Graph Pad Prism® Version 2.01 software. The statistical significance of any difference in each parameter among the groups was evaluated by one-way analysis of variance (ANOVA) followed by Tukey test. The Mann–Whitney *U*-test was chosen for non-parametric values. *P*-values of <0.05 were considered statistically significant. In the experiment involving histology or immunohistochemistry, the figures shown are representative of at least six experiments performed on different days.

3. Results

Fourteen days after intracolonic administration of TNBS, the control animals underwent severe anorexia with a marked body weight loss. Colitis gave rise to diarrhoea in the majority of animals. The inflammatory changes of the intestinal tract were associated with a significant increase of weight/length of the rat colon, an indicator of inflammation, and presence of adhesions to adjacent organs. Macroscopic inspection of the colon showed a flaccid appearance and evidence of bowel wall thickening, inflammation and ulcers. Lesions in the distal colon were quantified using a macroscopic damage score (mean: 6.5 ± 0.3) (Fig. 1). Treatment of TNBS-rats with rosiglitazone reduced the loss in body weight and the presence of adhesions to adjacent organs. No significant increase in the weight/length of the rat colon, was observed in TNBS rats, which had been treated with rosiglitazone (Table 1). In addition, the TZD at the doses used 1 and 5 mg/kg p.o., significantly attenuated the extent and severity of the colonic injury (Fig. 1). In fact, rosiglitazone was able to reduce the macroscopic damage score down to 3.62 ± 0.5 ($P < 0.001$) with the highest dose.

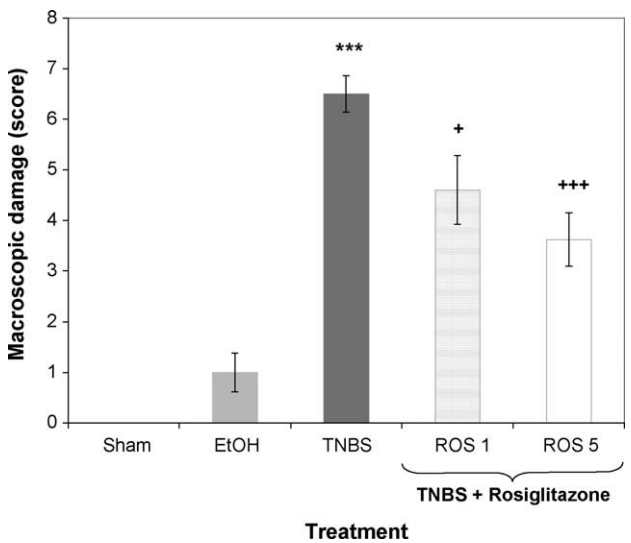

Fig. 1. Effects of chronic administration of rosiglitazone on the colonic damage score. Colonic macroscopic damage resulting from TNBS (30 mg/animal) instilled into rat colon was scored, as indicated in Section 2. Scores were quantified in the absence of treatment, but with daily administration of the vehicle saline solution (sham, ethanol and TNBS groups), or in the presence of rosiglitazone (Ros: 1 and 5 mg/kg/day p.o.). Data are expressed as the mean ± S.E.M. (***) $P < 0.001$ vs. sham, (+) $P < 0.05$ and (+++) $P < 0.001$ vs. TNBS group.

Table 1
Quantified parameters after administration of rosiglitazone (ROS: 1 and 5 mg/kg p.o.) in rats with chronic colitis induced by TNBS intracolonic instillation (30 mg/animal)

Group	*n*	Body weight changes (g)	Adhesions (score 0–2)	Diarrhea (score 0–1)	Colon weight/colon length (g/cm)
Sham	14	96.2 ± 9.39	0	0	0.12 ± 0.08
EtOH	14	82.0 ± 10.1	0.6 ± 0.2[d]	0	0.15 ± 0.02[d]
TNBS	8	47.14 ± 11.8[b]	1.83 ± 0.2[a]	0.86 ± 0.14[b]	0.57 ± 0.11[a]
TNBS + ROS 1	10	83.8 ± 6.4[c]	0.75 ± 0.2[c]	0.12 ± 0.12[c]	0.19 ± 0.03[d]
TNBS + ROS 5	10	97.5 ± 9.5[d]	0.8 ± 0.3[c]	0.17 ± 0.16[c]	0.25 ± 0.02[d]

Colonic parameters were quantified in the sham group ($n = 14$), which received saline instillation. TNBS group ($n = 14$) received TNBS intracolonically in a vehicle of 50% (v/v) ethanol; ethanol group ($n = 14$) received 50% (v/v) ethanol intracolonic injection. Data are expressed as mean ± S.E.M.

[a] $P < 0.001$, significantly different from sham.
[b] $P < 0.01$, significantly different from sham.
[c] $P < 0.05$, significantly different from TNBS.
[d] $P < 0.01$, significantly different from TNBS.

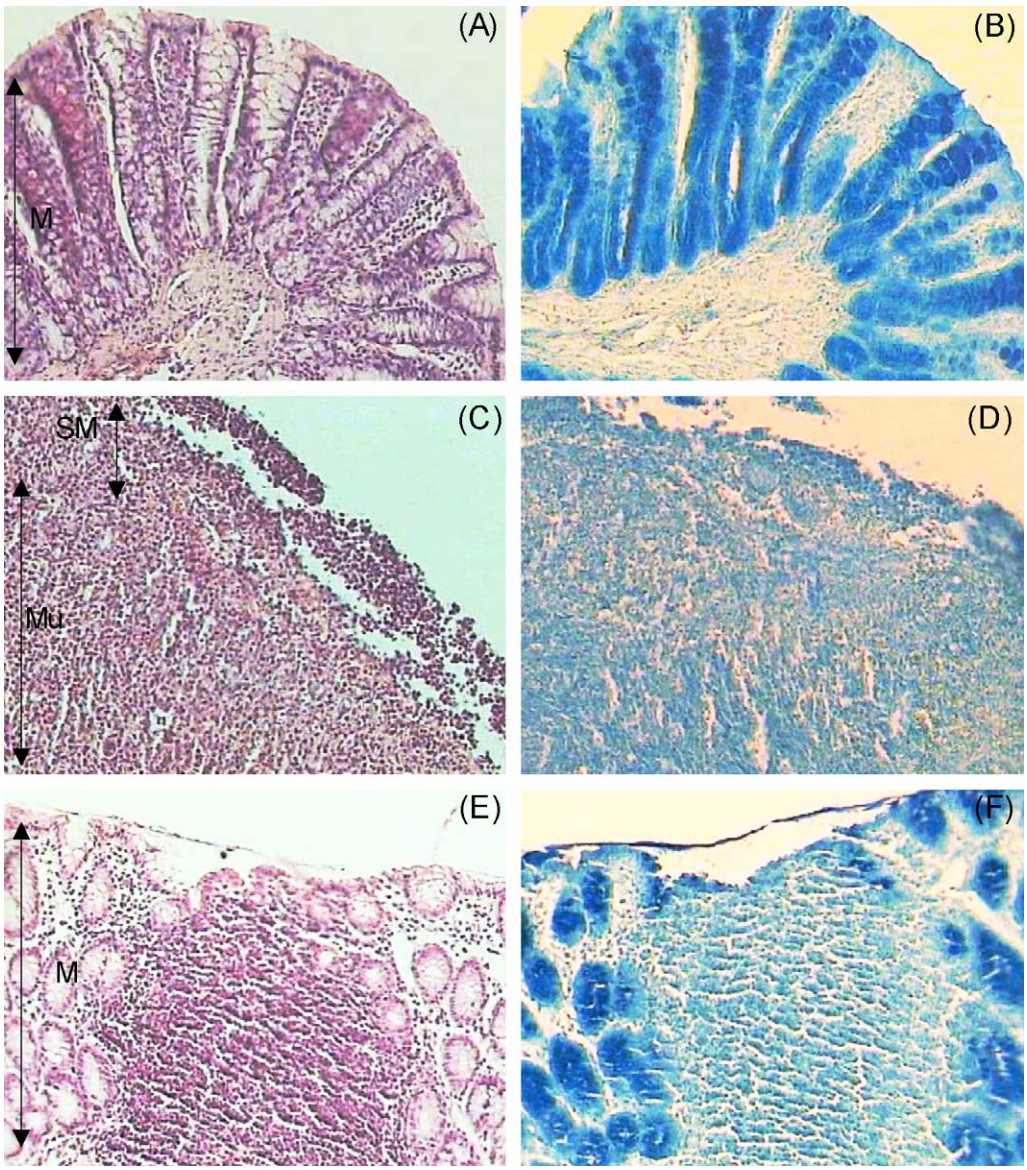

Fig. 2. Chronic colitis model induced by TNBS: effect of rosiglitazone on colon injury. Histological appearance of rat colonic mucosa after hematoxylin and eosin stain (H-E), Alcian blue stain (AB): sham (A and B), and treated with TNBS 30 mg/animal (C and D), and rosiglitazone 5 mg/kg p.o. (E and F). (A and B) No histological modification was present in the sham animals. (C and D) Mucosal injury was produced after TNBS administration, characterized by extensive granulation tissue with the presence of diffuse inflammatory infiltrates in the mucosa and submucosa. (E) Treatment with rosiglitazone 5 mg/kg p.o. reduced the morphological alterations associated with TNBS administration showing ulcers in the process of healing. (F) Some areas showed accumulation of mucus and cell remnants, however, Alcian blue positive-cells were less numerous, and the mucin layer of the epithelium was missing. Original magnification 20×.

On histological examination of the colon from sham-treated rats, the histological features of the colon were typical of a normal structure (Fig. 2A and B). In TNBS-treated rats, the inflammation extended through the mucosa, muscularis mucosae and submucosa. Extensive granulation tissue with the presence of fibroblasts and lymphocytes and diffuse inflammatory infiltrates was apparent. In some sections of ulcerated areas necrotic tissue adjacent to surface cells could be observed. The mucosa adjacent to ulcers showed extensive crypt distortion. Globet cells were totally absent at the surface epithelium (Figs. 2C, D, 3C and D) compared to sham-treated rats (Figs. 2A, B, 3A and B). After administration of rosiglitazone histologically, there was an attenuation of morphological signs of cell damage, the colonic mucosa showed ulcers in the process of healing, evolution to a more chronic inflammatory infiltrate, with mononuclear predominance and initiation of a repair process (Figs. 2E, 3C–E). In regions with reepithelization of the mucosal layer, globet cells with Alcian blue-positive cells (acid glucoproteins such as sialomucins) were clearly visible, whereas in ulcerative areas, an important mucin depletion was observed (Fig. 2F).

As shown in Table 2, a marked increase in MPO activity, an indicator of the infiltration of the colon with polymorphonuclear leukocytes also characterized the colitis caused by TNBS. This result was consistent with the histological findings. Treatment of TNBS-treated rats with the PPARγ agonist significantly ($P < 0.001$) reduced the degree of polymorphonuclear neutrophil infiltration. Colonic injury by TNBS administration was also characterized by an increase of the proinflammatory cytokine TNF-α. In contrast, the levels of this cytokine were significantly lower ($P < 0.05$ and $P < 0.01$) in rats treated with rosiglitazone.

Table 2
Myeloperoxidase activity (MPO, U/mg tissue) and tumour necrosis factor alpha levels (TNF-α, pg/mg tissue) after rosiglitazone (1 and 5 mg/kg p.o., respectively) in rats with chronic colitis produced by TNBS intracolonic instillation (30 mg/kg)

Group	*n*	MPO (U/mg tissue)	*n*	TNF-α (pg/mg tissue)
Sham	14	0.29 ± 0.02	14	350.49 ± 102.68
EtOH	14	0.43 ± 0.03	14	473.69 ± 77.46
TNBS	8	0.71 ± 0.07[a]	8	766.95 ± 155.27[a]
TNBS + ROS 1	10	0.32 ± 0.07[b]	10	367.76 ± 42.33[c]
TNBS + ROS 5	10	0.28 ± 0.04[b]	10	246.03 ± 32.63[d]

Colonic mucosal MPO activity (U/mg tissue) and TNF-α levels (pg/mg tissue) were quantified in the absence of treatment, but with daily administration of the vehicle saline solution (sham, ethanol and TNBS groups), or in the presence of rosiglitazone (1 and 5 mg/kg/day p.o.). Data are expressed as the mean ± S.E.M.

[a] $P < 0.001$ significantly different from sham.
[b] $P < 0.001$ significantly different from TNBS.
[c] $P < 0.05$ significantly different from TNBS.
[d] $P < 0.01$ significantly different from TNBS.

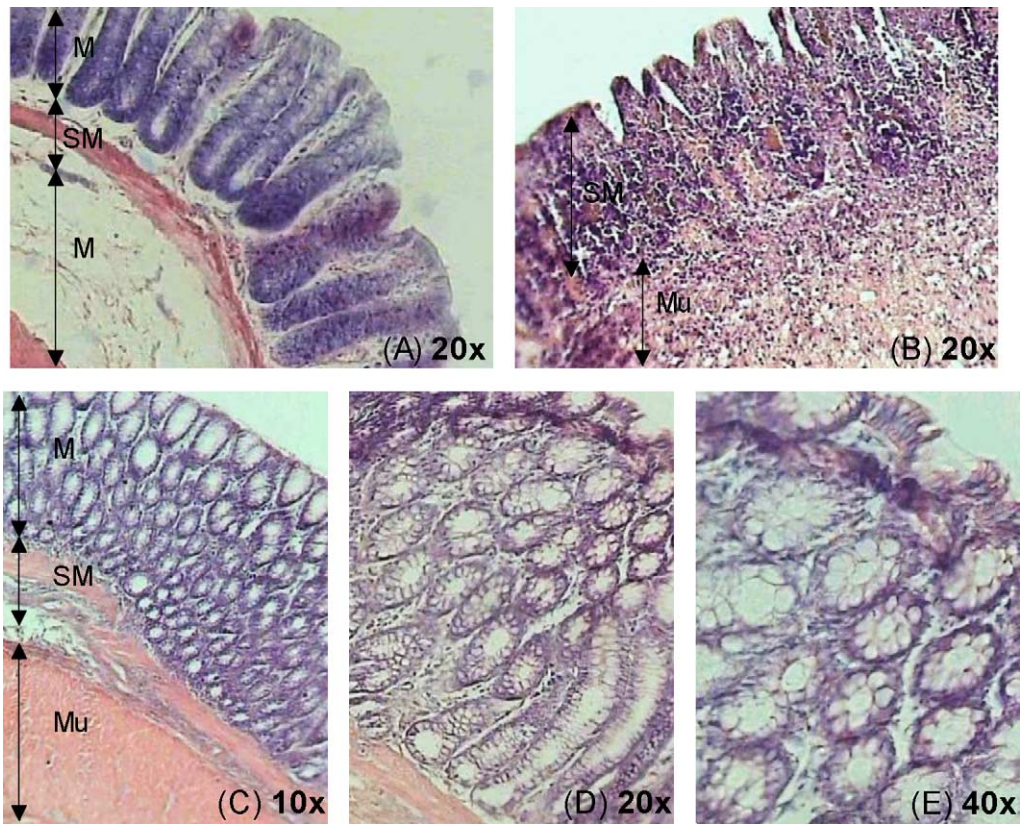

Fig. 3. Rat colon segments stained with Giemsa: sham (A), and treated with TNBS 30 mg/animal (B), and rosiglitazone 5 mg/kg p.o. (C–E). Infiltration of inflammatory cells was highly observed in the colonic mucosa of TNBS-treated animals. Rosiglitazone prevented development of inflammatory changes. Original magnifications 10, 20 and 40×.

The levels of expression of NF-κB p 65 and cyclooxygenases were measured by western blotting of nuclear and cytosolic extracts, respectively, from colonic mucosa. (Fig. 4) NF-κB p65 protein was not detected in nuclei of normal colon mucosa whereas a high expression of nuclear factor appeared in colon mucosa from control TNBS-treated rats. Nonetheless, upon treatment with rosiglitazone, the protein expression of NF-κB p65 was drastically decreased. As shown in this figure, the levels of COX-1 protein remained unchanged in all groups, however rosiglitazone induced upregulation of COX-2 in the treated groups versus TNBS control.

Our data showed that PGE_2 content decreased significantly ($P < 0.001$) in colonic mucosa of TNBS group compared with that of sham animals. In addition, under our experimental conditions, treatment with rosiglitazone (1 and 5 mg/kg p.o.) significantly increased ($P < 0.01$ and $P < 0.001$ versus TNBS, respectively) the rise in the PGE_2 generation (Fig. 5). As shown in this figure there was a significant ($P < 0.05$) increase in the mucosal generation of PGD_2 after TNBS administration. However, treatment with the PPARγ agonist returned the increased PGD_2 values to basal levels.

In normal colons, specific immunosignals for COX-1 were obtained in surface epithelium as well as in the upper half of the crypts. Mononuclear cells of the lamina propria and the regional lymphatic nodules as well as cells of the muscularis mucosae showed COX-1 specific immunosignals (Fig. 6B) in relation with control negative (Fig. 6A). In the basal part of the crypts, COX-1 expression was restricted to individual cells, which according to morphological criteria are endocrine cells, a specialized epithelial cell type of the lower crypt (Fig. 6C). COX-2 specific immunolabelling was occasionally observed in

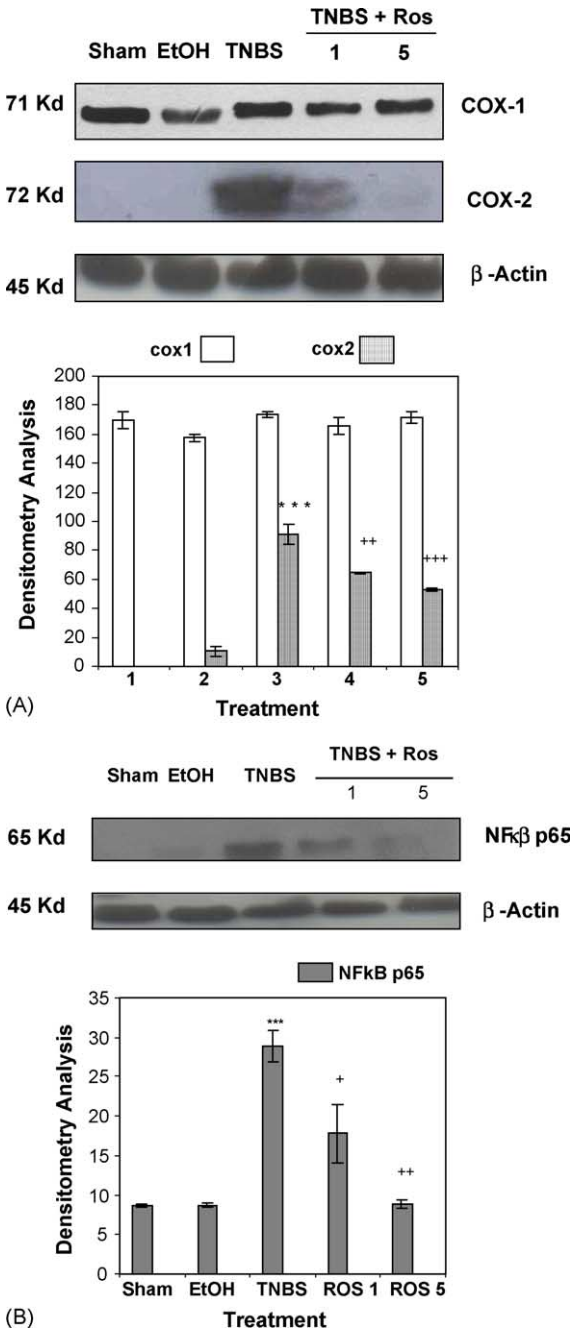

Fig. 4. Representative western blot analysis comparing cytoplasmic (A) and nuclear (B) proteins. COX–1 protein remained unchanged in all groups (sham, TNBS and TNBS + Rosiglitazone (ROS 1 and 5 mg/kg p.o.)), however rosiglitazone induced downregulation of COX-2 in the treated groups vs. TNBS control (A). The protein expression of NF-κB p65 was drastically decreased in TNBS+ rosiglitazone (ROS 1 and 5 mg/kg) groups p.o. Densitometric data were studied following normalisation to the control (house-keeping gene). The results are representative of three experiments performed on different samples and data are expressed as the mean ± S.E.M. (***) $P < 0.001$ vs. sham. (+) $P < 0.05$, (++) $P < 0.01$ and (+++) $P < 0.001$ vs. TNBS group.

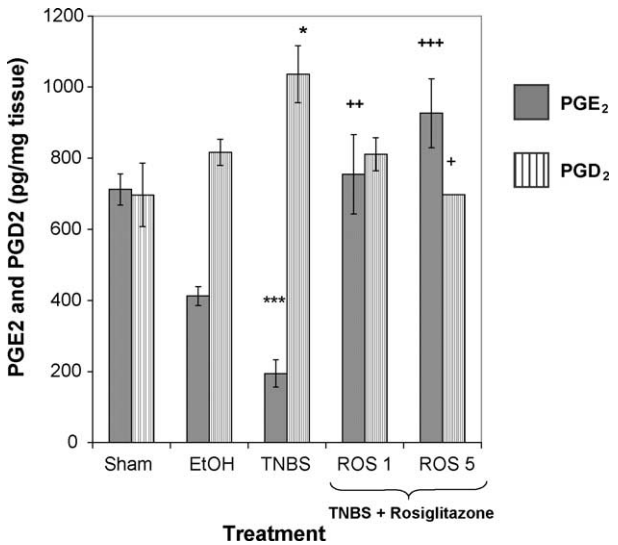

Fig. 5. Prostaglandin E_2 (PGE_2, pg/mg tissue) and Prostaglandin D_2 (PGD_2, pg/mg tissue) after rosiglitazone administration (1 and 5 mg/kg p.o.) in rats with chronic colitis produced by TNBS intracolonic instillation (30 mg/animal). Prostanoids synthesis in the colonic tissue was quantified in the absence of treatment, but with daily administration of the vehicle saline solution (sham, ethanol and TNBS groups), or in the presence of rosiglitazone (1 and 5 mg/kg/day p.o.). Data are expressed as the mean ± S.E.M. (*) $P < 0.05$ and (***) $P < 0.001$ vs. sham. (+) $P < 0.05$, (++) $P < 0.01$ and (+++) $P < 0.001$ vs. TNBS.

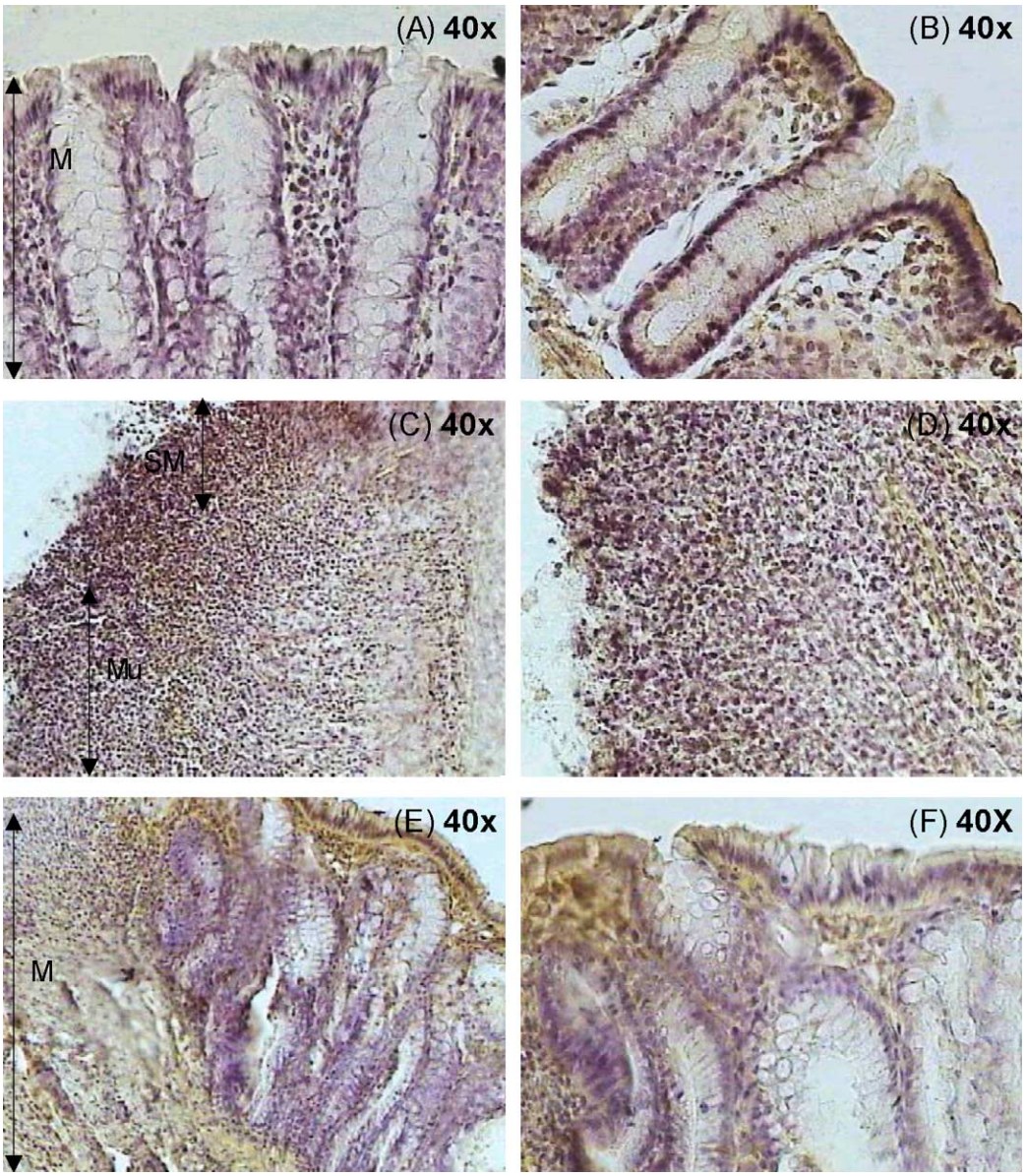

Fig. 6. Immunohistochemical localization of COX-1 isoenzyme in sections of colon. Negative control (A). In normal colon, colonocytes of the upper half of the crypts were found to be COX-1-positive (B). COX-1 immunosignal was weakly observed in the colon of TNBS-control rats (C and D). COX-1 expression of inflamed colon treated with rosiglitazone 5 mg/kg p.o. (E and F) reflected no important changes in relation to sham group. Original magnifications 20 and 40×.

colonocytes of the normal surface epithelium of matched control colon and mononuclear cells of lamina propria, as shown in Fig. 7A.

In animals treated with TNBS and vehicle, COX-1 inmmunosignal was weakly observed in surface epithelium cells and in the granulation tissue of mucosa (Fig. 6C and D), whereas prominent COX-2 expression was found in cells of surface epithelium and in cells of the inflammatory infiltrate (Fig. 7C and D). On the contrary, COX-2 was scarcely found in mucosa of the sham group (Fig. 7B). Compared with normal colon, COX-1 expression reflected no important differences in the cellular localization and the degree of positive staining for COX-1 in colon mucosa from rosiglitazone-treated rats after 14 days (Fig. 6E and F). At this time, rosiglitazone-treated rats showed a lower level of expression of the inducible isoform in apical epithelial cells of inflamed colon (Fig. 7E and F).

Since PPARγ agonists have been found to modulate apoptosis, we wished to know what their effects in colonic mucosa were under chronic inflammatory conditions by an ELISA that specifically detected cytoplasmic histone-associated DNA fragments, mononucleosomes, and oligonucleosomes. As shown in Fig. 8 apoptosis was observed in the colonic mucosa of sham animals. DNA fragmentation was dramatically increased in TNBS-treated rats. Furthermore, treatment of rats with the PPARγ agonist (1 and 5 mg/kg p.o.) caused a significant ($P < 0.05$ and $P < 0.001$, respectively) increase of TNBS-induced apoptosis.

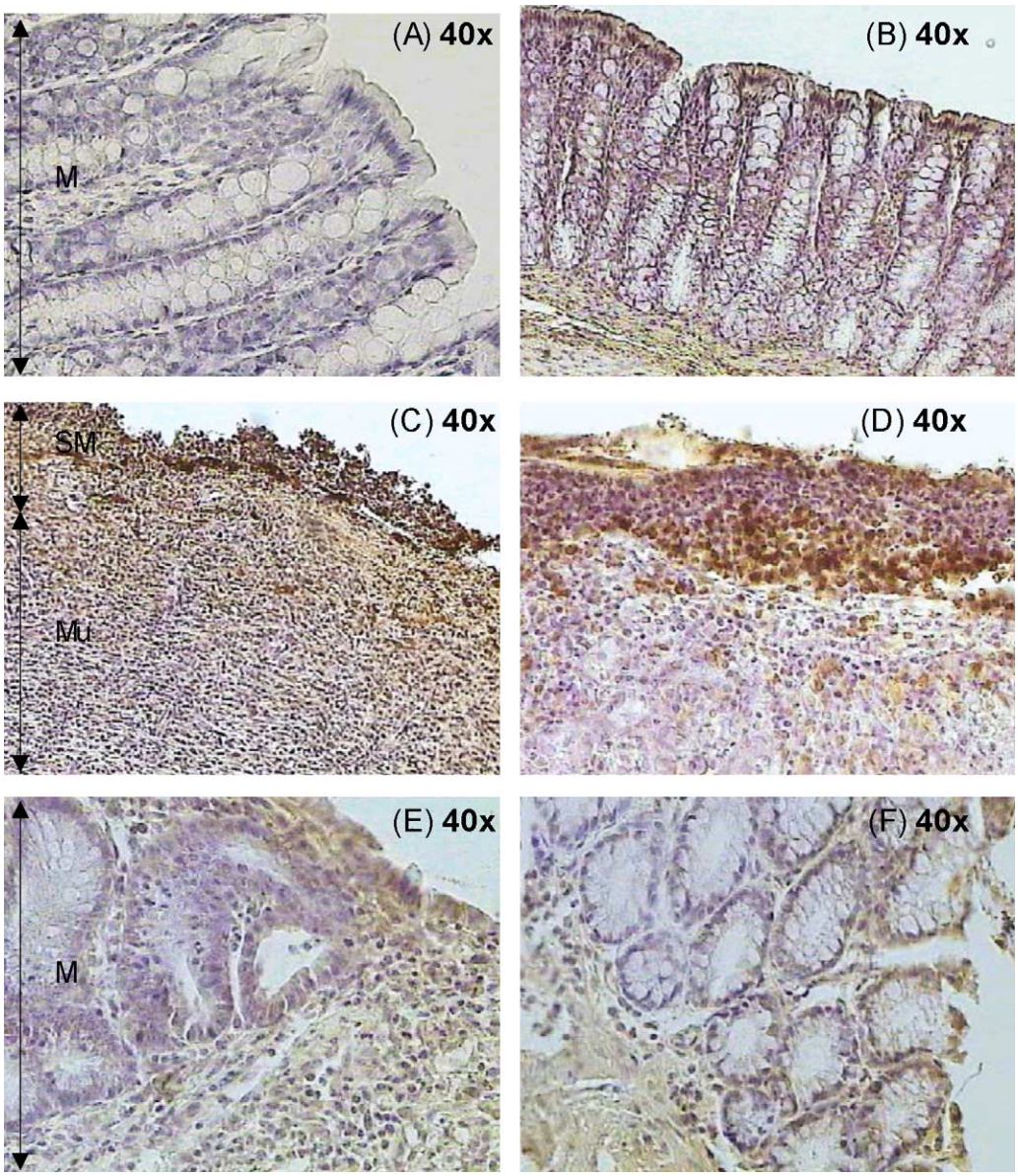

Fig. 7. Immunohistochemical localization of COX-2 isoenzyme in sections of colon. Negative control (A). COX-2 expression in normal colonic mucosa (B). COX-2 is strongly expressed in the colon of TNBS-control rats (C and D). COX-2 expression was decreased in apical epithelial cells of inflamed colon treated with rosiglitazone 5 mg/kg p.o. (E and F). Original magnifications 20 and 40×.

4. Discussion

The results of the present study indicate that rosiglitazone at the doses of 1 and 5 mg/kg p.o. reduced the severity and extension of chronic colonic damage induced by TNBS. The decrease in the extent of colitis was accompanied by a lower incidence of diarrhea, weight loss and a decrease in the incidence of adhesions. These beneficial effects seem not to be dose-dependent. Moreover, there was an attenuation of morphological signs of cell damage, the colonic mucosa showed ulcers in the healing process, evolution to a more chronic inflammatory infiltrate, with mononuclear predominance and initiation of a repair process.

Rosiglitazone also increased the amount of mucus stained by Alcian blue in colon mucosa. The protective effect of mucus as an active barrier may be largely attributed to its viscous and gel-forming properties which are derived from mucin glycoprotein constituents. Alcian blue-positive cells seem to be associated with regenerative mucosa processes [33] while reduction in the amount stained has been related to decreased mucosa resistance and paralleled by alterations in the normal maturation pattern of globbet cells mucine [34].

This study also shows that MPO activity and the proinflammatory cytokine TNF-α production were increased in colonic mucosa after 14 days of TNBS instillation. However, pretreatment with rosiglitazone significantly reduced

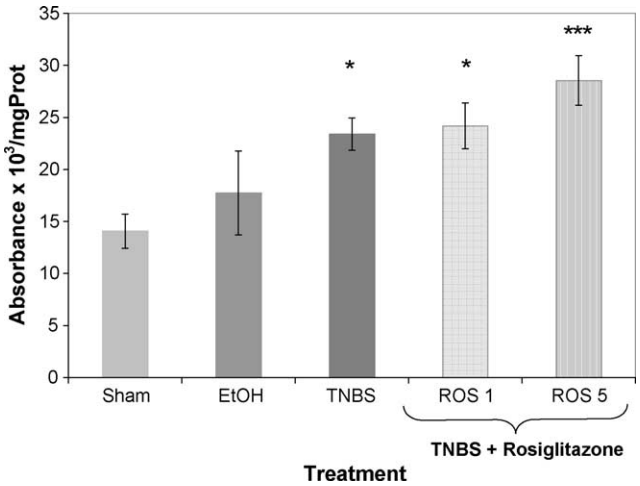

Fig. 8. Apoptosis observed in the colonic mucosa after chronic colitis induced by TNBS (30 mg/animal). Apoptosis was quantified in the absence of treatment, but with daily administration of the vehicle saline solution (sham, ethanol and TNBS groups), or in the presence of rosiglitazone (1 and 5 mg/kg/day p.o.). Data are expressed as the mean ± S.E.M. (*) $P < 0.05$ and (***) $P < 0.001$ vs. sham.

neutrophil infiltration and inhibited the cytokine production.

Leukocytes infiltration into the mucosa has been suggested to contribute significantly to tissue necrosis and mucosal dysfunction associated with colitis as they represent a major source of reactive oxygen and nitrogen species in the inflamed colonic mucosa [5,6]. Reactive oxygen species and peroxynitrite induce cellular injury and necrosis via several mechanisms including lipidic peroxidation, protein denaturation and DNA damage. Activated neutrophils produce superoxide anion, through NADPH oxidase, which reduces molecular oxygen to the superoxide anion radical, and through the enzyme myeloperoxidase which produces neutrophils which can also release proteases, lactoferrin and lipid mediators that can contribute to gastric injury [7a,b].

It has been suggested that the main chemoattractants for neutrophils are proinflammatory cytokines, such as IL-1β, interferon-γ and TNF-α that regulate endothelial molecule expression (ICAM-1) on vascular endothelial cells and promotes neutrophil adherence to these. Reports from other studies [7b,35] indicated that TNF-α production plays an important role in TNBS-induced chronic colitis, as in our findings. Interestingly, the levels of this proinflammatory cytokine were significantly lower in animals which were treated with rosiglitazone.

In our study, chronic inflammatory conditions were accompanied by the presence in the nuclear extracts of detectable quantities of NF-κB p65 whereas the nuclear protein expression of NF-κB p65 was drastically decreased upon treatment with rosiglitazone to rats. The p65 subunit activation has significance in IBD because it is highly activated in the mucosal biopsy specimens of patients with ulcerative colitis and Crohn's disease [36]. The p65 antisense oligonucleotide treatment also aborts chronic intestinal inflammation in a murine model of inflammation [35].

NF-κB has been shown to activate, via transcription the genes encoding pro-inflammatory cytokines (TNF-α, IL-1β, IL-12, and IL-6) in different cell-types, the expression of enzymes (e.g. inducible nitric oxide synthase and COX-2) and monocyte-chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-1), and intracellular adhesion molecule (ICAM) interfering with leukocyte chemo-attraction and cellular adhesion to endothelial cells [17].

Although the exact action mechanisms by which PPARγ agonists exert their anti-inflammatory effects in the colonic mucosa remain unclear, some authors have suggested that PPARγ activation seems to interfere with the activities of stress kinases, signal transducers and activators of transcription (STAT), activating protein 1 (AP-1), and the nuclear factor of activated T-cells (NFAT), all of which regulate cytokine gene expression [15]. PPARγ activation also results in the NF-κB nuclear transcription factor repression signalling by various proposed mechanisms such as PPARγ interaction with either p65 or p50 subunits or both together, inhibition of IκB protein inhibitory degradation, thus preventing the nuclear translocation of NF-κB [37–39], or interaction of PPARγ with cyclic AMP response element binding protein, a coactivator interacting with p65 [17]. Nagy et al. [40] have also suggested that PPARγ may suppress NF-κB activity via competition for co-activators and co-receptors, because both use many of the same co-factors and compete for them to initiate gene regulation [15]. Thus, it is possible that a mechanism underlying the protective effects of rosiglitazone involves a reduction of neutrophil infiltration into the colonic mucosa, possibly via inhibition of TNF-α production and NF-κB activation, although further investigation is needed to confirm this.

We have also demonstrated that (1) a decrease in PGE_2 colonic levels and an increase in PGD_2 production are associated with macroscopic damage; (2) COX-2 expression is increased in colon 14 days after the induction of colitis; rosiglitazone treatment increased PGE_2 production, returned PGD_2 to basal levels and reduced COX-2 protein expression.

These results are consistent with the observation of Allgayer et al. [41] and Tessner et al. [9] who demonstrated that exogenously applied PGE_2 attenuated experimental colitis and reduced the inflammatory response suggesting that endogenous PGE_2 is the mediator of mucosal protection. PGE_2 exerts anti-inflammatory effects including suppression of neutrophil function or prevention of mast cell degranulation [11] as well as suppressor T-cell induction and inhibition of the production and release of proinflammatory cytokines such as TNF-α, IL-1β and IL-12 by macrophages and neutrophils [42]. In that study, the decrease of PGE_2 in the inflamed colon is consistent with a recent observation that PGE_2 in cecal tissues of TNBS-

treated rats increases at 1 week post-TNBS, but returns to basal values by 2 weeks post-TNBS [43].

Importantly, several different reports have shown that PGD_2 plays an important role in the resolution of acute colonic inflammation in rats, possibly by downregulating neutrophil infiltration into the mucosa. It has further been suggested that this prostanoid is primarily derived from COX-2 [10]. Our results are also in accordance with Zamuner et al. [43] who observed increases in PGD_2 synthesis and COX-2 expression in relation to basal levels during the healing period of colonic injury.

Likewise, an important reduction of COX-2 expression in rosiglitazone-TNBS-treated animals was shown. PPARγ has been implicated in inhibition of the COX-2 expression in fetal hepatocytes [44] and macrophage-like differentiated U937 cells [45], and although the underlying mechanism of this suppressing action is still a matter of controversy, one might possibly be mechanism by preventing the NF-κB transactivation [46]. We suggest that the decrease of NF-κB p65 nuclear expression by rosiglitazone could explain the reduced COX-2 protein expression and PGD_2 production.

Previous studies have shown significant apoptosis in colonic epithelial cells during mild inflammation induced by dextran sulphate sodium [47] and TNBS-induced colitis [48]. These findings agree with the present study, in which colonic cell death was associated with apoptosis in the colon lesion 14 days after intracolonic administration of TNBS. In addition, treatment of rats with rosiglitazone caused a significant increase of TNBS-induced apoptosis. An increase in apoptosis linked to PPARγ agonists has already been documented in in vitro studies, for instance, human monocyte-derived macrophages [49] and various cell lines including HT-29 human colon cancer cells [50,51].

It is important to note that COX-2 overexpression plays important roles in cell adhesion, angiogenesis and apoptosis. Yang and Frucht [50] showed that activation of PPAR pathway by ciglitazone induced apoptosis and inhibition of COX-2 expression in human colon cells HT-29. Similar data were reported by Li et al. [52]. Furthermore, it is well documented that NF-κB plays a pivotal role in regulating programmed cell death, being able to activate both pro- and anti-apoptotic genes. Taking these data together, we suggest that induction of apoptosis by rosiglitazone could be mediated through down-regulation of COX-2.

In summary, we have shown that rosiglitazone, a PPARγ agonist, exerts protective effects in chronic experimental colitis. The anti-inflammatory effects seem to be related to impairment of neutrophil function, absence of up-regulation of TNF-α and decrease of nuclear NF-κB p65 expression. Our results also suggest that activation of the PPARγ pathway reduces COX-2 overexpression, returns the increased PGD_2 values to basal levels and induces a significant increase of TNBS-induced apoptosis. We conclude that rosiglitazone represents a novel approach to UC treatment.

References

[1] Van Assche G, Rutgeerts P. Antiadhesion molecule therapy in inflammatory bowel disease. Inflamm Bowel Dis 2002;8:291–300.

[2] Sandborn WJ, Yednock TA. Novel approaches to treating inflammatory bowel disease: targeting alpha-4 integrin. Am J Gastroenterol 2003;98:2372–82.

[3] Crofford LJ. COX-1 and COX-2 expression: Implications and predictions. J Rheumatol 1997;24:15–9.

[4] Warner TD, Mitchell JA. Cyclooxygenases: new forms, new inhibitors, and lesson from the clinic. Faseb J 2004;18:790–804.

[5] Martín AR, Villegas I, La Casa C, Alarcon De la Lastra C. The cyclooxygenase-2 inhibitor, rofecoxib, attenuates mucosal damage due to colitis induced by trinitrobenzene sulphonic acid in rats. Eur J Pharmacol 2003;481:281–91.

[6] Martín AR, Villegas I, La Casa C, Alarcon De La Lastra C. Resveratrol, a polyphenol found in grapes, suppresses oxidative damage and stimulates apoptosis during early colonic inflammation in rats. Biochem Pharmacol 2004;67:1399–410.

[7] (a) Villegas I, Alarcon De la Lastra C, Orjales A, La Casa C. A new flavonoid derivative, dosmalfate, attenuates the development of dextran sulphate sodium-induced colitis in mice. Int Immunopharmacol 2003;3:1731–41;
(b) Villegas I, Alarcon De la Lastra C, et al. A new flavonoid derivative, dosmalfate, attenuates the development of dextran sulphate sodium-induced colitis in mice. Int Immunopharmacol 2003;24:209–18.

[8] Fedorak RN, Empey LR, MacArthur C, Jewell LD. Misoprostol provides a colonic mucosal protective effect during acetic acid-induced colitis in rats. Gastroenterology 1990;98:615–25.

[9] Tessner TG, Schloemann S, Cohn SM, Stenson WF. Prostaglandins prevent decreased epithelial cell proliferation associated with dextran sodium sulfate injury in mice. Gastroenterology 1998;115:874–82.

[10] Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA. Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med 1999;5:698–701.

[11] Ajuebor MN, Singh A, Wallace JL. Cyclooxygenase-2-derived prostaglandin D(2) is an early anti-inflammatory signal in experimental colitis. Am J Physiol Gastrointest Liver Physiol 2000;279:G238–44.

[12] Berenguer B, Alarcón de la Lastra C, Moreno FJ, Martín MaJ. Chronic gastric ulcer healing in rats subjected to selective and non-selective cyclooxygenase-2 inhibitors. Eur J Pharmacol 2002;3:125–35.

[13] Cabrero A, Laguna JC, Vazquez M. Peroxisome proliferator-activated receptors and the control of inflammation. Curr Drug Targets Inflamm Allergy 2002;3:243–8.

[14] Na HK, Surh YJ. Peroxisome proliferator-activated receptor gamma (PPARgamma) ligands as bifunctional regulators of cell proliferation. Biochem Pharmacol 2003;66:1381–91.

[15] Alarcón de la Lastra C, Sánchez-Fidalgo S, Villegas I, Motilva V. New pharmacological perspectives and therapeutic potential of PPARγ agonists. Curr Pharm Design 2004;10(28):3505–24.

[16] Pershadsingh HA. Peroxisome proliferator-activated receptor-gamma: therapeutic target for diseases beyond diabetes: quo vadis? Expert Opin Investig Drugs 2004;13:215–28.

[17] Dubuquoy L, Dharancy S, Nutten S, Pettersson S, Auwerx J, Desreumaux P. Role of peroxisome proliferator-activated receptor gamma and retinoid X receptor heterodimer in hepatogastroenterological diseases. Lancet 2002;2:1410–8.

[18] Dubois M, Vantyghem MC, Schoonjans K, Pattou F. Thiazolidinediones in type 2 diabetes Role of peroxisome proliferator-activated receptor gamma (PPARgamma)]. Ann Endocrinol 2002;63:511–23.

[19] Adamson DJ, Frew D, Tatoud R, Wolf CR, Palmer CN. Diclofenac antagonizes peroxisome proliferator-activated receptor-gamma signaling. Mol Pharmacol 2002;61:7–12.

[20] Desreumaux P, Dubuquoy L, Nutten S, Peuchmauer M, Englaro W, Schoonjans K, et al. Attenuation of colon inflammation through

activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor γ (PPARγ) heterodimer: a basis for new therapeutic strategies. J Exp Med 2001;193:827–38.

[21] Lefebvre M, Paulweber B, Fajas L, Woods J, McCrary C, Colombel JF, et al. Peroxisome proliferator-activated receptor gamma is induced during differentiation of colon epithelium cells. J Endocrinol 1999; 162:331–40.

[22] Katayama K, Wada K, Nakajima A, Mizuguchi H, Hayakawa T, Nakagawa S, et al. A novel PPAR gamma gene therapy to control inflammation associated with inflammatory bowel disease in a murine model. Gastroenterology 2003;124:1315–24.

[23] Dubuquoy L, Jansson EA, Deeb S, Rakotobe S, Karoui M, Colombel JF, et al. Impaired expression of peroxisome proliferator-activated receptor gamma in ulcerative colitis. Gastroenterology 2003;124: 1265–76.

[24] Cuzzocrea S, Ianaro A, Wayman NS, Mazzon E, Pisano B, Dugo L, et al. The cyclopentenone prostaglandin 15-deoxy-delta(12,14)-PGJ2 attenuates the development of colon injury caused by dinitrobenzene sulphonic acid in the rat. Br J Pharmacol 2003;8:8–88.

[25] Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, et al. A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. J Clin Invest 1999;104:383–9.

[26] Takagi T, Naito Y, Tomatsuri N, Handa O, Ichikawa H, Yoshida N, et al. Pioglitazone, a PPAR-gamma ligand, provides protection from dextran sulfate sodium-induced colitis in mice in association with inhibition of the NF-kappaB-cytokine cascade. Redox Rep 2002;7:283–9.

[27] Saubermann LJ, Nakajima A, Wada K, Zhao S, Terauchi Y, Kadowaki T, et al. Peroxisome proliferator-activated receptor gamma agonist ligands stimulate a Th2 cytokine response and prevent acute colitis. Inflamm Bowel Dis 2002;8:330–9.

[28] Kohno H, Yoshitani S, Takashima S, Okumura A, Hosokawa M, Yamaguchi N, et al. a ligand for peroxisome proliferator-activated receptor gamma, inhibits chemically-induced aberrant crypt foci in rats. Jpn J Cancer Res 2001;92:396–403.

[29] Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 1989;96(3): 795–803.

[30] Bobin-Dubigeon C, Collin X, Grimaud N, Robert JM, Le Baut G, Petit JY. Effects of tumour necrosis factor-α synthesis inhibitors on rat trinitrobenzene sulphonic acid-induced chronic colitis. Eur J Pharmacol 2001;421:103–10.

[31] Grisham MB, Beniot JN, Granger DN. Assessment of leukocyte involvement during ischemia and reperfusion of intestine. Methods Enzymol 1990;186:729–42.

[32] Helenius M, Hanninen M, Lehtinek SK, Salminen A. Changes associate with aging and replicative senescence in the regulation of transcription factor nuclear factor-κB. Biochem J 1996;318: 603–8.

[33] Alarcón de la Lastra C, Martín MJ, Motilva V. Antiulcer and gastroprotective effect of quercetin: a gross and histologic study. Pharmacology 1994;48:56–62.

[34] Torres MI, García-Martín M, Fernández MI, Nieto N, Gil A, Ríos A. Experimental colitis induced by trinitrobenzenesulfonic acid. An ultrastructural and histochemical study. Dig Dis Sci 1999;44: 2523–9.

[35] Lawrance IC, Wu F, Leite AZ, Willis J, West GA, Fiocchi C, et al. A murine model of chronic inflammation-induced intestinal fibrosis down-regulated by antisense NF-kappa B. Gastroenterology 2003;125: 1750–61.

[36] Jobin C, Sartor RB. NF-kappaB signaling proteins as therapeutic targets for inflammatory bowel diseases. Inflamm Bowel Dis 2000;6:206–13.

[37] Chung SW, Kang BY, Kim SH, Pak YK, Cho D, Trinchieri G, et al. Oxidized low density lipoprotein inhibits interleukin-12 production in lipopolysaccharide-activated mouse macrophages via direct interactions between peroxisome proliferator-activated receptor-γ and nuclear factor-κ B. J Biol Chem 2000;275:32681–7.

[38] Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, et al. Anti-inflammatory cyclopentene prostaglandins are direct inhibitors of IκB kinase. Nature 2000;403:103–8.

[39] Castrillo A, Diaz-Guerra MJ, Hortelano S, Martin-Sanz P, Bosca L. Inhibition of IκB kinase and IκB phosphorylation by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 in activated murine macrophages. Mol Cell Biol 2000;20:1692–8.

[40] Nagy L, Lao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, et al. Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell 1997;89:373–80.

[41] Allgayer H, Deschryver K, Stenson WF. Treatment with 16,16′-dimethyl prostaglandin E_2 before and after induction of colitis with trinitrobenzenesulfonic acid in rats decreases inflammation. Gastroenterology 1989;96:1290–300.

[42] Kabashima K, Saji T, Murata T, Nagamachi M, Matsuoka T, Segui E, et al. The prostaglandin receptor EP4 supresses colitis, mucosal damage and CD4 cell activation in the gut. J Invest 2002;109:883–93.

[43] Zamuner SR, Warrier N, Bureo AG, MacNaughton WK, Wallace JL. Cyclooxygenase 2 mediates post-inflammatory colonic secretory and barrier dysfunction. Gut 2003;52:1714–20.

[44] Callejas NA, Catrillo A, Bosca L, Martín-Sanz PJ. Inhibition of prostaglandin synthesis up-regulates cyclooxygenase-2 induced by lipopolysaccharide and peroxisomal proliferators. Pharmacol Exp Ther 1999;288:1235–41.

[45] Inoue H, Tanabe T, Umeson K. Feedback control of cyclooxygenase-2 expression through PPARγ. J Biol Chem 2000;275:28028–32.

[46] Subbaramaiah K, Lin DT, Hart JC, Dannenberg AJ. Peroxisome proliferator-activated receptor γ ligands suppress the transcriptional activation of cyclooxygenase-2: evidence for involvement of activator protein-1 and CREB-binding protein/p300. J Biol Chem 2001;276: 12440–8.

[47] Tardieu D, Jaeg JP, Deloly A, Corpet DE, Cadet J, Petit CR. The COX-2 inhibitor nimesulide suppresses superoxide and 8-hydroxy-deoxyguanosine formation, and stimulates apoptosis in mucosa during early colonic inflammation in rats. Carcinogenesis 2000;21:973–6.

[48] Yue G, Lai PS, Yin K, Sun FF, Nagele RG, Liu X, et al. Colon epithelial cell death in 2,4 6-trinitrobenzenesulfonic acid-induced colitis is associated with increased inducible nitric-oxide synthase expression and peroxynitrite production. J Pharmacol Exp Ther 2001;297:915–25;
Colon epithelial cell death in 2,4,6-trinitrobenzenesulfonic acid-induced colitis is associated with increased inducible nitric-oxide synthase expression and peroxynitrite production. J Pharmacol Exp Ther 2001;21:973–6.

[49] Chinetti G, Griglio S, Antonucci M, Torra IP, Delerive P, Majd Z, et al. Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages. J Biol Chem 1998;273:25573–80.

[50] Yang WL, Frucht H. Activation of the PPAR pathway induces apoptosis and COX-2 inhibition in HT-29 human colon cancer cells. Carcinogenesis 2001;22:1379–83.

[51] Chen GG, Lee JF, Wang SH, Chan UP, Ip PC, Au WY. Apoptosis induced by activation of peroxisome-proliferator activated receptor-gamma is associated with Bcl-2 and NF-kappaB in human colon cancer. Life Sci 2002;70:2631–46.

[52] Li MY, Deng H, Zhao JM, Dai D, Tan XY. PPARgamma pathway activation results in apoptosis and COX-2 inhibition in HepG2 cells. World J Gastroenterol 2003;9:1220–6.