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Activated and inactivated PPARs-γ modulate experimentally induced colitis in rats

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Krzysztof Celiński[1 ABCDEF], Tomasz Dworzański[1 ABCDEF], Agnieszka Korolczuk[2 ABDF], Maria Słomka[1 BDF], Sebastian Radej[3 BDF], Halina Cichoż-Lach[1 BCDF], Agnieszka Mądro[1 BCDF]

[1] Department of Gastroenterology, Medical University of Lublin, Lublin, Poland
[2] Department of Pathomorphology, Medical University of Lublin, Lublin, Poland
[3] Department of Clinical Immunology, Medical University of Lublin, Lublin, Poland

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Summary

Background: This study sought to define the mechanism by which PPAR-γ ligands affect the course of experimentally induced colitis in rats.

Material/Methods: Inflammation was induced in Wistar rats by a single rectal administration of 2,4,6,-trinitrobenzene sulfonic acid (TNBS). The antagonist of PPARγ antagonist, bisphenol A diglycidyl ether (BADGE), was administrated intraperitoneally 120 mg/kg 4 times every other day. Rosiglitazone 8 mg/kg was administrated by gastric tube 4 times. Body weight was measured daily. After killing, the large intestinal tissue was weighed and collected for histopathologic and immunoenzymatic tests. Levels of IL-6, IL-10, and myeloperoxidase (MPO) were determined in serum and in intestinal homogenates.

Results: Rats receiving rosiglitazone had higher body weight, whereas large intestine weight/length ratio was lower; histology showed fewer inflammatory markers. Rats receiving TNBS and TNBS along with BADGE had more intensive inflammatory changes. Rosiglitazone alone decreased expression of IL-6; used with TNBS it decreased expression of MPO in intestinal tissue, yet did not increase the expression of IL-10. Decreased levels of MPO indicate reduced neutrophil-dependent immune response. The antagonist of PPAR-γ increased IL-6 in serum and decreased IL-10 in intestinal homogenates. Bisphenol A diglycidyl ether administrated to healthy animals increases serum IL-6 levels.

Conclusions: Rosiglitazone inhibits experimental inflammation; administration of its selective antagonist abolishes this protective influence. Rosiglitazone inhibits expression of proinflammatory IL-6 and does not affect IL-10. Agonists of PPARs-γ are possibilities for inflammatory bowel disease prevention. Exogenous substances blocking PPARs-γ may contribute to development or relapse of nonspecific inflammatory bowel diseases.

key words: PPAR-γ • rosiglitazone • BADGE • IL-6 • IL-10

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Author's address: Krzysztof Celiński, Jaczewskiego 8 Str., 20-954 Lublin, Poland, e-mail: celinski@mp.pl

Background

Peroxisome proliferator-activated receptors (PPARs) belong to the group of nuclear steroid receptors acting as transcription factors, which regulate the expression of genes. Recent studies have shown a wide spectrum of PPAR action [1–5]. They have been demonstrated to be involved in differentiation, proliferation, and programmed death of adipocytes [6–8], metabolism of lipids [9–12], regulation of insulin sensitivity, and conversion of monocytes into macrophages [13–15]. Moreover, PPARs play a crucial role in the development of immune response, mainly by affecting the inflammatory reaction [16–19]. The highest concentration of PPAR-γ protein is observed in the large intestine [20]; lower concentrations are found in the kidneys, liver, small intestine, and bone marrow [21]. The PPAR-γ protein is also found in immune system structures such as red and white pulp of the spleen [22], Peyer patches [22], T lymphocytes, monocytes [23–25], macrophages [26–29], hematopoietic stem cells of bone marrow [13], and numerous accessory cells [29]. Recently, the relevance of these receptors in alimentary diseases has been suggested in numerous reports [2,3,5,9,16,17].

The exact mechanism of action of PPARs-γ remains unknown. They may affect the inflammation process via the regulation of transcription of genes responsible for encoding cytokines involved in interactions between cells, adhesive factors, and other inflammatory mediators. Moreover, activated PPARs-γ likely block expression of iNOS, metalloproteinase, or scavenger receptor A (SR-A) genes, thus inhibiting synthesis of mRNA of the aforementioned factors. Most likely, PPAR-γ inhibits expression of these genes by blocking the transcription factors such as AP-1, STAT, and NF-αB [14,30,31], which are essential for regulating the immune response in many cells [30,31]. Expression of PPARs-γ increases significantly during the differentiation of monocytes into macrophages [25]. The activation of PPAR-γ in macrophages inhibits formation of cytokines IL-1β, IL-6, and TNF-α [32], and may cause their apoptosis by blocking the NF-κB pathway [31]. Moreover, it leads to increased expression of their surface receptor CD36+, involved in phagocytosis of the cells that underwent apoptosis. Inhibition of selectin E expression in the vascular endothelium, responsible for recruiting of immune-competitive cells to the site of infection, depends on the activation of PPARs-γ [33]. In the initiation of the secondary immune response, the IL-2-dependent activation and differentiation of T lymphocytes is essential. The presence of PPAR-γ mRNA has been confirmed in T lymphocytes in peripheral blood [32]. Ligands of PPAR-γ inhibit production of IL-2 and proliferation of T lymphocytes by blocking the NF-AT transcription factor crucial for activation of IL-2 gene transcription [34,35]. Thus, the immunosuppressive action of PPARs-γ occurs at the level of the initiation of immune response, as well as inhibition of T lymphocyte recruitment to the inflammation site [31]. According to some studies, PPAR-γ ligands also are likely to block proliferation of B lymphocytes or induce their apoptosis (cytotoxic effects) [36,37].

PPAR-γ agonists down-regulate synthesis of monocyte chemotactic protein 1 (MCP-1) and are involved in production of interleukin 8 (IL-8) by endothelial cells. Agonists of PPAR-γ (eg, troglitazone and 15d-PGJ2) decrease the cytokine-induced expression of VCAM-1 and ICAM-1. Consequently, chemotaxis, adhesion, and permeation of monocytes to the endothelium decrease, and the severity of inflammation is limited [37]. Therefore, PPARs-γ is a relevant immunoregulatory factor, and its ligands may have therapeutic potential for the treatment of inflammatory diseases.

In the present study, to evaluate the effects of activated PPARs-γ on colitis in rats, rosiglitazone (a thiazolidinedione), their agonist, was used. Rosiglitazone has already been used to treat or prevent nonspecific inflammatory intestinal diseases. The results of experimental studies in rats were published by Sanchez Hidalgo et al in 2005 and 2007 [38,39], demonstrating that the anti-inflammatory properties of this drug resulted from blocking of NF-αB signaling pathway (reduced expression of TNFα and concentration of MPO). Rosiglitazone markedly improved the general health status of animals with rectal TNBS-induced colitis. The drug was used by Adachi et al in models of transgenic mice deprived of the PPAR-γ gene, revealing that PPARs-γ played a key role in endogenous prevention of experimental colitis [40]. The first clinical trials with rosiglitazone were presented by Lewis et al in 2009, demonstrating its efficacy in mild and moderately severe ulcerative colitis [41].

Because the incidence of inflammatory bowel disease (IBD) in highly developed countries is high and increasing, the search for environmental factors involved has become critical. Among the factors implicated in the pathogenesis of IBD are substances inhibiting the activity of PPARs-γ. In our study, the antagonist of PPAR-γ – bisphenol A diglycidyl ether (BADGE), earlier used for production of plastic food containers and aluminum cans, was administered. According to the Directive of the European Committee of 2000, BADGE is included on the list of toxic substances that should not be found in food [42]. At the end of the 1990's, numerous reports were published indicating that BADGE from plastic containers and internal layers of cans permeated food products [43,44]. Radioimmunologic assays demonstrated that BADGE was the PPAR-γ ligand (antagonist) of micromolar affinity. The above data acquire new significance when confronted with increasingly accurate information concerning the effects of PPARs on proper functioning of many tissues and organs.

The aim of this study was to determine the mechanism of action of PPARs-γ activated by rosiglitazone and inactivated by BADGE in experimentally induced colitis in rats.

Material and Methods

Experimental animals

The experimental study was done on 96 Wistar rats weighing 200–220 g, in accordance with approval No. 23/2008 given by the local bioethics commission. Rats were put into laboratory hutches, 4 animals each. Ambient temperature was 22°C to 24°C, and humidity was maintained at 70% to 75%. A 12-hour day-light cycle was used. Animals had unlimited access to water and feed.

Induction of colitis

Animals were anaesthetized with intraperitoneal ketamine 50 mg/kg. Inflammation was induced by a single rectal

administration of TNBS 10 mg (Sigma-Aldrich Corp. St. Louis, MO, USA) diluted in 50% ethanol up to the volume of 0.25 mL. 2,4,6,-trinitrobenzene sulfonic acid was administrated by a rectally-introduced catheter (2 mm in diameter) to the depth of 8 cm from the rectal sphincter. To evaluate the effects of PPAR-γ agonist, the study groups, which received rosiglitazone at the dose of 8 mg/kg/bw, were created. Rosiglitazone, diluted in 0.9% NaCl up to the volume of 1 mL, was administrated by gastric tube, without previously induced sleep. To evaluate the effects of PPAR-γ antagonist, BADGE was given intraperitoneally 4 times at the dose of 120 mg/kg/bw. One group of animals was given rosiglitazone along with TNBS, and another group received BADGE along with TNBS. Additionally, the control group was given 50% ethanol (TNBS solvent) up the volume of 0.25 mL, administrated by rectal probe. In the last group of animals, rosiglitazone, together with BADGE and TNBS, was administrated. Animals were under daily observation of behavior patterns, changes in body weight or diarrhea. Rats were decapitated.

Division of experimental groups (12 rats, each)

I. Control group received only feed and water. Rats were killed after 7 days.
II. Received rosiglitazone – 8 mg/kg/bw diluted in 0.9% NaCl (up to the total volume of 1 mL) – given in 4 doses, on days 1, 2, 3, and 4 of the experiment. Rats were killed on day 7.
III. Received intraperitoneal BADGE in 4 doses (120 mg/kg/bw) on days 1, 3, 4, and 5. Rats were killed on day 7.
IV. Received TNBS by single rectal administration at the dose of 10 mg/kg/bw diluted in 50% ethanol up to the volume of 0.25 mL. Rats were killed on day 7.
V. Received TNBS and rosiglitazone – 8 mg/kg/bw 48, 24, and 1 hour before the induction of inflammation (rectal administration of TNBS) and 24 hours after induction. Rats were killed on day 7.
VI. Received TNBS and BADGE – 120 mg/kg/bw administrated intraperitoneally in 4 doses on days 1, 3, 5, and 6. The last dose was given on the day of rectal administration of TNBS. Rats were killed on day 8.
VII. Received TNBS, BADGE, and rosiglitazone. BADGE – 120 mg/kg was given on days 1, 3, 5, and 6. Rosiglitazone – 8 mg/kg/bw was administrated on days 2, 3, 5, and 7. 2,4,6,-trinitrobenzene sulfonic acid along with BADGE was given on day 6. Rats were killed on day 8.
VIII. Received 50% ethanol (TNBS solvent) by single rectal administration in the volume of 0.25 mL. Rats were killed on day 7.

Evaluation of colitis

Macroscopic evaluation was carried out in accordance with the procedures described by Sanchez Hidalgo et al. The intestine was divided for the purposes of histopathologic examination and enzyme-linked immunosorbent assay (ELISA).

Histopathologic examination

Tissue sections for microscopic examination were sampled at 2.5 cm, 5 cm and 7 cm from the colon and stained with hematoxylin and eosin stain, mucicarmine, and Masson's trichrome. The following parameters were evaluated: edema of the mucosa, extent and depth of inflammation, inflammatory activity, follicle aggregates, ulceration, mucosal necrosis, and crypt blunting. The above parameters were determined according to the following scale: 0 (lack of changes); 1 (slight focal and superficial mucosal lesions); 2 (more diffused, moderate lesions reaching the muscular lamina); 3 (severe changes affecting more than 1 section, reaching the muscular layer).

Enzyme-linked immunosorbent assay

Immediately after decapitation, 8–10 mL of blood was sampled. Blood samples were centrifuged at 5000 r/minute for 15 minutes, and the obtained serum was frozen at –80°C.

The large intestine section used for immunoenzymatic analysis was weighed and ground using Medicons (Consul TS). The material was diluted with 2 mL of Ca2+ and Mg2+-free PBS. The solution was centrifuged at 2000 r/minutes for 5 minutes, and the supernatant was frozen at –80°C. Levels of IL-6, IL-10, and MPO were determined in serum and in intestinal homogenates. IL-6 and IL-10 levels were evaluated using ELISA plates (R & D Systems Inc., Minneapolis, MN, USA); ELISA plates (Hycult Biotechnology b.v., The Netherlands) were applied for MPO determinations. Results were read using the ELISA Victor 3 scanner (Perkin-Elmer, Waltham, MA, USA).

Statistical analysis

The Kolmogorov-Smirnov test was applied to check for normal distribution of variables. Since normality was not demonstrated, the Mann-Whitney test was used to compare 2 groups and find significant differences.

The 5% error risk was assumed; thus, $P<.05$ was considered statistically significant (*); $P<.01$, more significant (**); and $P<.001$, highly significant (***).

RESULTS

Histopathologic results in groups I, II, and III

No microscopic lesions in the intestinal wall were observed. In the mucosa, signs of inflammation or edema were not found, and the structure of intestinal crypts was preserved. Infiltration of neutrophils and mononuclear cells was not demonstrated (Figures 1, 2).

Histopathologic results in group IV

Transmural inflammatory changes were observed in most parts of the intestinal surface. Marked epithelium necrosis and intestinal wall edema were visualized. Diffuse, intensive, inflammatory infiltration of neutrophils and less-intensive infiltration of mononuclear cells were found within the mucosa, submucosa, and muscular layer. Moreover, crypt abscesses and numerous deep ulcerations, exceeding the muscular lamina, were noted. Slight fibroma within the submucosal layer, local atrophy of crypt structures, and decreased amounts of mucus also were observed. The mucus layer covering the epithelium was lacking (Figure 3).

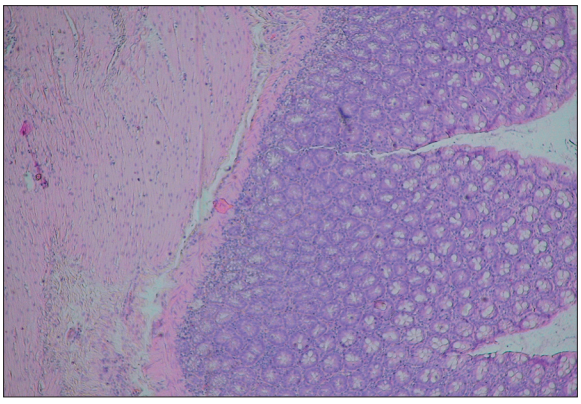

Figure 1. H+E ×100. Regular histopathological picture of the large intestine in group I (control group).

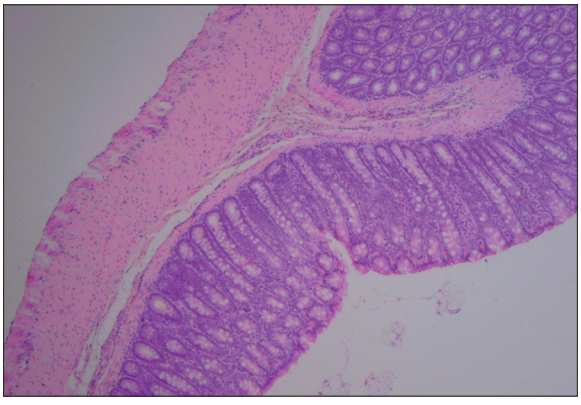

Figure 2. Histopathological picture of the large intestine in II group (Rosiglitazone). H+E ×50. Regular mucosa, submucosa and muscular layer of the large intestine. Structure of intestinal crypts preserved. No infiltrations of neutrophils and nuclear cells.

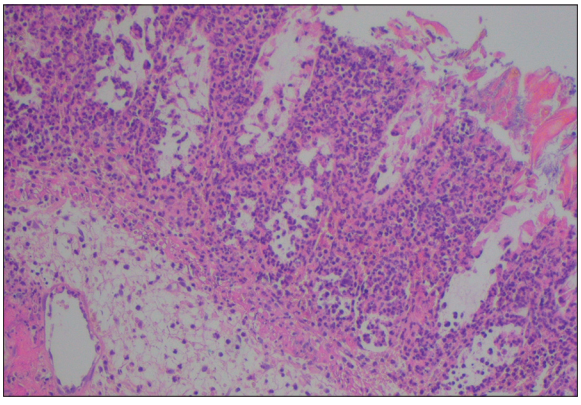

Figure 3. Histopathological picture of the large intestine in group IV (TNBS). H+E ×200. Ulcerations of intestinal musoca covered with the fibrin pseudomembrane reaching its muscular lamina, very intensive inflammatory neutrophil infiltration, submucosal oedema.

Histopathologic results in group V

Compared with group IV, inflammatory changes in group V were less severe and less extensive. Slight, focally moderate edema of the intestinal mucosa and submucosa was observed.

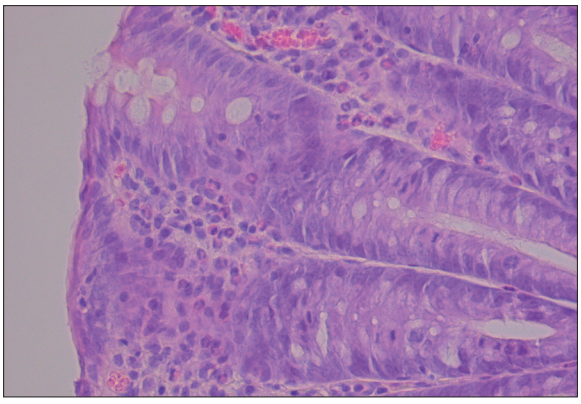

Figure 4. Histopathological picture of the large intestine in group V (TNBS + rosiglitazone). H+E ×200. Few neutrophils detected in the lamina propria, single cells penetrating into the epithelium of intestinal crypts.

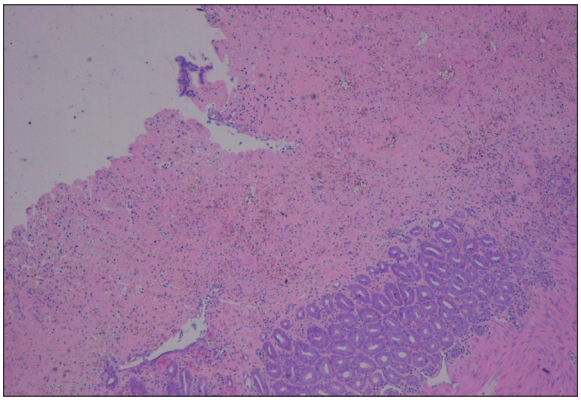

Figure 5. Histopathological picture of the large intestine in group VI (TNBS + BADGE). H+E ×200. Extensive ulceration of the intestinal mucosa along with total necrosis of the mucosal layer.

Moreover, slight focal inflammatory infiltrations of nucleus cells were noted. Slight atrophy of intestinal crypts also was visible. Single neutrophils in the lamina propria and a few aggregates of lymphatic follicles were found. No ulceration within the mucous membrane was noticed; the mucus layer covering the mucous membrane was visible (Figure 4).

Histopathologic results in group VI

The presence of transmural inflammatory changes locally reached the serous membrane within the entire intestine. Intensive necrosis, edema, and numerous infiltrations of neutrophils, lymphocytes, and monocytes within the mucosa and submucosal layers were observed. Furthermore, extensive ulcerations with fibroma in the submucosal layer exceeding the muscular mucosal lamina were found. Marked structural abnormalities of intestinal crypts, with decreased amounts of mucus, were also noted. The submucosal layer was found to be thicker, particularly in substantially changed intestinal crypts (Figure 5).

Histopathologic results in group VII

Histopathologic changes corresponded to the intensity of inflammation observed in groups IV and VI.

Table 1. Body weight changes and colon weight/length ratios in the experimental groups.

	Group	n	Body weight changes (g)	Colon weight/colon length (g/cm)
I	Control	8	15.375±2.97	0.103±0.016
II	Rosiglitazone	8	19.75±5.06[2]	0.122±0.018[2]
III	BADGE	8	11.75±2.25[2]	0.142±0.032
IV	TNBS	8	–28.37±13.09[1]	0.35±0.056[1]
V	Rosiglitazone + TNBS	8	10.375±4.75[2,3]	0.202±0.035[1,3]
VI	BADGE + TNBS.	8	–38.00±14.12*[,1]	0.37±0.052*[,1]
VII	Rosigl. + BADGE + TNBS	8	–17.00±8.55*[,1]	0.349±0.064*[,1]
VIII	Ethanol 50%	8	8.125±2.64[1,3]	0.149±0.024[1,3]

Data are expressed as a mean ±SD. [1] P<0.001; [2] P<0.05 significant compared to control group; [3] P<0.001 significant compared TNBS * P<0.001 significant compared to rosiglitazone + TNBS.

Table 2. Serum concentrations of IL-6, IL-10 and MPO in the experimental groups.

		Serum concentration of cytokines		
	Group	IL-6 pg/ml	IL-10 pg/ml	MPO ng/ml
I.	Control	11.424±3.19	6.27±2.32	931.39±194.04
II.	Rosiglitazone	6.895±4.65[2]	9.882±2.40[2]	814.56±168.78
III.	BADGE	27.908±12.90	10.806±1.23	1010.04±178.44
IV.	TNBS	26.109±8.36[1]	11.081±3.41	977.24±124.73
V.	Rosiglitazone +TNBS	21.271±11.23[1]	11.654±1.68[3]	987.001±280.26
VI	BADGE + TNBS.	48.010±12.42[3,*]	14.286±5.12[1]	967.54±220.83
VII.	Rosig. + BADGE + TNBS	29.296±13.93	11.416±4.23	1024.71±175.33
VIII	Ethanol 50%	16.466±11.11	11.902±5.68	900.73±149.18

Data are expressed as a mean ±SD. [1] P<0.01; [2] P<0.05; [3] P<0.001 significant compared to control group.

Histopathologic results in group VIII

Slight focal inflammatory changes and superficial erosions of the mucous membrane were noted. Within the mucosa, a slight local inflammatory infiltration of neutrophils and lymphocytes was observed. The submucosal layer was characterized by focally minor edema; structures of intestinal crypts with proper amounts of mucus were found to be preserved.

Findings concerning body weight changes and large intestine length/weight ratios

The body weight slightly decreased in the group receiving TNBS and TNBS along with the PPAR-γ antagonist – BADGE. Rosiglitazone administrated together with TNBS had protective effects, reflected by the increased body weight observed in this group. Animals receiving only rosiglitazone showed a significant increase in body weight without developing inflammation.

The intestinal length/weight ratio was analyzed. Its increase, when compared to the control group, is a relevant factor and marker of the severity of inflammation. The ratio found demonstrates that rosiglitazone had a positive effect on histopathologically confirmed inhibition of rats' colitis.

Table 1 presents the detailed results of changes in body weight in individual groups, and values of intestinal length/weight ratios.

IL-6, IL-10, and MPO levels

The level of IL-6, which is the major proinflammatory cytokine involved in the pathogenesis of IBD, and the level of its antagonistically acting IL-10, were determined in serum and large intestinal homogenates. Additionally, concentration of MPO, a marker of neutrophil infiltrations in the colitis-affected intestine, was evaluated. Table 2 presents serum levels of the these variables.

Statistical analyzis

Statistically significantly increased levels of IL-6 were observed in groups IV, V, VI, and VII; that is, those receiving TNBS (the inducer of inflammation). The highest level of IL-6 was found in group VI, in which TNBS was administrated

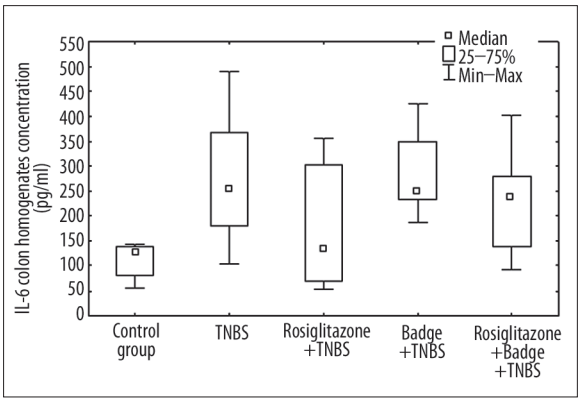

Figure 6. Colon homogenate IL-6 concentrations in the experimental groups.

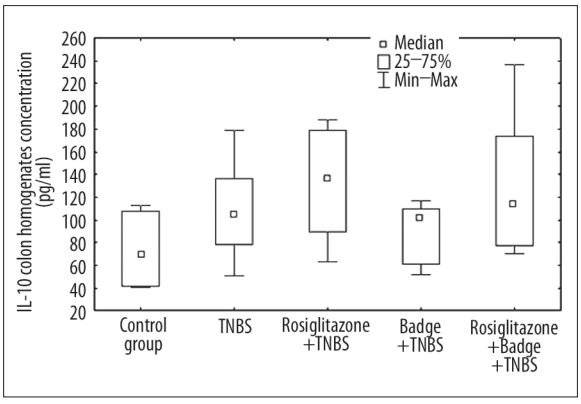

Figure 7. Colon homogenate IL-10 colon concentrations in the experimental groups.

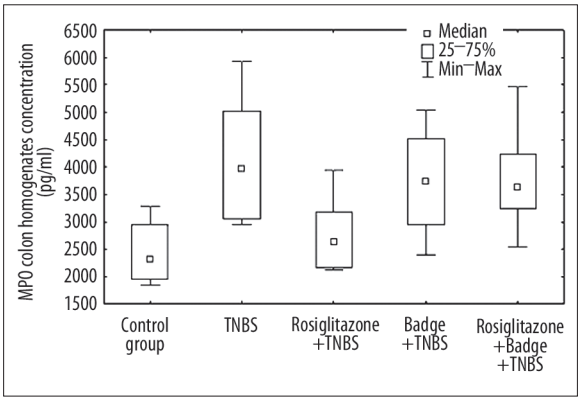

Figure 8. Colon homogenate MPO concentrations in the experimental groups.

along with BADGE. In group V, receiving rosiglitazone with TNBS, the IL-6 level was not significantly lower compared with group IV. IL-6 was decreased in group II (treated with rosiglitazone) and increased in group III (receiving BADGE), and in both of these groups colitis was not induced.

Besides increased levels of IL-6 in groups receiving TNBS compared with controls, no significant intergroup differences were demonstrated in the concentration of this cytokine in intestinal homogenates. Figure 6 presents the levels of IL-6 in colon homogenates.

No relevant effects of rosiglitazone and BADGE on serum IL-10 levels were observed in groups IV, V, VI, and VII. Compared with controls, a statistically significant increase in IL-10 was found in group II (rosiglitazone).

IL-10 levels determined in intestinal homogenates did not show positive effects of rosiglitazone administrated concurrently with TNBS on significant increase in IL-10 expression. Compared with the group receiving only TNBS, BADGE administrated along with TNBS decreased the level of IL-10. Figure 7 shows the levels of IL-10 in colon homogenates. In individual groups, no significant changes in serum MPO levels were observed.

Anti-inflammatory effects of rosiglitazone were confirmed by MPO determinations in colon homogenates. In group V (receiving TNBS together with rosiglitazone), neutrophil chemotaxis was found to be inhibited, which was reflected by the decreased level of MPO compared to group VI (TNBS). Figure 8 presents the MPO levels in colon homogenates

Discussion

In recent years numerous studies have focused on immunologic mechanisms involved in colitis, and the findings of such studies should result in better management of the immunologic processes of the pathogenesis of IBD. The detection of PPARs and their potential immunomodulatory function in inflammations make PPARs potentially useful tools in the treatment of ulcerative colitis and Crohn's disease. Our findings regarding the effects of rosiglitazone and BADGE on experimentally induced colitis demonstrate that the course and severity of inflammation may be modulated using the PPAR ligands. Rosiglitazone administrated concurrently with the colitis-inducing substance decreased the severity of inflammation, which was confirmed by analysis of changes in animals' body weights, colon weight/length ratios, and histopathologic findings. A statistically significant loss in body weight resulting from intensive diarrhea and decreased feed intake was observed in group IV (TNBS), VI (BADGE + TNBS), and VII (rosiglitazone + BADGE + TNBS). Animals receiving the PPAR-γ agonist along with inflammation-inducer (TNBS) gained not much less weight than healthy controls. The most significant decrease in body weight was observed among rats from the group given the PPAR-γ antagonist together with TNBS, which is likely associated with higher sensitivity to destructive effects of TNBS resulting from inhibited basic activity of PPARs-γ induced by numerous endogenous ligands. The beneficial influence of rosiglitazone on inhibition of inflammation was confirmed by the intestinal weight/length ratio, which usefully reflects the intensity of inflammatory intestinal infiltrations. In the group with TNBS and the antagonist of PPAR-γ receptor, no increase in the above variable was noted.

Our results concerning the effect of rosiglitazone on rats' acute colitis agree with data published by Sanchez-Hidalgo et al in 2007, demonstrating positive effects of rosiglitazone on both macroscopic inflammatory parameters (such as changes in body weight, presence of abdominal adhesions, increased diarrhea) and on the microscopic condition of the large intestine and concentrations of TNF-α and MPO.

Additionally, the effect of activated PPAR-γ on limited expression of COX 2 and PGE2 was observed. The authors suggested that the inhibition of expression of proinflammatory factors results from blockage of NF-κB and MAPK-signaling pathway by PPARs-γ, which was confirmed by decreased concentrations of p65 and p38 proteins [38]. Positive, anti-inflammatory effects of rosiglitazone were also reported by Sanchez-Hidalgo in 2005 in a study focused on the influence of PPAR-γ agonist on acute colitis [39].

Studies conducted by Takaki et al., based on the differently induced colitis model (DSS) and the use of different thiazolidinedione derivatives (pioglitazone, netoglitazone), demonstrated the anti-inflammatory properties of PPAR-γ activators [45]. In their study of troglitazone administrated as the PPAR-γ agonist, no differences were found in animal body weight between the control group, the group receiving DSS, and the group treated with troglitazone. However, histopathologic examinations revealed less severe inflammation in the group receiving troglitazone along with DSS [46].

The efficacy of troglitazone in the anti-inflammatory treatment of IBD was questioned by Ramaker et al, who demonstrated that prophylactic administration of rosiglitazone over several days before induction of colitis can intensify the course of inflammation. Their observations were associated with higher permeability of the intestinal mucosa membrane resulting from prolonged administration of rosiglitazone [47].

The majority of the available literature confirms positive anti-inflammatory effects of PPAR-γ agonists on colitis; however, the mechanism of their action is disputed. According to most of authors, the crucial mechanism reducing the inflammation is the inhibition of expression of proinflammatory factors. Some authors suggest that higher expression of anti-inflammatory factors results from the activation of PPARs-γ. The effects of the PPAR-γ antagonists on inflammation have not been elucidated. To clarify the doubtful issues, the present study analyzed the effects of both agonists and antagonists of PPAR-γ on the expression of proinflammatory IL-6, anti-inflammatory IL-10 and MPO.

Interleukin-6 is the major factor responsible for controlling immunologic protection. Interleukin-6 is primarily produced by monocytes and macrophages, and the essential factors inducing its formation are IL-1, INF-γ, TNF-α, and LPS. Interleukin-6 stimulates differentiation of B lymphocytes toward plasmatic cells and production of acute-phase proteins found in the initial stage of inflammation [48,49]. In the pathogenesis of IBD, IL-6, as the derivative of Th2 lymphocyte subpopulation, plays an essential role in the development of ulcerative colitis and Crohn's disease.

Our findings do not confirm the positive effects of rosiglitazone on IL-6 expression in animals with induced colitis. Although in the group with colitis receiving rosiglitazone, the mean level of IL-6 was markedly lower than in the group receiving TNBS, the difference was not statistically significant. Inhibition of IL-6 expression by activated PPAR-γ is shown by a 2-fold increase in IL-6 after the PPAR antagonist administration.

The results reported by Takaka et al., investigating the prophylactic and therapeutic effects of netoglitazone and pioglitazone on DSS-based colitis, are promising. Immunoenzymatic analysis of IL-6 levels in intestinal homogenates, as well as Western blot immunoassay for the expression of STAT3, the factor that activates transcription of proinflammatory cytokines and whose expression is PPAR-γ activation-dependent, showed a significant reduction in these parameters after administering pioglitazone at a dose of 150 mg/kg [45].

According to Ramakers et al, the PPAR-γ agonist had no favorable inhibiting effects on expressing IL-6. Despite a significantly intensified inflammation observed in the group of animals treated with rosiglitazone, levels of TNF-α and IFN-γ in intestinal homogenates were decreased. This appears to confirm the immunomodulatory effects of induced PPARs-γ on the expression of proinflammatory cytokines, and suggests sensitization of the intestinal mucosa as the destructive factor induced by long-term administration of rosiglitazone [47].

Yamamoto et al implicated inhibited expression of IL-6 genes resulting from the induced PPAR-γ. Based on this model, positive effects of 4-OHDHA on colitis intensity were reported. Additionally, 4-OHDHA has been demonstrated to inhibit IL-6 expression and induce synthetase of nitric oxide (Nos2/iNOS) in lipopolysaccharide (LPS)-activated macrophages [50].

The relations between PPAR-γ ligands and expression of IL-10 seem to be crucial in explaining the mechanisms controlling the inflammatory process. The main function of IL-10 is inhibition of the production of cytokines produced by Th1 lymphocytes – IFN-γ and IL-2, in particular. Moreover, it inhibits the production of IL-1α, IL-6, IL-8, IL-12, G-CSF, GM-CSF, TNF-α, as well as oxides and nitric oxide. It also limits expression of class II MHC on monocytes and their capacity to present antigens [51,52].

Analysis of serum IL-10 levels did not show its intensified expression resulting from activation of PPARs-γ by rosiglitazone. Compared with the control group, serum levels of IL-10 significantly increased in groups with induced colitis (groups IV, V, VI, and VII). However, no differences in its levels between those groups were reported. Thus, increased concentration of IL-10 was not associated with activation or blockage of PPARs-γ after administration of rosiglitazone or BADGE. Most likely, the increase in IL-10 was caused by activation of anti-inflammatory factors, which counterbalanced the effects of cytokines enhancing the inflammatory response.

The key role of IL-10 in the pathogenesis of IBD was described by Lytle et al., who studied transgenic mice without IL-10 genes but with spontaneous colitis. Rosiglitazone inhibited expression of mRNA for TNFα, INFγ, IL-17, and induced synthetase of nitric oxide. Lack of IL-10 genes did not impair the immunomodulatory properties of the PPAR-γ-inducing substances. The authors suggested that IL-10 was essential for maintaining intestinal immune balance [53]. Our findings did not confirm the effects of rosiglitazone on increased expression of IL-10.

Effects of PPAR-γ agonists such as rosiglitazone, pioglitazone, and troglitazone on lymphocyte Th1- and Th2-dependent

immunologic processes involved in inflammation were studied by Saubermann et al. Administration of PPAR-γ agonist resulted in reduced concentrations of proinflammatory Th-1-dependent cytokines (decrease in TNF-α and INF-γ) and increased concentrations of Th-2 based cytokines such as IL-10 and IL-4. According to these authors, activated PPARs-γ limits Th-1-based processes and stimulates Th-2-based immunologic mechanisms [54]. Further studies involving determinations of IL-1, IL-2, IL-4, and IFN should allow us to assess the results of Saubermann et al. Myeloperoxidase is an enzyme belonging to the family of peroxidases, which is characterized by potent antiviral and antibacterial action. Myeloperoxidase is found mainly in azurophilic granules of neutrophils, and its determination is therefore an important marker of neutrophil infiltrations in the affected tissue [55,56].

The results of immunoenzymatic examinations of intestinal tissues revealed increased concentrations of MPO in groups with the most severe symptoms of inflammation. Levels of MPO in intestinal homogenates from animals with experimentally induced colitis treated with rosiglitazone were substantially lower than in the group without the PPAR-γ agonist, which demonstrates less intensive neutrophil infiltrations, which in turn is likely to result from limited expression of neutrophil chemotactic factors. This is confirmed by histopathologic results in the group treated with rosiglitazone, which revealed only single neutrophils in the lamina propria of mucosa.

Sanchez-Hidalgo et al. in their studies of 2005 and 2007 showed the correlation between the concentration of MPO and administration of rosiglitazone in acute and chronic experimental models of colitis [38,39]. Rosiglitazone (regardless of the dosage administered) was found to reduce infiltrations in intestinal tissues (MPO level) in both models. Their results correspond to the results obtained in the present study.

Similar observations, that is, reduced inflammatory infiltrations, were reported by Cuzzocrea et al. [57] (decreased MPO concentration and expression of intracellular cell adhesion molecule [ICAM] involved in adherence of leukocytes to the vascular endothelium and their migration to the site of inflammation).

Our results concerning the influence of rosiglitazone on neutrophil infiltration in the affected large intestine (MPO concentration) differ from those published by Ramaker et al., according to whom there were no differences in MPO levels in intestinal homogenates and serum between animals with experimentally induced colitis provided only with feed and those who received feed with the PPAR-γ agonist [47]. The administration of PPAR-γ antagonist along with TNBS compared with TNBS alone did not result in increased levels of MPO.

Conclusions

1. Rosiglitazone reduced severity of experimental colitis in rats, improving their general status as well as macro- and microscopic parameters of the large intestine.

2. Rosiglitazone administered to animals with colitis did not decrease expression of proinflammatory IL-6, but limited neutrophil infiltrations, which was shown by decreased tissue levels of MPO.

3. Anti-inflammatory properties of rosiglitazone were not associated with increased expression of anti-inflammatory IL-10.

4. The PPAR-γ antagonist (BADGE) given to rats with colitis enhanced the serum expression of Il-6; histopathologic inflammatory parameters, however, were not increased.

5. The application of BADGE along with rosiglitazone inhibited anti-inflammatory action of PPARs-γ.

References:

1. Sokołowska M, Kowalski ML, Pawliczak R: Peroxisome proliferator-activated receptors-gamma (PPAR-gamma) and their role in immunoregulation and inflammation control. Postepy Hig Med Dosw, 2005; 59: 472–84
2. Celiński K, Dworzański T, Prozorow-Król B, Korolczuk A: The role of PPAR-γ receptors in gastrointestinal inflammation diseases. Gastroenterol Pol, 2009; 16(1): 51–56
3. Celiński K, Mądro A, Prozorow-Król B et al: Rosiglitazone, a peroxisome proliferator-activated receptor gamma (PPAR-γ)-specific agonist, as a modulator in experimental acute pancreatitis. Med Sci Monit, 2009; 15(1): BR21–29
4. Stulc T, Ceska R: rosiglitazone in the prevention of diabetes and cardiovascular disease: dream or reality? Med Sci Monit, 2008; 14(4): RA45–47
5. Giaginis C, Katsamangou E, Tsourouflis G et al: Peroxisome proliferator-activated receptor-gamma and retinoid X receptor-alpha expression in pancreatic ductal adenocarcinoma: association with clinicopathological parameters, tumor proliferative capacity, and patients' survival. Med Sci Monit, 2009; 15(5): BR148–56
6. Chawla A, Schwarz EJ, Dimaculangan DD, Lazer MA: Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. Endocrinology, 1994; 135(2): 798–800
7. Brun RP, Tontonoz P, Forman BM et al: Differential activation of adipogenesis by multiple PPAR isoforms. Genes Dev, 1996; 10(8): 974–84
8. Okuno A, Tamemoto H, Tobe K et al: Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. J Clin Invest, 1998; 101(6): 1354–61
9. Chinetti G, Fruchart JC, Staels B: Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflamm Res, 2000; 49(10): 497–505
10. Debril MB, Renaud JP, Fajas L, Auwerx J: The pleiotropic functions of peroxisome proliferator-activated receptor gamma. J Mol Med, 2001; 79(1): 30–47
11. Li AC, Binder CJ, Gutierrez A et al: Differential inhibition of macrophage foam-cell formation and atherosclerosis in mice by PPARalpha, beta/delta, and gamma. J Clin Invest, 2004; 114(11): 1564–76
12. Chawla A, Barak Y, Nagy L et al: PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. Nat Med, 2001; 7(1): 48–52
13. Greene ME, Blumberg B, McBride OW et al: Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. Gene Expr, 1995; 4(4–5): 281–99
14. Ricote M, Huang JT, Welch JS, Glass CK: The peroxisome proliferator-activated receptor(PPARgamma) as a regulator of monocyte/macrophage function. J Leukoc Biol, 1999; 66(5): 733–39
15. Tontonoz P, Nagy L, Alvarez JG et al: PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell, 1998; 93(2): 241–52
16. Konturek PC, Dembinski A, Warzecha Z et al: Pioglitazone, a specific ligand of peroxisome proliferator-activated receptor-gamma, protects pancreas against acute cerulein-induced pancreatitis. World J Gastroenterol, 2005; 11(40): 6322–29
17. Konturek PC, Brzozowski T, Engel M et al: Ghrelin ameliorates colonic inflammation. Role of nitric oxide and sensory nerves. J Physiol Pharmacol, 2009; 60(2): 41–47

18. Kieć-Wilk B, Dembińska-Kieć A, Olszanecka A et al: The selected pathophysiological aspects of PPARs activation. J Physiol Pharmacol, 2005; 56(2): 149–62
19. Ptak-Belowska A, Pawlik MW, Krzysiek-Maczka G et al: Transcriptional upregulation of gastrin in response to peroxisome proliferator-activated receptor gamma agonist triggers cell survival pathways. J Physiol Pharmacol, 2007; 58(4): 793–801
20. Fajas L, Auboeuf D, Raspe E et al: The organization, promotor analysis and exspression of the human PPAR gamma gen. J Biol Chem, 1997; 272: 18779–89
21. Elbrecht MJ, Chen Y, Cullian CA et al: Molecular cloning, expression and characterization of human peroxisome proliferators activated receptors gamma 1 and gamma 2. Biochem Biophys Res Commun, 1996; 224: 431–37
22. Vidal-Puig A, Jimenez-Liñan M, Lowell BB et al: Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. J Clin Invest, 1996; 97(11): 2553–61
23. Lambe KG, Tugwood JD: A human peroxisome proliferators-activated receptor gamma is activated by inducers of adipogenesis including thiazolidedinedione drugs. Eur J Biochem, 1996; 239: 1–7
24. Pontsler AV, St Hilaire A, Marathe GK et al: Cyclooxygenase-2 is induced in monocytes by peroxisome proliferator activated receptor gamma and oxidized alkyl phospholipids from oxidized low density lipoprotein. J Biol Chem, 2002; 277(15): 13029–36
25. Thieringer R, Fenyk-Melody JE, Le Grand CB et al: Activation of peroxisome proliferator-activated receptor gamma does not inhibit IL-6 or TNF-alpha responses of macrophages to lipopolysaccharide *in vitro* or *in vivo*. J Immunol, 2000; 164(2): 1046–54
26. Asada K, Sasaki S, Suda T et al: Antiinflammatory roles of peroxisome proliferator-activated receptor gamma in human alveolar macrophages. Am J Respir Crit Care Med, 2004; 169(2): 195–200
27. Chinetti G, Fruchart J.C, Staels B: Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflamm Res, 2000; 49(10): 497–505
28. Chinetti G, Lestavel S, Fruchart JC et al: Peroxisome proliferator-activated receptor alpha reduces cholesterol esterification in macrophages. Circ Res, 2003; 92(2): 212–17
29. Wang AC, Dai X, Luu B, Conrad DJ: Peroxisome proliferator-activated receptor-gamma regulates airway epithelial cell activation. Am J Respir Cell Mol Biol, 2001; 24(6): 688–93
30. Ricote M, Li AC, Willson TM et al: The peroxisome proliferators-activated receptor-gamma is a negative regulator of macrophage activation. Nature, 1998; 391: 79–82
31. Welch JS, Ricote M, Akiyama TE et al: PPARgamma and PPARdelta negatively regulate specific subsets of lipopolysaccharide and IFN-gamma target genes in macrophages. Proc Natl Acad Sci USA, 2003; 100(11): 6712–17
32. Jiang JG, Ting AT, Seed B: PPAR-gamma agonist inhibit production of monocyte inflammatory cytokines. Nature, 1998; 391: 82–86
33. Nawa T, Nawa MT, Cai Y et al: Repression of TNF-alpha-induced E-selectin expression by PPAR activators: involvement of transcriptional repressor LRF-1/ATF3. Biochem Biophys Res Commun, 2000; 275(2): 406–11
34. Yang XY, Wang LH, Chen T et al: Activation of human T lymphocytes is inhibited by peroxisome proliferator-activated receptor gamma (PPARgamma) agonists. PPARgamma co-association with transcription factor NFAT. J Biol Chem, 2000; 275(7): 4541–44
35. Clark RB, Bishop-Bailey D, Estrada-Hernandez T et al: The nuclear receptor PPAR gamma and immunoregulation: PPAR gamma mediates inhibition of helper T cell responses. J Immunol, 2000; 164(3): 1364–71
36. Padilla J, Kaur K, Cao HJ et al: Peroxisome proliferator activator receptor-gamma agonists and 15-deoxy Delta(12,14)(12,14)-PGJ(2) induce apoptosis in normal and malignant B-lineage cells. J Immunol, 2000; 165(12): 6941–48
37. Padilla J, Kaur K, Harris SG, Phipps RP: PPAR-gamma-mediated regulation of normal and malignant B lineage cells. Ann NY Acad Sci, 2000; 905: 97–109
38. Sánchez-Hidalgo M, Martín AR, Villegas I, Alarcón de la Lastra C: Rosiglitazone, a PPARγ ligand, modulates signal transduction pathways during the development of acute TNBS-induced colitis in rats. Eur J Pharmacol, 2007; 562: 247–58
39. Sánchez-Hidalgo M, Martín AR, Villegas I, Alarcón de la Lastra C: Rosiglitazone, an agonist of peroxisome proliferator-activated receptor gamma, reduces chronic colonic inflammation in rats. Biochem Pharmacol, 2005; 69(12): 1733–44
40. Adachi M, Kurotani R, Morimura K et al: Peroxisome proliferator activated receptor gamma in colonic epithelial cells protects against experimental inflammatory bowel disease. Gut, 2006; 55(8): 1104–13
41. Lewis JD, Lichtenstein GR, Deren JJ et al: Rosiglitazone for active ulcerative colitis: a randomized placebo-controlled trial.; rosiglitazone for Ulcerative Colitis Study Group. Gastroenterology, 2008; 134(3): 688–95
42. Wright H, Clish C, Mikamii T et al: A Synthetic Antagonist for the Peroxisome Proliferator-activated Receptor g Inhibits Adipocyte Differentiation. J Biol Chem. 2000; 275(3): 1873–77
43. Poole A, van Herwijnen P, Weideli H et al: Review of the toxicology, human exposure and safety assessment for bisphenol A diglycidylether (BADGE). Food Addit Contam, 2004; 21(9): 905–19
44. Cabado AG, Aldea S, Porro C et al: Migration of BADGE (bisphenol A diglycidyl-ether) and BFDGE (bisphenol F diglycidyl-ether) in canned seafood. Food Chem Toxicol, 2008; 46(5): 1674–80
45. Takaki K, Mitsuyama K, Tsuruta O et al: Attenuation of experimental colonic injury by thiazolidinedione agents. Inflamm Res, 2006; 55(1): 10–15
46. Tanaka T, Kohno H, Yoshitani S et al: Ligands for peroxisome proliferator-activated receptors alpha and gamma inhibit chemically induced colitis and formation of aberrant crypt foci in rats. Cancer Res, 2001; 61(6): 2424–28
47. Ramakers JD, Verstege MI, Thuijls G et al: The PPARgamma agonist rosiglitazone impairs colonic inflammation in mice with experimental colitis. J Clin Immunol, 2007; 27(3): 275–83
48. Gołąb J, Jakóbisiak M, Lasek W, Stokłosa T: Immunologia. 2007; 118–20
49. Noguchi D, Wakita D, Tajima M et al: Blocking of IL-6 signaling pathway prevents CD4+ T cell-mediated colitis in a T(h)17-independent manner. Int Immunol, 2007; 19(12): 1431–40
50. Yamamoto K, Ninomiya Y, Iseki M et al: 4-Hydroxydocosahexaenoic acid, a potent peroxisome proliferator-activated receptor c agonist alleviates the symptoms of DSS-induced colitis. Biochem Biophys Res Commun, 2008; 367: 566–72
51. Matharu KS, Mizoguchi E, Cotoner CA et al: Toll-like receptor 4-mediated regulation of spontaneous Helicobacter-dependent colitis in IL-10-deficient mice. Gastroenterology, 2009; 137(4): 1380–90
52. Gołąb J, Jakóbisiak M, Lasek W, Stokłosa T: Immunologia, 2007; 121–23
53. Lytle C, Tod BS, Kathy T et al: The Peroxisome Proliferator-Activated Receptor γ Ligand rosiglitazone Delays the Onset of Inflammatory Bowel Disease in Mice with Interleukin 10 Deficiency. Inflamm Bowel Dis, 2005; 11: 231–43
54. Saubermann LJ, Nakajima A, Wada K et al: Peroxisome proliferator-activated receptor gamma agonist ligands stimulate a Th2 cytokine response and prevent acute colitis. Inflamm Bowel Dis, 2002; 8(5): 330–39
55. Van Schooten FJ, Boots AW, Knaapen AM et al: Myeloperoxidase (MPO) -463G->A reduces MPO activity and DNA adduct levels in bronchoalveolar lavages of smokers. Cancer Epidemiol Biomarkers Prev, 2004; 13(5): 828–33
56. Zhang AH, Chen M, Gao Y et al: Inhibition of oxidation activity of myeloperoxidase (MPO) by propylthiouracil (PTU) and anti-MPO antibodies from patients with PTU-induced vasculitis. Clin Immunol, 2007; 122(2): 187–93
57. Cuzzocrea S, Di Paola R, Mazzon E et al: Role of endogenous and exogenous ligands for the peroxisome proliferators activated receptors alpha (PPAR-α) in the development of inflammatory bowel disease in mice. Lab Invest, 2004; 84: 1643–54