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EFFECTS OF PEROXISOME PROLIFERATOR-ACTVATED RECEPTORS-GAMMA LIGANDS ON DEXTRAN SODIUM SULPHATE-INDUCED COLITIS IN RATS

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Recent studies indicate the involvement of peroxisone proliferator-activated receptor-γ (PPAR-γ) in the inflammatory reaction. The exact mechanism of PPAR-γ action has not been elucidated. It is supposed that PPAR-γ regulates transcription of genes responsible for encoding cytokines involved in the inflammatory response. The latest studies, carried out to explain the pathogenesis of non-specific colitis, confirm beneficial effects of PPAR-γ agonists on attenuation of colon inflammation. The aim of the present study was to assess the effects of nuclear PPAR-γ activity on the course of experimental acute colitis induced by intragastric administration of dextran sodium sulphate (DSS) using the PPAR-γ agonist rosiglitazone and the antagonist BADGE in rats. Colitis in Wistar rats was induced by 1.5% DSS administered in drinking water for 8 days. Animals with induced colitis received rosiglitazone, bisphenol A diglycidyl ether (BADGE) or both substances. After decapitation, colons were macroscopically and histopathologically evaluated. Levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α) and myeloperoxidase (MPO) were determined in serum and colon homogenates using ELISA. In rats with experimentally induced colitis receiving rosiglitazone, the inflammatory reaction was found to be markedly limited; ulceration, oedema and infiltration activity were reduced. The activated PPAR-γ inhibit the expression of proinflammatory factors, such as IL-6, TNF-α, and neutrophil chemotaxis, which was evidenced by MPO reduction in serum and colon homogenates mediated by rosiglitazone. The positive effects of rosiglitazone on expression of IL-10 were also demonstrated. During the short period of observation, BADGE did not increase histopathological inflammatory markers.

Key words: *bisphenol A diglycidyl ether, inflammatory bowel disease, dextran sodium sulphate, peroxisome proliferator-activated receptor-γ, rosiglitazone, ulcerative colitis*

INTRODUCTION

The etiology of non-specific inflammatory bowel diseases (IBDs) has not been fully elucidated. IBDs are chronic, recurrent diseases that pose relevant clinical problems in everyday medical practice. Despite vast advances in identification of factors underlying these diseases, the pathogenesis of non-specific colitis and other inflammatory gastrointestinal diseases remains unclear. Numerous recent studies confirm the involvement of immunological mechanisms in colitis (1-4). The identification of peroxisome proliferator-activated receptors (PPARs) sheds new light and expands therapeutic options in non-specific inflammatory diseases. Many recent studies dealt with the effects of peroxisome proliferator-activated receptor-γ (PPAR-γ) ligands on colitis (5, 6). The majority of them confirmed beneficial effects of PPAR-γ agonists on colitis; however, some publications demonstrated their unfavourable influence. The issue is undoubtedly interesting, both theoretically and practically with respect to novel therapeutic methods. The exact mechanism of PPAR-γ action remains unknown. It is implicated that effects they exert on the inflammatory process include the regulation of transcription of genes encoding cytokines involved in common interactions between cells, adhesive factors and other inflammatory mediators (7). Moreover, activated PPAR-γ might block the expression of inducible nitric oxide synthase (iNOS) genes, metalloproteinases and SR-A (scavenger receptor A). Due to the above action, synthesis of mRNA for aforementioned factors is inhibited. PPAR-γ receptor is most likely to inhibit the expression of these genes by blocking transcription factors such as AP-1, STAT, and nuclear factor (NF)-κB (8-10). The experiment described in the present study regarded experimental colitis in rats induced by the administration of 1.5% dextran sodium sulfate (DSS) in drinking water. In various studies, experimental models of acute colitis were induced by DSS administered for different periods of time (from several to <20 days) (11-13). The results are consistent with the majority of literature findings using this model of colitis to study the pathogenesis of non-specific IBDs. In our experiment, the agonist rosiglitazone and the antagonist bisphenol A diglycidyl ether (BADGE) were applied as the substances either stimulating or blocking PPAR-γ receptors and their effects on experimentally induced colitis were evaluated.

The aim of the present study was to assess the effects of the nuclear PPAR-γ receptor agonist and antagonist on experimental acute colitis induced by administration of 1.5% DSS in rats.

MATERIAL AND METHODS

Experiments followed a protocol approved by the local Animal Ethics Committee.

Eighty Wistar rats weighing 200-220 g were used. Colitis was induced using 1.5% dextran sodium sulphate (DSS) (Sigma Aldrich Company) administered in drinking water for 8 days. Rosiglitazone (Avandia - GlaxoSmithKline), 8 mg/kg body weight, dissolved in 0.9% NaCl to the volume of 1 ml, was given four times through a gastric tube. The dose was chosen based on literature data (14). Bisphenol A diglycidyl ether (BADGE) (Sigma Aldrich Company), a PPAR-γ antagonist, was administered intraperitoneally four times at the dose of 120 mg/kg body weight. BADGE was defined by Wright *et al*. (15) as a PPAR-γ selective antagonist. The individual groups of animals received DSS and rosiglitazone, or DSS and BADGE or DSS combined with rosiglitazone and BADGE. The behaviour of animals during the experiment as well as pre- and post-experiment body weights were assessed. After decapitation, macroscopic evaluation was performed; the collected intestinal material was examined histopathologically and immunoenzymatically (ELISA). The levels of interleukin (IL)-1β , IL-6, IL-10, TNF-α and MPO were determined in serum and intestinal homogenates.

The division of experimental groups (10 rats each) is presented in *Table 1*.

Histopathological examination

Tissue sections for microscopic examination were sampled at the distance of 2.5 cm, 5 cm and 7 cm from the colon of each animal in the experimental groups. H+E, mucicarmine and Masson's trichrome staining was carried out. In the microscopic picture, the following parameters were evaluated: oedema of the mucosa, range, intensity and depth of inflammation, inflammatory activity, follicle aggregates, ulceration, mucosa necrosis and crypt blunting. The above parameters were scored according to the following scale: 0- lack of changes, 1- slight focal and superficial changes of the mucosa, 2- more diffuse, moderate lesions reaching the muscular lamina, 3- lesions of significant intensity, affecting more than one section, reaching the muscular layer.

Enzyme-linked immunosorbent assay - ELISA

Levels of IL-1β, IL-6, IL-10, TNF-α and MPO were determined both in serum and in intestinal homogenates. For IL-1β, IL-6 and IL-10 determinations, ELISA plates from R&D System Inc. (USA) were used. For MPO determinations, ELISA plates from Hycult Biotechnology b.v. (Netherlands) were applied. Results were read using the ELISA scanner (Victor 3, Perkin Elmer, USA).

Statistical analysis

The Kolmogorov-Smirnov test was applied to check whether variables had normal distribution. Since it was not demonstrated, the Mann-Whitney test was used to compare two groups and find significant differences. The 5% error risk was assumed; thus, $p<0.05$ was considered statistically significant (*), $p<0.01$ - more significant (**), and $p<0.001$ - highly significant (***) *vs*. Group A (Control) or Group E (DSS) (description below the tables).

RESULTS

Histopathological evaluation of the large intestine

Histopathological findings in group A, B and D are very similar. The microscopic picture of the colon wall - normal; the mucosa - without features of inflammatory reaction or oedema; the structure of crypts preserved with proper mucus amount. There were no infiltrations with granulocytes and mononuclear cells observed. Single aggregates of lymphatic follicles were observed. The presence of the mucous layer covering the epithelium (*Fig. 1*).

Histopathological findings in group E: the inflammatory lesions were present at most of the colonic surface. In the microscopic picture, marked oedema of the intestinal wall was seen. In the mucous, submucous and muscular membrane (yet focally) - diffuse inflammatory infiltration with neutrophilic granulocytes of marked intensity and, to a lesser degree, with mononuclear cells was observed. Moreover, neutrophils penetrating the crypt epithelium and crypt abscesses were present. Diffuse, deep ulcerations invading the muscular lamina of the mucosa were also detected. In the submucosa, at the level of ulcerations, slight fibrosis is observed. Abnormal architectonics of

Table 1. Experimental groups, substances administered, doses and routes of their administration.

Group	substance	Routes of administration
A	control	Only fodder and water. Decapitation after 7 days.
B	rosiglitazone	In the dose of 8 mg/ kg b.w. dissolved in 0.9% NaCl (up to the total volume of 1ml) - given intragastric in 4 doses, on day 1, 2, 3 and 4. Decapitation on day 7.
C	BADGE	Intraperitoneal administration in 4 doses (120 mg/kg b.w.) on day 1, 3, 4 and 5. Decapitation - on day 7.
D	0.9% NaCl intragastric	Intragastric administration by a gastric tube in volume of 1ml in 4 doses, on day 1, 2, 3, 4. Decapitation - on day 7.
E	1.5% DSS	In drinking water *ad libitum* for 8 days.
F	1.5% DSS+rosiglitazone	In drinking water *ad libitum* for 8 days and 4 doses of Rosiglitazone (8mg/kg/b.w.) through a gastric tube on day 2, 4, 6, and 8 of DSS administration.
G	1.5% DSS+BADGE	1.5% DSS in drinking water *ad libitum* for 8 days and 4 doses of BADGE on day 2, 4, 6 and 8 of DSS administration.
H	1.5% DSS+rosiglitazone +BADGE	1.5% DSS in drinking water *ad libitum* for 8 days, rosiglitazone administered in 4 doses on day 1, 3, 5, 7 of the experiment, BADGE also in 4 doses on days 2, 4, 6 and 8. Decapitation on day 8.

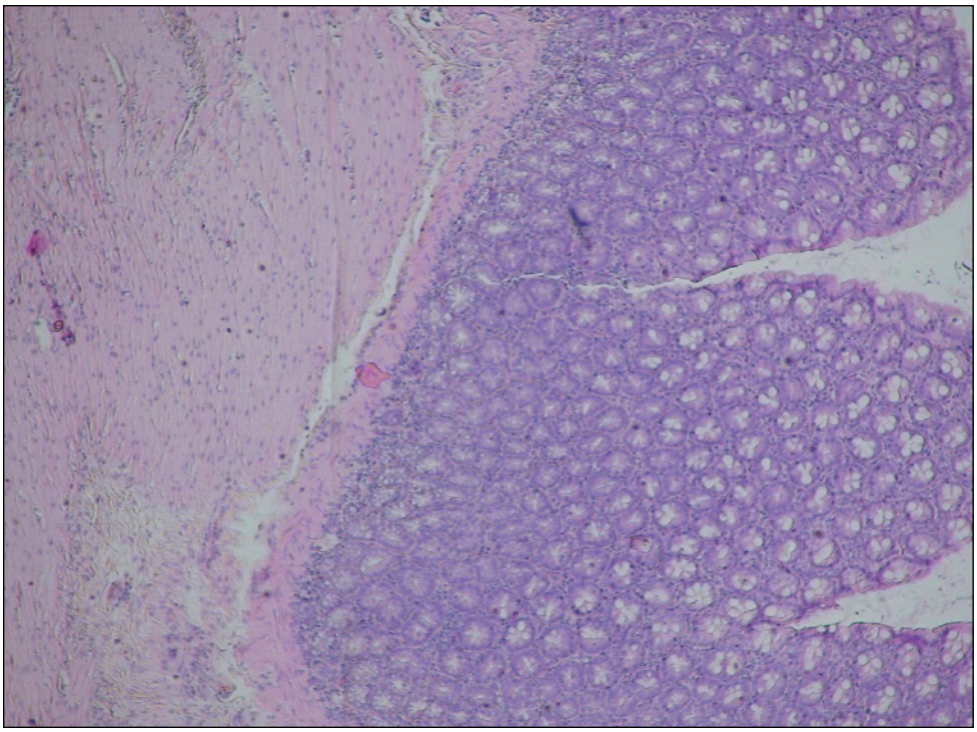

Fig. 1. Group A H+E x 50. The colonic mucosa shows normal appearance.

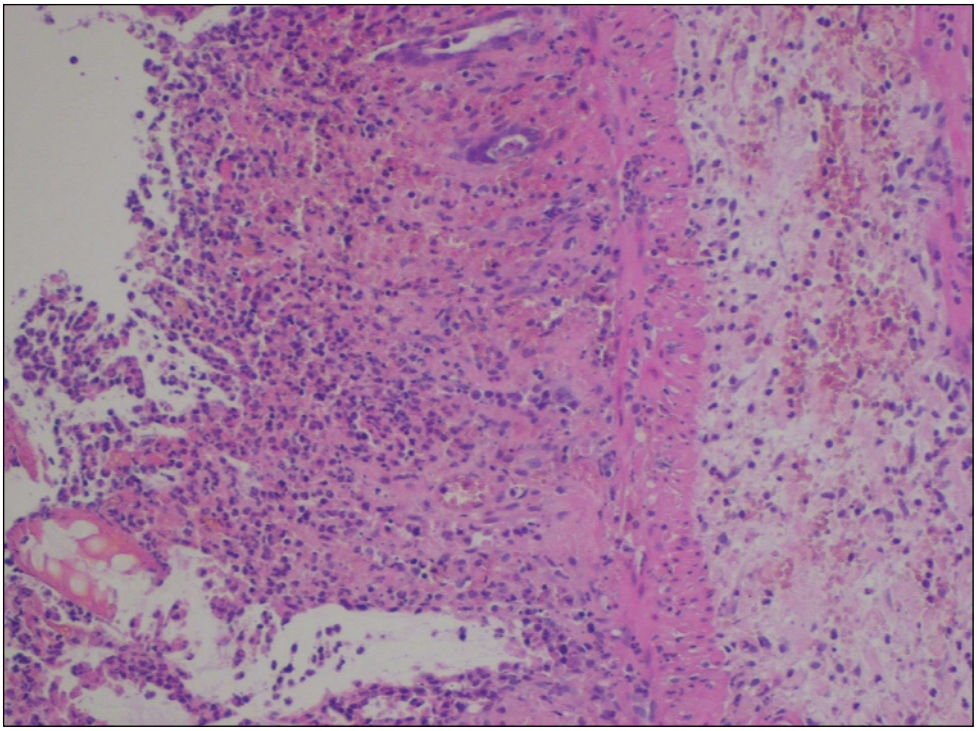

Fig. 2. Group E, H+E x 200. Ulceration of the mucosa with enhanced necrosis of intestinal crypts accompanied by intense inflammatory mucosal infiltration with neutrophils.

intestinal crypts with local atrophy and decreased amount of mucus is found. Furthermore, lack of the mucous layer covering the epithelium is observed (*Fig. 2*).

Histopathological findings in group F: the microscopic picture of the large intestinal wall shows inflammatory lesions of markedly decreased severity and extent. Slight oedema of the mucosa and submucosa are present. Moreover, slight atrophy of intestinal crypts are observed. Numerous aggregates of lymphatic follicles; no ulcerations within the mucous membrane were also observed (*Fig. 3*).

Histopathological findings in group G and H: the inflammatory lesions in the large intestine of similar severity as in group E were observed (*Fig. 4*). The mechanisms of action of agonist-activated and antagonist-deactivated PPAR-γ are complex and involve the effects on expression of selected genes. To explain them more clearly, the levels of some cytokines involved in the pathogenesis of non-specific colitis were analysed. In the experiment, the levels of IL-1β, IL-6, IL-10, TNF-α and MPO were determined in individual groups of animals in blood serum and colon homogenates using ELISA.

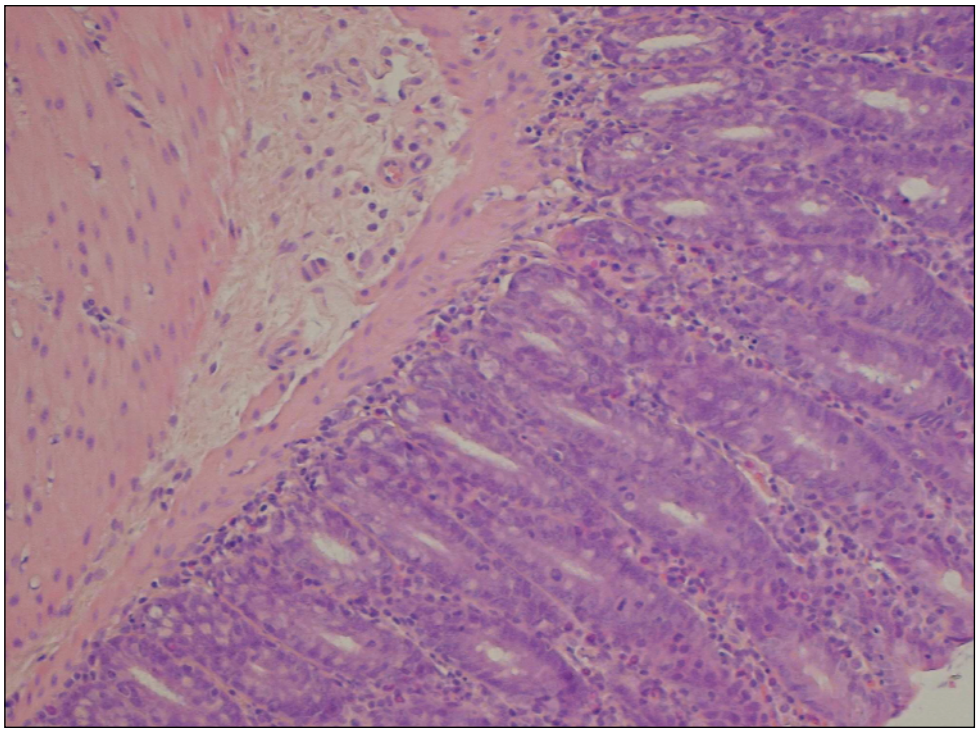

Fig. 3. Group F, H+E x 200. Slight oedema of the mucosa and submucosa. Moderate inflammatory infiltration of the mucosa with lymphocytes and few neutrophils focally penetrating the crypt epithelium.

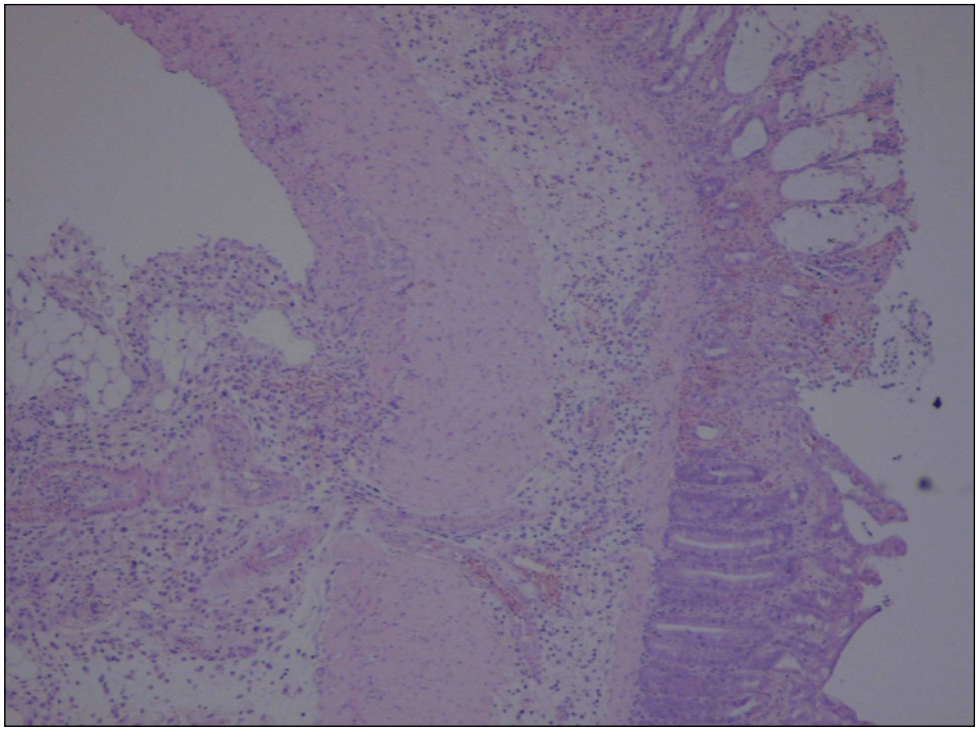

Fig. 4. Group H, H+E x 200. Ulceration of the colonic mucosa, oedema and intense inflammatory infiltration extending into the serosa.

Results of immune-enzymatic determinations

The *Table 2* presents the results of determinations of IL-1β, IL-6, TNF-α levels in serum expressed in pg/ml. The analysis showed statistically significantly increased levels of IL-1β, IL-6, TNF-α and MPO in the group receiving 1.5% DSS compared to the control group. The level of IL-10 in this group did not change significantly. The treatment with rosiglitazone in the group with colitis resulted in a significant decrease in TNF-α and MPO levels and significant increase in the level of IL-10 compared to the group receiving DSS alone. This seems to indicate multi-directional anti-inflammatory action of activated PPARs, *i.e.* inhibition of the expression of pro-inflammatory cytokines and intensification of factors limiting the inflammation such as IL-10. In the group H receiving DSS, rosiglitazone and BADGE, the level of TNF-α was significantly higher than in group F whereas the level of IL-10 did not change. Interestingly, the expression of TNF-α in the group receiving DSS with BADGE was significantly higher compared to the group administered DSS and rosiglitazone. This appears to result from BADGE-induced inhibition of PPAR activity manifesting itself in suppressed expression of proinflammatory factors and in enhanced synthesis of antiinflammatory factors (*Figs. 5-7*).

Table 2. Serum concentration of IL-1β, IL-6, IL-10, TNF-α and MPO in the experimental groups. Data expressed as a mean±S.D. Groups B, C, D: [1]p<0.05; [2]p<0.01; significantly different from the control group A; Groups F, G, H: [1]p<0.05; [2]p<0.01 significantly different from group E (DSS).

Group		Serum concentration of cytokines (pg/ml)				
		IL-1β	IL-6	IL-10	TNF-α	MPO
A	control group	159.7298	11.42433	6.26981	1.634288	931.388
B	rosiglitazone	89.7670[1]	6.89467[1]	9.88194[1]	1.944684	814.562
C	BADGE	471.1199[2]	27.90795[2]	10.80605[2]	2.433815	1010.037
D	0.9% NaCl intragastric	169.8054	14.93089	6.92886	2.373550	961.171
E	1.5% DSS	526.9715	34.77056	7.55502	3.941086	1253.970
F	1.5% DSS+rosiglitazone	349.3989	26.98454	12.99254[1]	2.242115[1]	813.210[2]
G	1.5% DSS+BADGE	612.7335	32.07852	11.64336	5.044326	1194.010
H	1.5% DSS+rosiglitazone +BADGE	400.1138	30.43177	7.60210	6.049179	1003.868

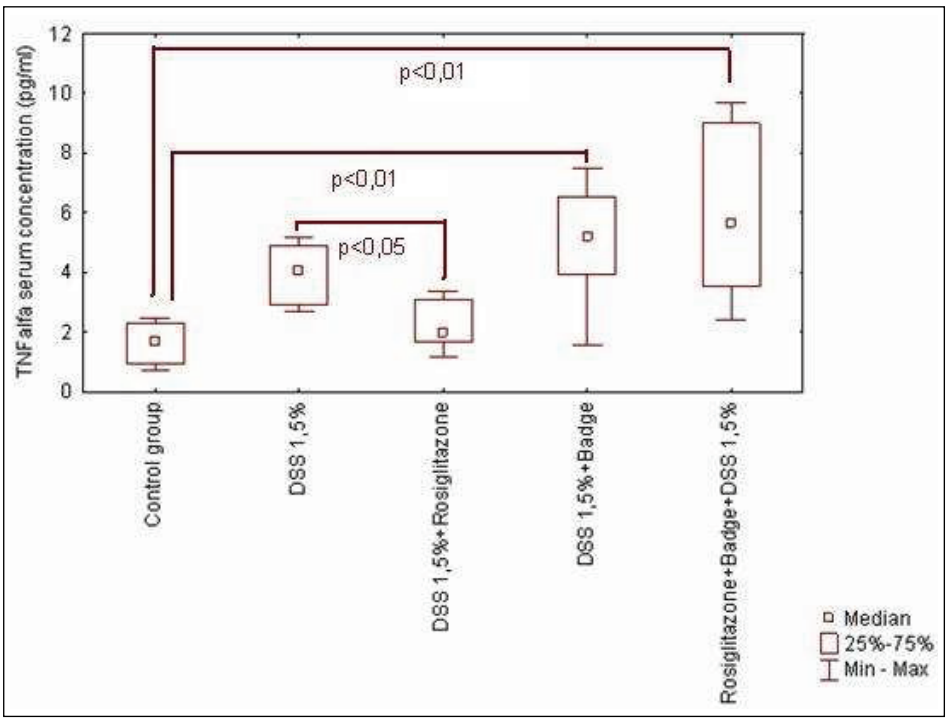

Fig. 5. Serum TNF-α concentration in the experimental groups.

The *Table 3* presents the results of determinations of IL-1β, IL-6 and TNF-α levels in colon homogenates expressed in pg/ml. The analysis of levels of the selected cytokines in colon homogenates revealed a statistically significant increase in IL-6, IL-10, TNF-α and MPO in the group receiving DSS compared to the control group. The use of rosiglitazone in rats with colitis resulted in a statistically significant decrease in the level of IL-6 and tissue MPO. In group F (rosiglitazone+DSS), no statistically significant alterations in TNF-α and Il-6 were observed. In group G (DS+BADGE), a significant reduction in the level of IL-10 was observed, which demonstrates that blocking PPAR activity results in reduced expression of IL-10. A statistically significant IL-6 reduction in the group B without colitis receiving intragastric rosiglitazone is worth emphasizing (*Fig. 7, 8*).

DISCUSSION

Effective therapy of inflammatory bowel diseases (IBDs) is the target of scientists and physicians worldwide. Novel, more efficacious and safer therapeutic options are continuously being searched for. Animal experiments still play an important role in this search; thanks to them, a given preparation may be included in or excluded from further clinical trials. The study results presented above with substances modulating the activity of PPAR-γ receptors are promising despite imperfections of each animal model in imitating the real pathophysiological processes in patients with IBDs. Our experiment was carried out in the animal model of colitis induced by 1.5% DSS administered in drinking water for 8 days. In literature models, the duration of DSS administration varied from several to dozen or so days (11-

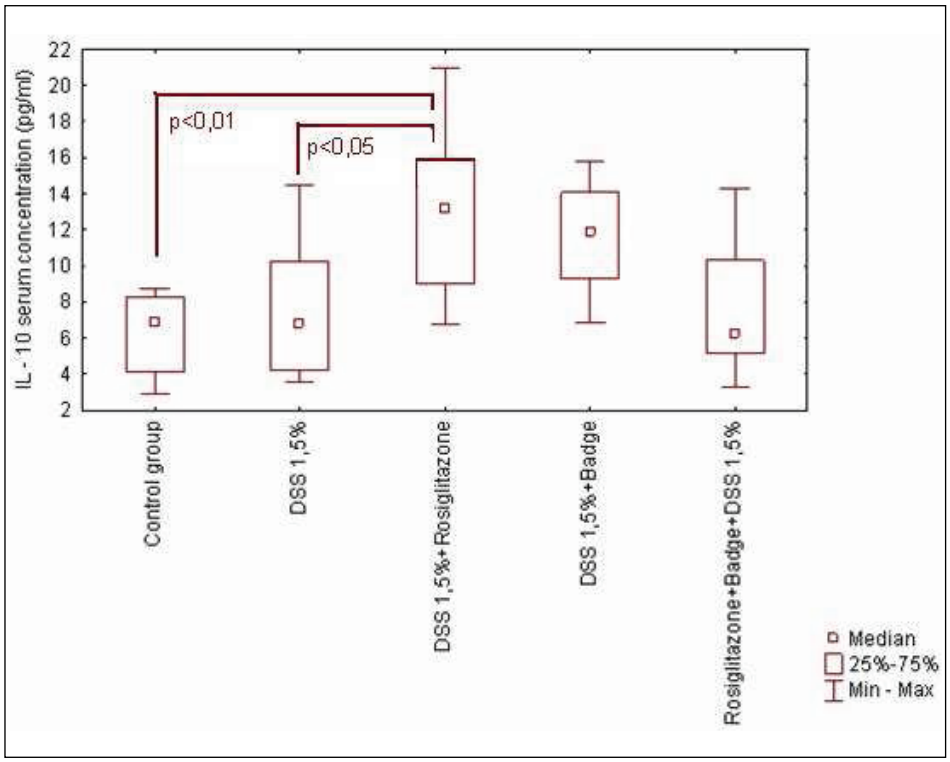

Fig. 6. Serum IL-10 concentration in the experimental groups.

Table 3. Colon homogenate concentration of IL-1β, IL-6, IL-10, TNF-α and MPO in the experimental groups. Data expressed as a mean±S.D. Groups B, C, D: [1]p<0.01 significantly different from the control group; Groups F, G, H: [1]p<0.05; [2]p<0.01 significantly different from group E (DSS).

Group		Colon homogenate concentration of cytokines (pg/ml)				
		IL-1β	IL-6	IL-10	TNF-α	MPO
A	control group	7317.37	111.4904	73.8243	70.2473	2447.783
B	rosiglitazone	8240.64	37.5236[1]	64.9671	78.5191	2719.491
C	BADGE	8216.41	125.1922	101.3393	73.6939	2687.193
D	0.9% NaCl intragastric	8394.77	118.2975	113.4314	91.1078	2816.031
E	1.5% DSS	11132.05	261.2906	114.9494	116.0834	4557.077
F	1.5% DSS+rosiglitazone	11340.93	146.2674[1]	119.1246	99.7347	2881.914[2]
G	1.5% DSS+BADGE	12611.53	137.1579[1]	84.9338[1]	131.8636	4269.870
H	1.5% DSS+rosiglitazone +BADGE	11966.08	243.0605	120.2294	98.1939	4298.156

13). Another model of colitis used most frequently is based on rectal administration of TNBS (16). In both models mice and rats are most commonly used.

The two most popular models of experimental colitis were compared by Celinski *et al*. (17). Their findings confirm the thesis that the model of acute colitis based on DSS in drinking water induces more severe inflammation resulting in higher body weight loss and more intensified inflammatory infiltration compared to the rectal TNBS model. They demonstrated that the DSS and TNBS model could accurately imitate the pathogenesis of Crohn`s disease and ulcerative colitis. The results of the present study were compared with the results of the doctoral thesis of Dworzanski (18), in which colitis was induced with rectal administration of TNBS in which histopathological lesions of the large intestine and levels of IL-1β, IL-6, IL-10, TNF-α and MPO were analysed. The comparative analysis demonstrated that rats receiving 1.5% DSS in drinking water developed more intensified histopathological lesions (larger extent of inflammatory changes, more severe oedema, more highly expressed abnormalities of intestinal crypt architectonics). The levels of IL-1β, IL-6, IL-10, TNF-α and MPO in serum and colon homogenates did not show statistically significant differences between the two models, with an exception of statistically significantly higher serum levels of MPO in the DSS model. Another confirmation of the usefulness of DSS model for evaluation of effects of pharmacological agents in patients with IBD and pathophysiological mechanisms accompanying these diseases are experimental study conducted by Cooper *et al*. (19). In their experiment, colitis was induced by oral administration of DSS for 7 days. Chronic inflammation was induced by DSS administered in three cycles, 7 days each. The

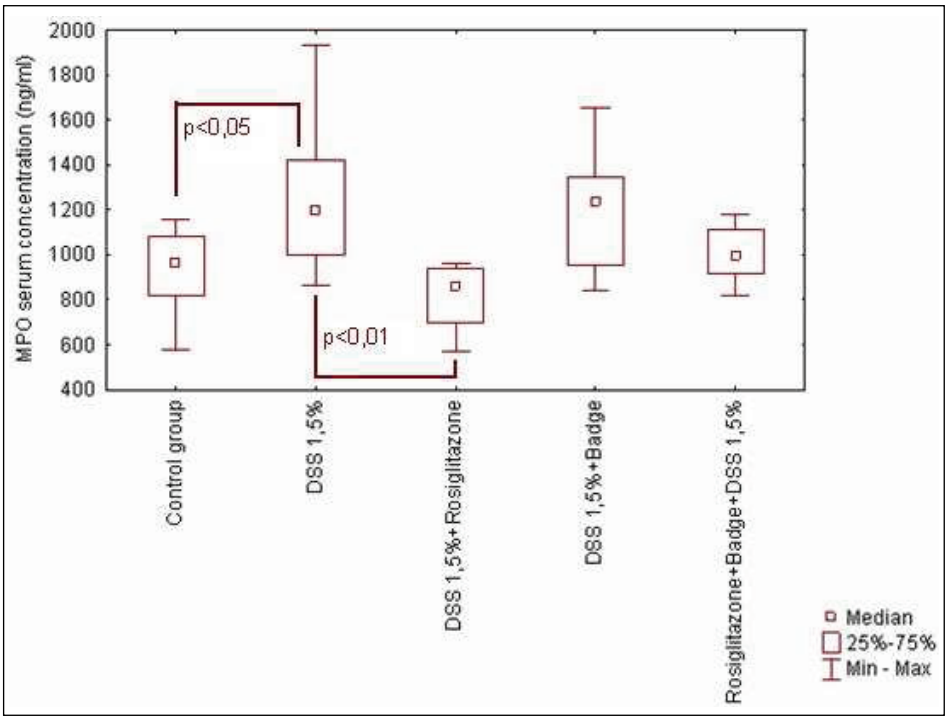

Fig. 7. Serum MPO concentration in the experimental groups.

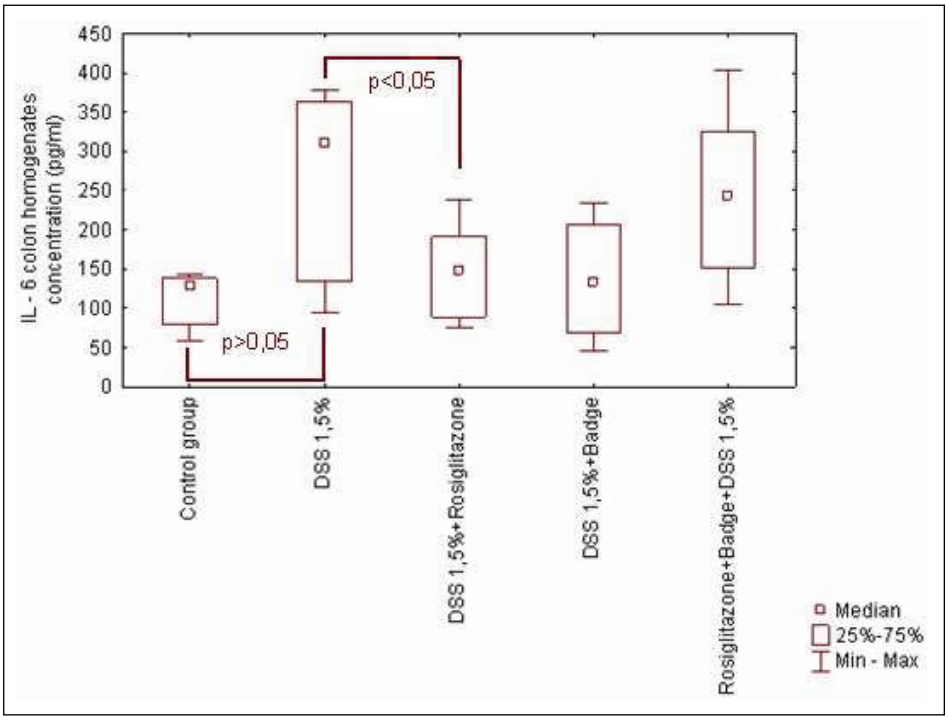

Fig. 8. Colon homogenate IL-6 concentration in the experimental groups.

authors conclude that this model enables also to study the conditions leading to dysplasia or even colorectal carcinoma. Recently, numerous reports were published concerning the effects of PPAR-γ agonists on limitation of colitis. On the other hand, studies regarding antagonists of this receptor are few. The main purpose for carrying out our study was to extend our knowledge on the effects of substances blocking and stimulating PPAR-γ on the course of colitis. Our findings demonstrating beneficial effects of rosiglitazone in the experimental model of colitis induced with DSS administered in drinking water are confirmed by the results reported by Saubermann *et al*. (20). The PPAR-γ agonists used by them included troglitazone, pioglitazone and rosiglitazone. In each case, the protective effects of PPAR-γ ligands on colitis were confirmed. Moreover, the results presented by Takaki *et al*. (21) demonstrate beneficial anti-inflammatory action of thiazolidinedione substances. In their study, intestinal inflammatory lesions were induced by 1% DSS administered to mice in drinking water. Pioglitazone and netoglitazone were used as PPAR-γ agonists. They employed PPAR-γ activating substances different from the ones used in our experiment; nonetheless, they confirm the anti-inflammatory effects of thiazolidinedione substances. Amongst the recent publications, the study by

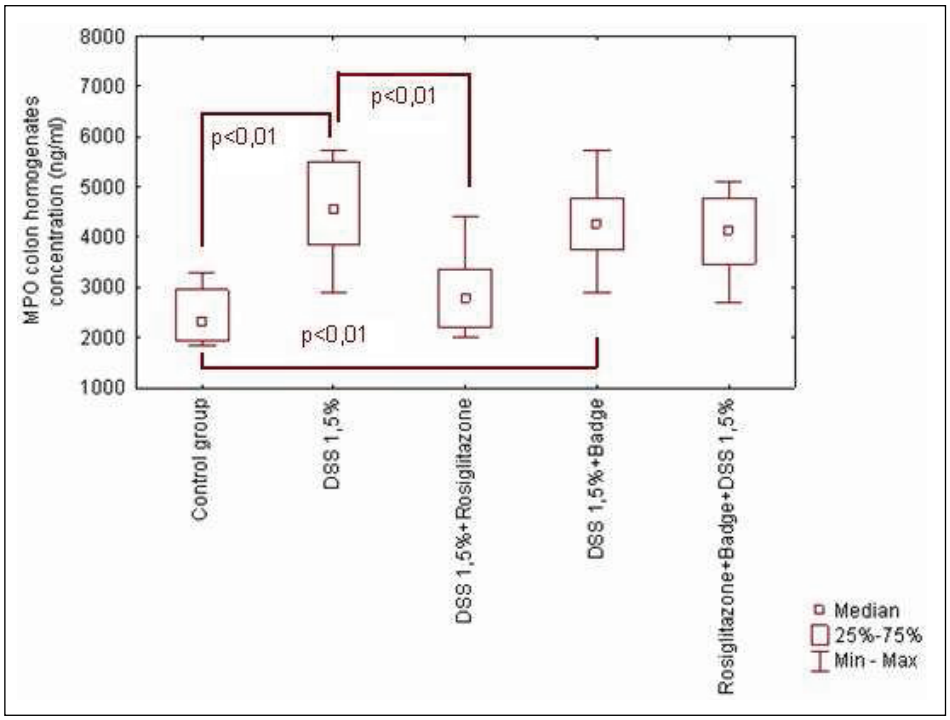

Fig. 9. Colon homogenate MPO concentration in the experimental groups.

Ramakers *et al*. (22) presents a slightly different opinion. In their experiment, rosiglitazone was administered to mice in fodder for 16 days before colitis was induced by 7-day administration of DSS. The authors demonstrated that rosiglitazone administered in such a way aggravated the course of induced inflammation. They suggest that exacerbated inflammation might have been the result of long-term exposure to rosiglitazone, which sensitised the mucous membrane to the injuring factor, *i.e.* DSS. The authors postulate that the enhanced inflammatory reaction resulted from increased permeability of the mucosa.

The results of IL-1β determinations do not demonstrate reduction in its serum concentration in rats receiving rosiglitazone together with 1.5% DSS inducing inflammation. The fact that in healthy rats without colitis rosiglitazone reduced the serum level of IL-1β is worth stressing. According to Shan *et al*. (23), activation of PPAR-γ inhibited the expression of pro-inflammatory cytokines, including IL-1β. Schaeffer *et al*. (24) conducted the study concerning the inhibitory effects of stimulated PPAR-γ on activation and inflow of lymphocytes Th1 to the bowel affected by the inflammatory process in mice. Pioglitazone decreased the concentration of chemotactic protein for lymphocytes Th1 and ultimately inhibited the inflammatory reaction, which was confirmed by analysis of TNF-α and INF-γ levels. Their findings are consistent with our results. Furthermore, Cuzzocrea *et al*. (25), evaluating the effect of rosiglitazone on experimentally induced acute phase of pleurisy in rats, demonstrated anti-inflammatory action of rosiglitazone, which administered intraperitoneally limited the expression of IL-1β and TNF-α. One of the experimental groups created by the authors received rosiglitazone concurrently with BADGE (25). The blocking of PPAR-γ activity did not result in decreased levels of pro-inflammatory parameters. The effects of rosiglitazone and BADGE on IL-1β concentration in our present study confirms the findings presented and discussed in their paper.

The analysis of IL-6 discloses that its colon homogenate level significantly decreased in group F with experimental colitis receiving rosiglitazone. The administration of BADGE together with DSS in group G and BADGE with DSS and rosiglitazone in group H did not affect the concentration of IL-6. This would not confirm blocking of endogenous, anti-inflammatory activity of PPAR-γ limiting the expression of this interleukin by BADGE. The study demonstrating the essential role of IL-6 in the inflammatory bowel process was carried out by Naito *et al*. (26). In their experiment, 4.5% DSS was given in drinking water for 8 days to wild-type mice and transgenic IL-6 knockout mice. The levels of TNF-α, IL-6, IL-10, iNOS mRNA were markedly lower in the group of transgenic mice compared to wild-type mice. Such data indicate that blocking of IL-6 expression has beneficial effects on colitis. Evidence demonstrating decreased expression of genes for IL-6 due to activation of PPAR-γ system is undoubtedly provided by Yamamoto *et al*. (27).

In order to understand the mechanisms of inflammation control, the possible relation between the action of PPAR-γ ligands and expression of anti-inflammatory IL-10 is essential. The analysis of results obtained in the present study shows the enhanced serum concentration IL-10 in the DSS+rosiglitazone group F. The serum level of this cytokine, compared to controls, increased statistically significantly in groups with colitis. The anti-inflammatory properties of PPAR agonists result not only from their ability to reduce the expression of pro-inflammatory factors but also to induce the synthesis of inflammation-limiting factors, *e.g.* IL-10. Literature findings are consistent with our results. Saubermann *et al*. (20) induced inflammation in mice using 2.5% DSS in drinking water and demonstrated the beneficial effects of rosiglitazone, pioglitazone and troglitazone on inhibition of the inflammatory reaction. PPAR agonist decreased levels of proinflammatory cytokines dependent on the activity of Th1 lymphocytes, *i.e.* TNF-α and INF-γ, and increased activity of Th2-dependent processes evidenced by the enhanced expression of IL-10 and IL-4, were observed. The conclusions presented by Kim Myung-Gyu *et al*. (28) are of interest. The aim of their study was to analyse the effect of rosiglitazone on nephritis in mice. Rosiglitazone was injected intraperitoneally over 3 successive days and followed by the injection of cisplatin 20 mg/kg/ b.w. causing renal damage. The results showed decreased levels of pro-inflammatory cytokines with simultaneously increased levels of IL-10. The authors suggested that activation of IL-10 was mediated by

rosiglitazone. Amongst the recent studies concerning the importance of IL-10 in the pathogenesis and course of non-specific colitis, the results reported by Lytle *et al*. (29) are worth mentioning. In their experiment transgenic IL-10 knockout mice with spontaneous colitis were used. The animals received normal laboratory fodder and fodder containing rosiglitazone for the period of 12 weeks. The PPAR-γ agonist was found to delay markedly the development of inflammatory reaction in the colon. Rosiglitazone inhibited the expression of mRNA for TNF-α, INF-γ, IL-17, and iNOS. The IL-10 deprivation did not affect the action of the PPAR-γ activating substance. Interestingly, in both groups of IL-10 knockout mice, the use of rosiglitazone 5 weeks after the development of colitis symptoms was ineffective. The data from this study are consistent with our findings, *e.g.* inhibitory effects of rosiglitazone on the expression of pro-inflammatory cytokines, such as TNF-α because the positive effects of PPAR-γ agonist on limitation of synthesis of this cytokine in animals with experimental colitis were demonstrated. The use of the antagonist BADGE did not significantly change the course of DSS-induced inflammation. The concurrent administration of the PPAR-γ agonist and antagonist in DSS group H resulted in statistically non-significant increase in serum TNF-α levels. The levels of this cytokine were also determined in colon homogenates and a statistically significant decrease in its expression after activation of PPAR-γ by rosiglitazone was observed. Our results are consistent with those reported by Saubermann *et al*. (20) and that the ligands of PPAR-γ *e.g.* rosiglitazone, had beneficial effects on DSS-induced colitis. Markedly decreased levels of interferon γ and TNF-α were observed. Shimizu *et al*. (30) studied the influence of taurine on DSS-induced inflammation and found that taurine inhibited the secretion of TNF-α, thus alleviating the inflammation. The properties of BADGE, also used in our study, are in keeping with results published by Harold *et al*. (31). According to them, the use of BADGE displaced rosiglitazone bound with PPAR-γ. This effect results most likely from blocking of PPAR-γ or displacement of agonists bound with the receptor. MPO is an enzyme of the peroxidase family of potent antiviral and bactericidal action. The immunoenzymatic assay of the intestinal tissue homogenates confirmed higher levels of MPO in the group E receiving DSS, group F receiving DSS and BADGE as well as in the group H receiving DSS, rosiglitazone and BADGE. In the colon homogenate with experimental colitis and rosiglitazone, the level of MPO was significantly lower compared to the group not receiving the PPAR-γ agonist, which evidences less intensified infiltration of neutrophilic granulocytes in the rosiglitazone group. The relation between MPO levels in the experimental model of colitis and the use of rosiglitazone or otherwise was discussed by Sanchez-Hidalgo *et al*. (14). These authors demonstrated beneficial effects of rosiglitazone on limitation of neutrophilic infiltration in the intestinal tissue (14), which is consistent with our findings. A similar effect, *i.e.* limitation of inflammatory infiltration, related to the decrease in MPO, was described by Cuzzocrea *et al*. (32). Anti-inflammatory effects of rosiglitazone were also confirmed by Adachie *et al*. (33). The authors induced experimental colitis with DSS. Their findings suggest that anti-inflammatory action of rosiglitazone resulted from activation of PPAR-γ receptors and from mechanisms independent of them.

In summary, the use of PPAR-γ antagonist rosiglitazone inhibited the inflammatory process in experimental DSS-induced colitis in rats. The activated PPAR-γ inhibited the expression of pro-inflammatory factors, such as IL-6, TNF-α, and neutrophil chemotaxis, which was evidenced by MPO reduction in serum and colon homogenates mediated by rosiglitazone. Moreover, positive effects of rosiglitazone on IL-10 expression were demonstrated. During the short period of observation, BADGE did not affect histopathological inflammatory markers. Furthermore, BADGE effects on blocking of anti-inflammatory activity of PPAR-γ were not observed.

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