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# INFLUENCE OF THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPAR-γ) AGONIST, ROSIGLITAZONE AND ANTAGONIST, BIPHENOL-A-DIGLICYDYL ETHER (BADGE) ON THE COURSE OF INFLAMMATION IN THE EXPERIMENTAL MODEL OF COLITIS IN RATS

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PPAR-γ plays a role in the development of immune response, particularly in inflammation. The inflammatory reaction may be stimulated or suppressed by the presence of PPAR ligands. Some researchers suggest positive influence of the PPAR-γ agonist on suppression of the intestinal inflammatory process, yet there has not been much evidence showing that the antagonist of PPAR-γ can affect the inflammatory process. The aim of the present study was to define the mechanism by which PPAR-γ ligands affect the course of experimentally induced colitis in rats. Colitis was induced in rats by rectal administration of TNBS (trinitrobenzene sulfonate). Rosiglitazone was administrated to animals at the dose of 8 mg/kg four times *via* an intra-gastric probe. Biphenol-A-diglicydyl ether (BADGE) was administrated intraperitoneally at the dose of 120 mg/kg, three times every second day. One group of animals received rosiglitazone together with BADGE before the induction of inflammation. Histological and ELISA examinations of large intestine samples were performed. Levels of IL-1β, IL-6, TNF-α cytokines were determined in serum and homogenates. Rats exposed to rosiglitazone had higher body weight yet lower large intestine weight. Histological findings showed less ulceration, lower expression of crypts' loss and smaller oedema. Animals, which did not receive rosiglitazone, and those receiving it together with BADGE, developed more severe inflammatory changes. Rosiglitazone decreased the expression of inflammatory cytokines, such as IL-6 and TNF-α, both in serum and in intestinal homogenates. BADGE used with TNBS did not increase the expression of inflammatory cytokines; however, applied together with rosiglitazone, it caused inflammation similar to that observed among rats with experimentally induced colitis. Rosiglitazone reduces inflammation by decreasing the expression of IL-6 and TNF-α. BADGE administered with rosiglitazone blocks the activity of PPAR-γ and abolishes the protective effects of PPAR-γ agonist.

Key words: *biphenol-A-diglicydyl ether (BADGE), peroxisome proliferator-activated receptor gamma, rosiglitazone, trinitrobenzene sulfonate (TNBS), tumor necrosis factor-α, interleukin-1β*

## INTRODUCTION

Nowadays, thousands of people in the world suffer from inflammatory bowel diseases (IBDs). The number of patients diagnosed with ulcerative colitis and Crohn's disease is increasingly high, especially in the developed countries. Despite continuous advances in medicine, IBDs remain incurable. Numerous groups of medicines applied in the treatment of IBDs are not fully effective to inhibit their aggravation or maintain long-term remission. Recent studies on immunological processes involved in colitis seem promising. The identification of peroxisome proliferator-activated receptors (PPARs) and their potential immune-modulating effects on the course of inflammation provides new therapeutic options for IBDs.

Peroxisome proliferator-activated receptors belong to a group of nuclear receptors activated by steroid hormones (1-5). As transcription factors regulating gene expression, they significantly affect many physiological and pathophysiological processes observed in living organisms (6-10). There are more and more reports suggesting that PPAR plays a crucial role in the development of immune reaction; particularly in inflammation (11-14). PPARs-γ present in the alimentary canal (the liver, pancreas, large intestine) are characterized by the widest spectrum of the effects mentioned (15-18). The receptors might be indirectly activated by synthetic ligands such as thiazolidinediones (troglitazone, ciglitazone, pioglitazone, rosiglitazone) or by fibrates (fenofibrate, clofibride, bezafibrate), which are PPAR-α agonists (19-22). Moreover, influence of non-steroidal anti-inflammatory drugs on PPAR activation has been reported. Many recent studies describe beneficial effects of PPAR-γ stimulation on inhibition of gastro-intestinal inflammation. The literature available, including studies in animal models, demonstrates decreased expression of inflammatory cytokines, such as TNF-α, IL-1β,

IL-6 and myeloperoxidase (MPO) in the intestinal mucosa mediated by PPAR agonists (23, 24). According to some other experimental studies, PPAR-γ ligands, through selective blockage of NF-κB signaling pathway *in vitro*, lead to a significant decrease in inflammatory IL-8 and MCP-1 expression (25, 26). Due to the immune regulatory functions of PPAR-γ, further studies on their possible application in inflammations have been carried out. The present study focuses on possible anti-inflammatory effects of the PPAR agonist (rosiglitazone) and its influence on the production of pro- and anti-inflammatory cytokines in both serum and intestinal tissues in the experimental model of colitis. Moreover, effects of the PPAR-γ antagonist on the course of inflammation were analyzed.

The aim of the study was to evaluate the effects of the PPAR-γ agonist (rosiglitazone) and antagonist (BADGE) on the course of TNBS-induced colitis in rats.

## MATERIALS AND METHODS

### *Experimental animals*

The study was conducted on Wistar rats weighing 200-220 g. Rats were placed in the laboratory cages, four animals each, at an ambient air temperature of 22-24°C and humidity of 70-75%. 12-hour daylight was provided. Animals had unlimited access to water and fodder.

Experiments followed the protocol approved by the local Animal Ethics Committee in Lublin no. 23/2008.

### *Induction of large intestine inflammation*

Animals were anaesthetized with intraperitoneal ketamine. Inflammation was induced by single rectal administration of TNBS (trinitrobenzene sulfonate) (Sigma Aldrich Company) at the dose of 10 mg dissolved in 50% ethanol to the volume of 0.25 ml. TNBS was administrated through a rectal catheter, 2 mm in diameter, introduced to the depth of 8 cm from the rectal sphincter. To assess the effects of the PPAR-γ agonist and antagonist, animals were divided into 10 groups. Rosiglitazone (Avandia GlaxoSmithKline) was administrated by a gastric tube at the dose of 8 mg/kg of b.w. after dilution in 0.9% NaCl to the volume of 1 ml. The dose was chosen based on literature data (in particular, Sanchez Hidalgo *et al*. (27)). BADGE (biphenol-A-diglicydyl ether) (Sigma Aldrich Company), defined by Wright *et al*. as a PPAR-γ pure antagonist, was used as their blocker (28). BADGE was given intraperitoneally four times at the dose of 120 mg/kg. Animals were under daily observation (behavior, body weight changes and diarrhea). Rats were sacrificed by guillotine decapitation.

### *Experimental groups*

*Table 1* presents the experimental groups of animals and substances administered.

### *Evaluation of large intestine inflammation*

Macroscopic evaluation of effects of modulating substances on PPAR activity involved: changes in body weight, adhesions in the abdomen and stool consistency. The presence of adhesions

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

| Group | N | Substance | Routes of administration |
|---|---|---|---|
| I | 8 | CONTROL GROUP | Only fodder and water. Decapitation after 7 days |
| II | 8 | ROSIGLITAZONE | In the dose of 8 mg/kg b.w dissolved in 0.9% NaCl (up to the total volume of 1ml) - given in 4 doses, on day 1, 2, 3 and 4 day. Decapitation on day 7. |
| III | 8 | BADGE | Intraperitoneal administration in 4 doses (120 mg/kg b.w.) on day 1, 3, 4 and 5. Decapitation - on day 7. |
| IV | 8 | 0.9%NaCl intrarectal | Single rectal administration, volume of 0.25 ml. Decapitation - on day 7. |
| V | 8 | 0.9% NaCl intragastric | Single intragastric administration by a gastric tube, volume of 1ml. Decapitation - on day 7. |
| VI | 8 | TNBS | Single rectal administration at the dose of 10 mg/kg b.w. dissolved in 50% ethanol up to the volume of 0.25 ml. Decapitation - on day 7. |
| VII | 8 | ROSIGLITAZONE + TNBS | Rosiglitazone at the dose of 8 mg/kg b.w. given 48, 24 and 1 hour before induction of inflammation (rectal administration of TNBS) as well as 24hours after induction. Decapitation - on day 7. |
| VIII | 8 | BADGE + TNBS | BADGE at the dose of 120 mg/kg b.w. administrated intraperitoneally in 4 doses on day 1, 3, 5 and 6. The final dose was given on the day of rectal administration of TNBS. Decapitation - on day 8. |
| IX | 8 | ETHANOL 50% intrarectal | (TNBS solvent) single rectal administration in the volume of 0.25 ml. Decapitation - 7 days after administration |
| X | 8 | ROSIGLITAZONE + BADGE + TNBS | BADGE (120 mg/kg) given on day 1, 3, 5 and 6. Rosiglitazone at the dose of 8 mg/kg b.w. administrated on day 2, 3, 5 and 7. TNBS along with BADGE given on day 6. Decapitation - on day 8. |

was assessed according to the 0-2 scale, whereas stool consistency according to the 0-1 scale. Furthermore, intestinal specimens were prepared for histopathological examinations and for the enzyme-linked immunosorbent assay (ELISA).

*Histopathological examination*

Tissue sections for microscopic examination were sampled at the distance of 2, 5 and 7 cm from the colon. Slides were stained with H&E, mucicarmine and T. Masson. The following parameters were evaluated: edema of the mucosa, range, intensity and depth of inflammation, inflammatory activity, follicle aggregates, ulceration, mucosal necrosis and crypt blunting. The scale used was as follows: 0 – lack of changes, 1 – slight focal and superficial mucosal changes, 2 – more diffused, moderate lesions reaching the muscular lamina, 3 – significantly intensive changes involving more than one section and reaching the muscular layer.

*Enzyme-linked immunosorbent assay - ELISA*

Levels of IL-1β, IL-6 and TNF-α were determined both in serum and in intestinal homogenates. IL-1β and IL-6 levels were evaluated using the ELISA plates (R&D System Inc. USA). Results were read with an ELISA Victor 3 scanner (PERKIN ELMER USA Company).

*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 (***).

## RESULTS

Changes in general condition and behavior of rats were not observed in group I (controls), II (rosiglitazone), as well as IV and V (0.9% NaCl administrated intragastrically and rectally). In these groups, body weight losses and adhesions were not observed. In groups VI, VIII and X, which received TNBS, TNBS + BADGE and TNBS + BADGE + rosiglitazone, respectively, decreased fodder intake, increased diarrhea and losses of body weight were found on the decapitation day compared to controls. Macroscopic examination of the large intestine indicated changes typical of acute inflammation were noticed: edema, ulceration and congestions. In these groups, stronger correlations between the intestine weight and its length as well as interintestinal adhesions were observed. In group VII (rosiglitazone at the dose of 8 mg/kg/b.w.), weight gain was reported; the adhesion and stool consistency scores were found lower compared to the TNBS group. *Table 2* presents detailed results of the parameters examined.

*Results of histopathological examinations*

In group I (control) and group IV, which received 0.9% NaCl per rectum, except for insignificant and focal mucosal edema, no microscopic changes in the large intestine were observed. Observations in group II (rosiglitasone) and group III (BADGE) were similar. No significant microscopic changes were found, except for mild edema and scattered lymphoid follicles within the mucosa present in 3 cases. In group VI receiving TNBS, the microscopic findings showed the changes of experimental colitis, *i.e*. mucosal ulcerations and transmural intense inflammatory infiltrates composed mostly of lymphocytes and less numerous plasmocytes. The inflammatory process was active with neutrophiles entering the glandular epithelium and the lumen of crypts. Inflammatory infiltratrations involved the full thickness of the large bowel wall, with involvement of the serosal surface in one case. Additionally, the following microscopic features were present: edema of the mucosa, formation of lymphoid follicles, focal loss of crypts and superficial epithelium. The lesions were associated with architectural distortion and focal fibrosis within the lamina propria and submucosa. The amount of mucin was decreased. (*Fig. 1, 2*).

In group VII (rosiglitazone + TNBS), microscopic changes were less intense and focal. In 2 animals, colonic mucosa

*Table 2.* Body weight changes, adhesions and diarrhea score in the experimental groups. Data expressed as mean ±S.D.

| GROUP | n | Body weight changes (g) | Adhesions (score 0–2) average | Diarrhoea (score 0–1) average |
|---|---|---|---|---|
| CONTROL GROUP | 8 | 15.375±2.97 | 0.0 | 0.0 |
| ROSIGLITAZONE | 8 | 19.75±5.06[2] | 0.0 | 0.0 |
| BADGE | 8 | 11.75±2.25[2] | 0.625 | 0.25 |
| 0.9% NaCl - INTRARECTAL | 8 | 14.5±2.2[3] | 0.0 | 0.125 |
| 0.9% NaCl INTRAGASTRIC | 8 | 15.625±2.87 | 0.0 | 0.0 |
| TNBS | 8 | -28.37±13.09[1] | 1.625 | 0.5 |
| ROSIGLITAZONE + TNBS | 8 | 10.375±4.75[23] | 0.875 | 0.375 |
| BADGE + TNBS | 8 | -38.00±14.12[°1] | 1.75 | 0.625 |
| ETHANOL 50% | 8 | 8.125±2.64[13] | 0.125 | 0.375 |
| ROSIGLITAZONE + BADGE + TNBS | 8 | -17.00±8.55[°1] | 1.625 | 0.625 |

[1]$P<0.001$; [2]$P< 0.05$ significantly different compared to controls;
[3]$P<0.001$ significantly different compared to TNBS group
[°]$P<0.001$ significantly different compared to Rosiglitazone + TNBS group

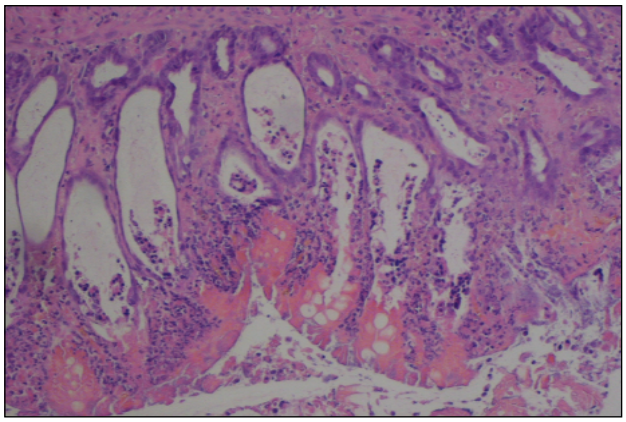

*Fig. 1.* Similar microscopic appearance was seen in group VIII administered with TNBS and BADGE. Histopathological picture of the large intestine in TNBS group (VI). H+E magnification x 200. Large bowel wall showing mucosal necrosis covered by fibrinous exudates, intense inflammatory infiltrates with neutrophils entering the lumen of crypts and focal hemorrhages within the lamina propria.

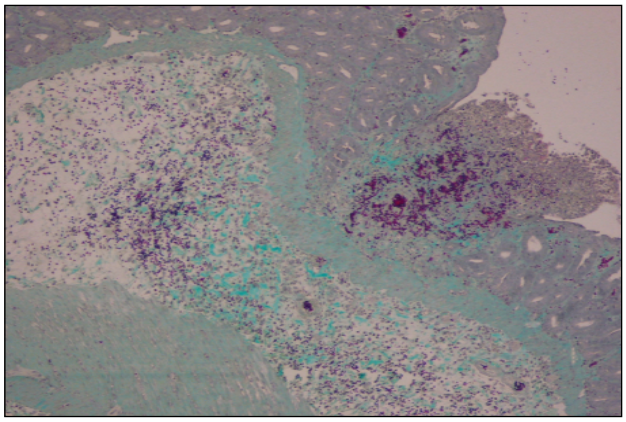

*Fig 2*. Histopathological picture of the large intestine in BADGE+TNBS group (VIII). Trichrom Masson staining, magnification x200. Ulceration of the colonic mucosa accompanied by mild fibrosis within the submucosa.

showed inflammatory changes present within more than one section of the intestine. The inflammatory infiltrations were mild to moderate and confined only to the mucosa with focal involvement of the submucosa. In 4 out of 8 animals, mild mucosal oedema was observed. Moreover, focal loss of crypts was found with decreased amount of mucin. No ulcerations were noted; however, focal loss of superficial epithelium was present in 2 out of 8 animals in this group (*Fig. 3*).

*Results of immune-enzymatic determinations (Table 3)*

Levels of IL- 1 β, IL-6, and TNF-α were determined both in serum and in intestinal homogenates, according to ELISA methodology. In group VI (TNBS), considerably increased levels of IL-1β and IL-6 were observed compared to controls. In group VII (TNBS + rosiglitazone), levels of those cytokines were higher than in the control group, yet significantly lower than in group VI. Interestingly, in the group receiving rosiglitazone alone, levels of cytokines were lower than in the control group. In the BADGE group, the level of IL-1β was higher compared to the control group, however, the increase observed was lower than in group VI (only TNBS); the level of IL-6 increased and reached

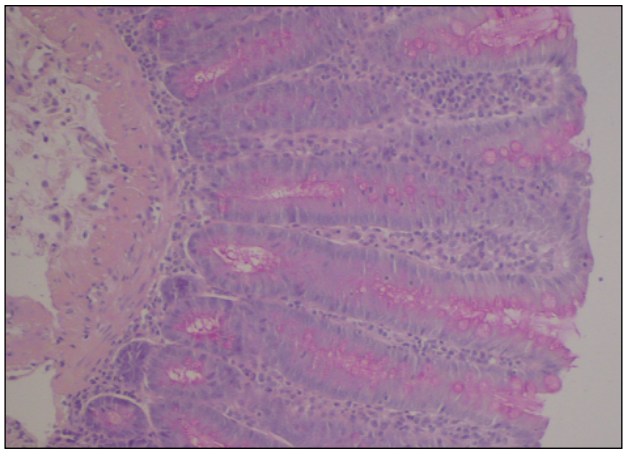

*Fig. 3*. Histopathological picture of colonic mucosa healing after therapy with rosiglitazone in group VII. Mucicarmine staining, magnification x200. Colonic mucosa showing very mild edema within the mucosa and submucosa. Glandular epithelial cells within the colonic crypts show normal amount of mucin.

the level observed in group VI. Moreover, in group X (rosiglitazone with BADGE and TNBS), increased values of cytokines were reported, indicating that beneficial anti-inflammatory effects of the agonist following earlier administration of the antagonist were abolished (*Figs. 4* and *5*).

Determinations of TNF-α levels in serum also confirmed anti-inflammatory effects of rosiglitazone - a significantly lower level in group VII compared to group VI. In the BADGE group, the level of TNF-α was higher than in the control group. The group administered 50% ethanol (solvent of TNBS) showed increased levels of IL-6, IL-10 and TNF-α. The level of IL-1β was not different from that in the control group (*Fig. 6*).

The *Table 4* below presents the results of determinations of IL-1β, IL-6, TNF-α levels in rats' intestinal homogenates (in pg/ml).

The levels of IL-1β in group VI (TNBS) and VIII (TNBS + BADGE) were significantly higher. A characteristic decrease in IL-1 level, similar to the level observed in the control group, was reported in group VII (rosiglitazone + TNBS) (*Fig. 7*).

Analysis of IL-6 levels in intestinal homogenates in all groups demonstrated a significant decrease in group II (rosiglitazone alone). In group VI, an increase in IL-6 was noticed, whereas in group VII its level was more normalized. No statistically significant differences between groups VI and X were observed (*Fig. 8*).

Levels of TNF-α in intestinal homogenates were similar. The increased level was observed in group VI, whereas normalization of its level was reported in group VII (rosiglitazone and TNBS). The level of TNF-α in group X was similar to that in group VI (TNBS alone). In the remaining groups, no differences were noticed compared to the control group.

## DISCUSSION

In recent years, many scientific reports describing the influence of PPAR-γ ligands on inflammation of intestines have been published. The results of some of them are very promising, particularly regarding the use of PPAR-γ agonist for the inhibition of colitis. However, some scientists are skeptical about such a therapy and claim that agonists of PPARs-γ might have negative effects on the course of inflammation. There are few reports on applications of PPAR-γ antagonists. Thus, the aim

*Table 3.* Serum concentration of IL-1β, IL-6, TNF-α in the experimental groups.

| Serum concentration of cytokines (in pg/ml) | | | | | |
|---|---|---|---|---|---|
| GROUP | | N | IL-1β | IL-6 | TNF-α |
| I. | CONTROL GROUP | 8 | 159.7298± 88.4991 | 11.42433± 3.19190 | 1.634288± 0.701483 |
| II. | ROSIGLITAZONE | 8 | 89.7670± 80.9915[2] | 6.89467± 4.64757[2] | 1.944684± 0.570707 |
| III. | BADGE | 8 | 471.1199± 153.3448[n] | 27.90795± 12.90284 | 2.433815± 1.160536 |
| IV. | 0.9%NaCl - INTRARECTAL | 8 | 168.3947± 143.3181 | 14.46444± 10.45514 | 2.632636± 0.821097 |
| V. | 0.9%NaCl INTRAGASTRIC | 8 | 169.8054± 140.3690 | 14.93089± 8.10582 | 2.373550± 1.414553 |
| VI. | TNBS | 8 | 536.3999± 196.0502[3] | 26.10943± 9.36104[1] | 3.839744± 1.202331[1] |
| VII. | ROSIGLITAZONE + TNBS | 8 | 261.3975± 85.2432[n°] | 21.27146± 11.22844[1] | 1.149454± 0.800587 |
| VIII. | BADGE + TNBS | 8 | 361.5786± 122.8530[1] | 48.01048± 12.41838[3] | 3.890731± 1.979335[2] |
| IX. | ETHANOL 50% | 8 | 173.5890± 104.9925[°] | 16.46571± 11.10690 | 2.328991± 0.899024 |
| X. | ROSIGLITAZONE + BADGE + TNBS | 8 | 312.8517± 149.3198[2] | 29.29657± 13.93307 | 3.325422± 1.671462[2] |

Data expressed as mean ±S.D.
[1]P<0.01; [2]P<0.05; [3]P<0.001 significantly different from the control group;
°P<0.001; [n]P<0.01 significantly different from TNBS.

*Table 4.* Colon homogenate concentration of IL-1β, IL-6 and TNF-α in the experimental groups.

| GROUP | | N | IL-1β (pg/ml) | IL-6 (pg/ml) | TNF-α (pg/ml) |
|---|---|---|---|---|---|
| I. | CONTROL GROUP | 8 | 7317.37± 2157.903 | 111.4904± 34.3061 | 70.2473± 25.21047 |
| II. | ROSIGLITAZONE | 8 | 8240.64± 1821.444 | 37.5236± 19.5447 | 78.5191± 35.13485 |
| III. | BADGE | 8 | 8216.41± 1700.951 | 125.1922± 53.4338 | 73.6939± 11.85034 |
| IV. | 0.9%NaCl - INTRARECTAL | 8 | 8129.41± 4265.516[°] | 153.1068± 69.9082[°] | 79.9981± 27.61858[°] |
| V. | 0.9%NaCl INTRAGASTRIC | 8 | 8394.77± 2525.353 | 118.2975± 66.4865 | 91.1078± 19.62079 |
| VI. | TNBS | 8 | 12558.61± 3282.295[1] | 275.5282± 130.4358[2] | 117.2186± 25.41235[1] |
| VII. | ROSIGLITAZONE + TNBS | 8 | 7502.12± 3736.327[°] | 178.8132± 126.9733 | 90.8503± 22.09415[°] |
| VIII. | BADGE + TNBS | 8 | 11219.61± 3289.113[2] | 285.4868± 81.4555[3] | 119.5864± 32.04618[1] |
| IX. | ETHANOL 50% | 8 | 11096.85± 4435.782[2] | 145.5836± 45.0662[°] | 88.8490± 20.54722[°] |
| X. | ROSIGLITAZONE + BADGE + TNBS | 8 | 13060.20± 3415.49[1] | 226.031± 102.64[2] | 96.654± 16.55 |

Data expressed as a mean ±S.D.
[1]P<0.01; [2]P<0.05; [3]P<0.001 significantly different from the control group;
°P<0.05; significantly different from the TNBS group.

of the present study was to evaluate the influence of substances stimulating or blocking PPAR-γ on the course of colitis. Research was conducted based on the experimental model of experimental colitis induced by single rectal administration of TNBS in rats. The model used enables to study the acute phase of inflammatory bowel disease (IBD).

Our findings are in accordance with the results reported by Sanchez-Hidalgo *et al*. (27), who demonstrated a significant decrease in rat body weight in the group treated with TNBS; in the control group and in groups receiving rosiglitazone, no such a decrease was observed. Rosiglitazone was found to reduce the severity of abdominal adhesions and diarrhea. Moreover, a

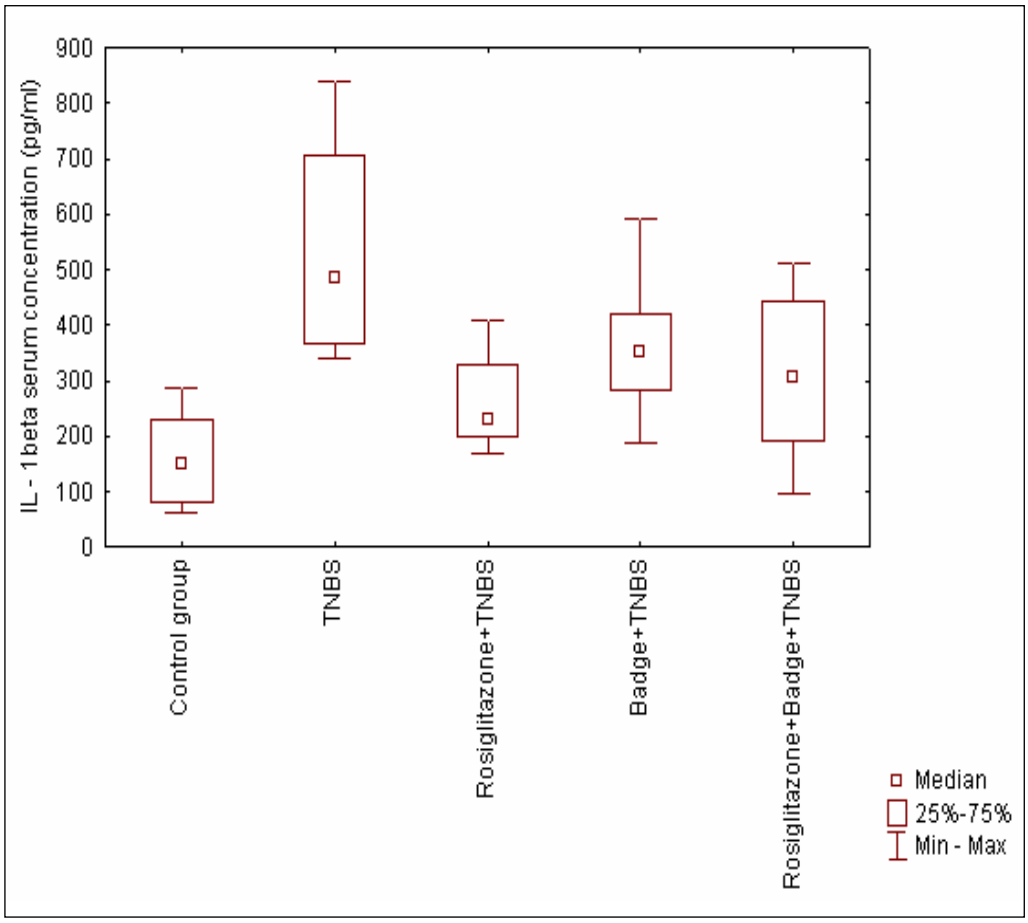


*Fig. 4*. Serum IL-1β serum concentration in the experimental groups.

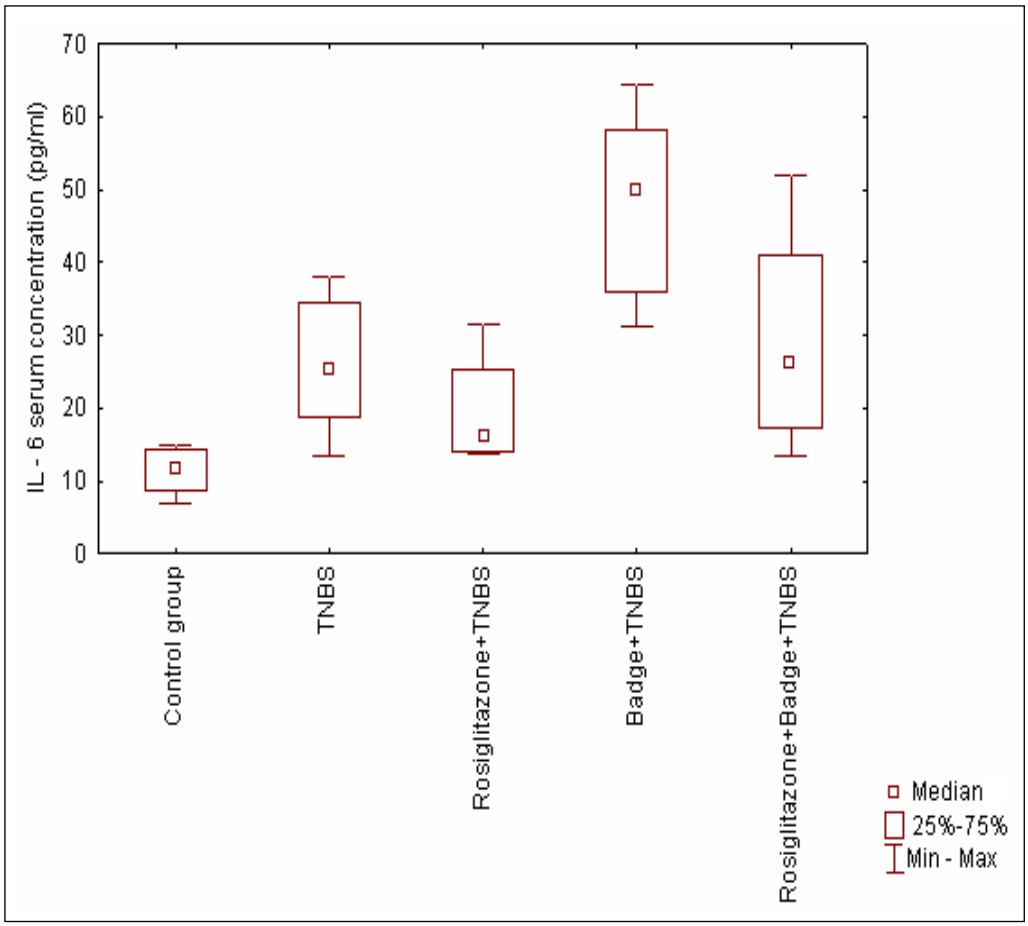


*Fig. 5*. Serum IL-6 concentration in the experimental groups.

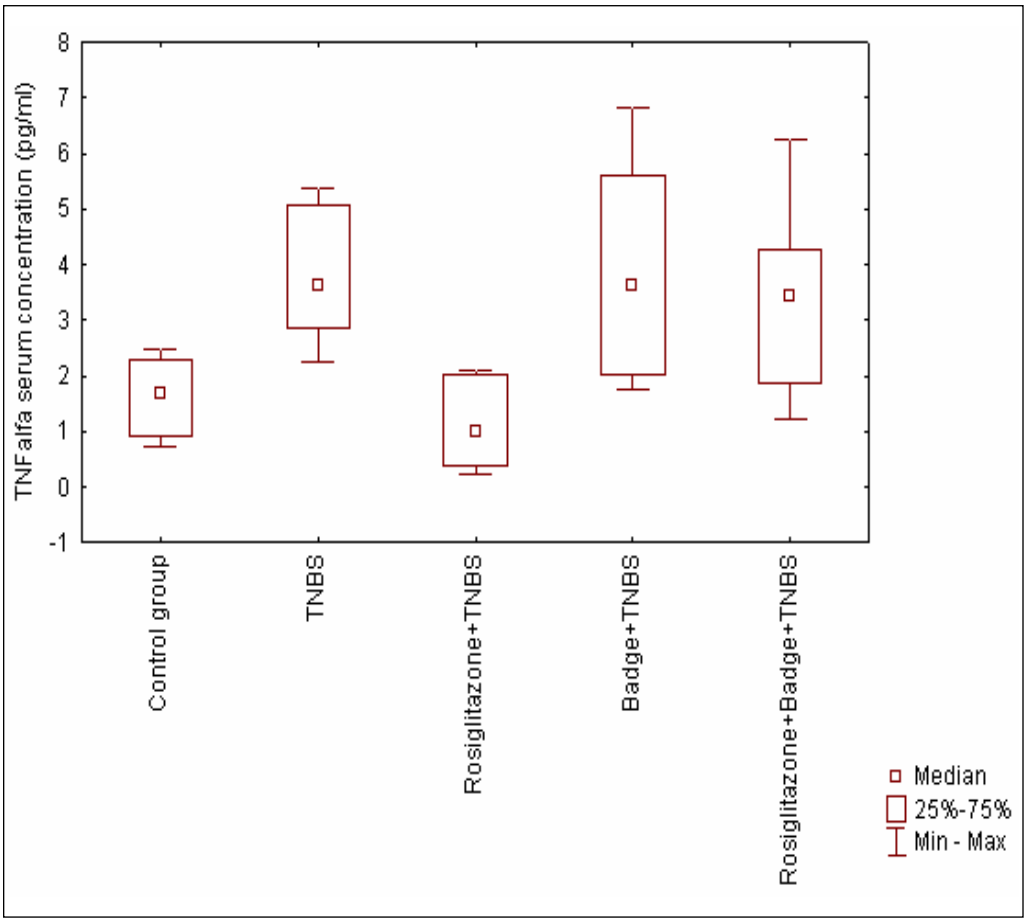


*Fig. 6*. Serum TNF-α concentration in the experimental groups.

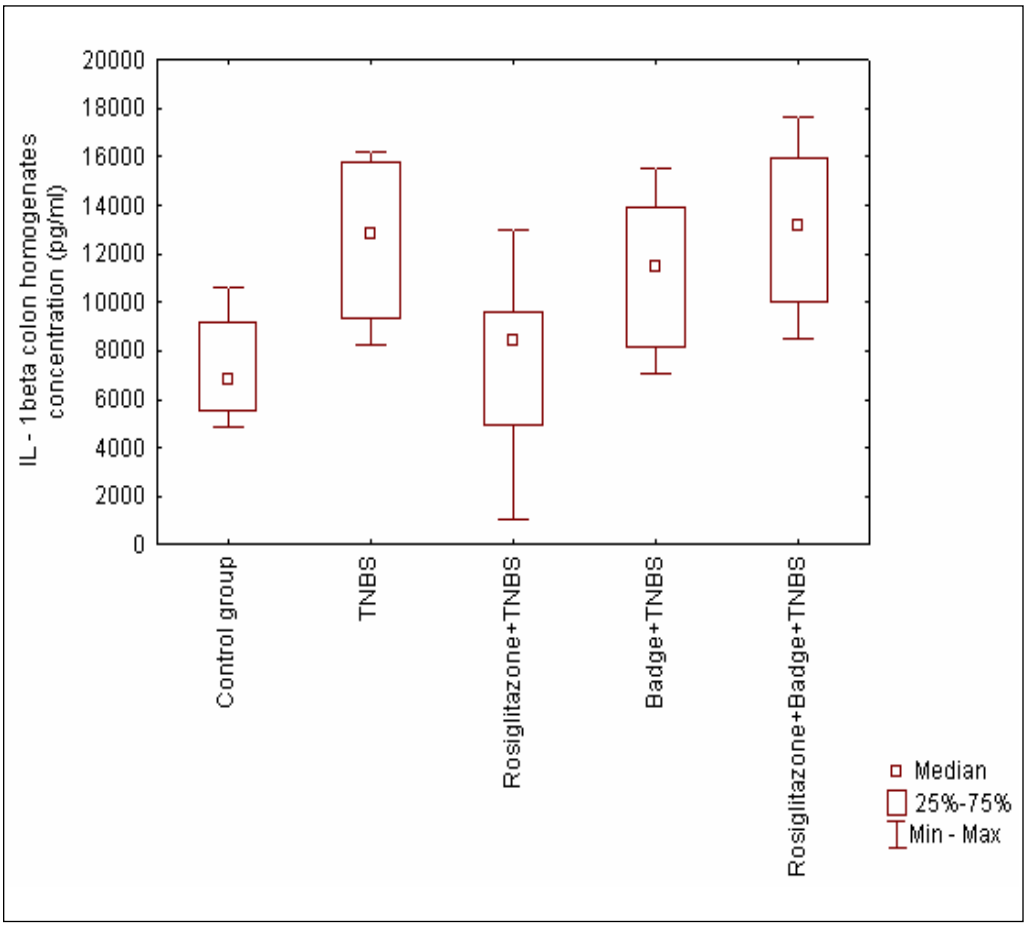


*Fig. 7*. Colon homogenate IL-1β concentration in the experimental groups.

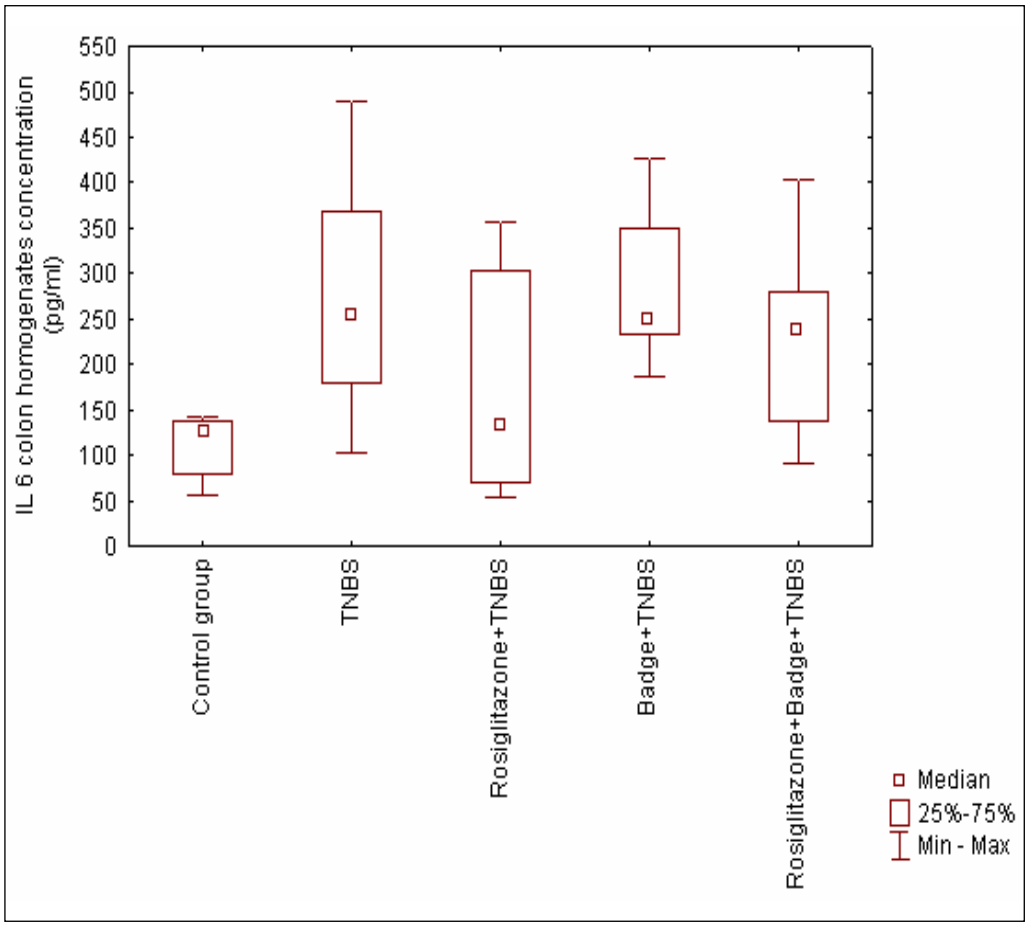


*Fig. 8*. Colon homogenate IL-6 concentration in the experimental groups.

statistically significant decrease in TNF-α level was demonstrated in the rosiglitazone group compared to the group treated only with TNBS. The administration of rosiglitazone to animals with colitis reduced COX-2 and $PGE_2$ expression yet did not affect the level of COX-1. The authors suggest that rosiglitazone reduces the expression of pro-inflammatory cytokines and COX-2 by down-regulating the expression of transcription factors NF-κB and MAPK.

Sanchez-Hidalgo *et al*. (27) studied the experimental model of chronic intestinal inflammation induced by rectal administration of TNBS in rats. Rosiglitazone was administrated 24 hours after induction of inflammation and daily over the two subsequent weeks. The authors observed positive effects of PPAR-γ agonist on body weight gain and diarrhea. Their findings were more spectacular than in the present study, most probably due to longer duration of the experiment. Rosiglitazone was also found to cause a dose-dependent reduction in TNF-α level in intestinal homogenates. Moreover, the multi-factorial anti-inflammatory activity of PPAR-γ agonist was demonstrated (decreased expression of COX-2, normalization of $PGD_2$ level and inhibition of NF-κB synthesis (29).

Unfortunately, not all reports describing the beneficial influence of PPAR-γ on inflammation are so encouraging.

Remaker *et al*. (30) administered rosiglitazone added to fodder to mice 16 days before the induction of inflammation. They found that the agent intensified colitis - loss of body weight, more severe diarrhea, higher weight of the spleen and large intestine, and shortened intestines were observed. The authors suggest that intensification of inflammation might have resulted from long-lasting activity of rosiglitazone, which sensitized the mucosa to DSS administered. Such an effect could be attributed to increased mucosa permeability resulting in easier shift of bowel bacteria, which causes inflammation. Paradoxically, in animals that received rosiglitazone and developed more intensive inflammatory changes, decreased levels of TNF-α in the large intestine and spleen as well as decreased levels of INF-γ in intestine were reported. Negative effects of rosiglitazone may be associated with different ways of PPAR-γ agonist administration. In the majority of studies confirming the anti-inflammatory action of substances which stimulate PPARs-γ, agonists were administrated only 1-2 days before (31-34), on the day of (31) or immediately after the induction of inflammation (29).

The findings published by Takaki *et al*. (34) confirm our results, *i.e*. positive anti-inflammatory effects of thiazolidinediones. In their study, pioglitazone and netoglitazone were administered preventively and therapeutically. Both substances were demonstrated to have significant anti-inflammatory action, with stronger effects of pioglitazone. Although they did not apply rosiglitazone and different models of experimental colitis were used, their findings confirm potentially therapeutic anti-inflammatory properties of thiazolidinediones.

Furthermore, Tanaka *et al*. (35) confirmed positive effects of PPAR agonists on experimentally induced colitis of rats. A statistically significant reduction in intestinal wall damage was observed in the group receiving troglitazone and bezafibrate in both doses used.

In the study by Desreumax *et al*. (31), PPAR-γ and its heterodimer heterozygote mice reacted more intensively to the factor inducing colitis (TNBS). They evaluated anti-inflammation properties of PPAR-γ and RXR agonists

(rosiglitazone and trioglitazone as well as LG101305, respectively). Anti-inflammatory effects were assessed using the macroscopic scoring scale. Both macroscopic and histopathological tests revealed a considerable reduction in inflammation in animals receiving PPAR-γ and RXR agonists. Moreover, similar survival rates were observed in those groups. Rosiglitazone was found more effective for inflammation inhibition compared to trioglitazone. Interestingly, simultaneous administration of rosiglitazone and LG101305 resulted in a synergistic effect (31).

In our research the immune-enzymatic tests showed almost doubled reduction in serum IL-1β concentration in rats administered rosiglitazone together with TNBS. This confirms inhibitory effects of activated PPAR-γ on the expression of this inflammatory cytokine. It is worth stressing that rosiglitazone administrated to healthy animals in group II (without induced colitis) also significantly reduced the serum concentration of IL-1β. Analysis of IL-1β levels in intestinal homogenates of animals with intestinal inflammation receiving rosiglitazone confirmed inhibitory effects of PPAR-γ agonist on expression of IL-1β. Contrary to reduced serum levels of IL-1β in rats given only rosiglitazone (group II), IL-1β levels in intestinal homogenates were not lower - which is likely to be related to short duration of our experiment.

Moreover, inhibitory effects of substances activating PPAR-γ have on expression of inflammatory cytokines, *e.g.* IL-1β were confirmed by Shan *et al*. (36), who studied anti-inflammatory properties of PPAR-γ expressed in macrophages. Their finding demonstrated that transgenic mice lacking the gene for macrophage PPAR-γ receptor developed more severe colitis induced by DSS compared to wild strains. Determinations of mRNA for IL-1β, MCP-1, iNOS, TNF-α and IFNγ using the PCR technique showed their higher levels in mice with experimentally induced inflammation and without genes for macrophage PPAR-γ. The authors suggest that PPAR-γ may affect the selection of macrophages involved in the pathogenesis of IBDs (34).

The pro-inflammatory interleukin IL-6 plays a crucial role in the pathogenesis of IBD and particularly in ulcerative colitis. Its levels in animals treated with rosiglitazone were not found to be statistically significantly lower; the average level of IL-6 in animals treated with rosiglitazone was lower both in serum and intestinal homogenate, however due to variability of results (high standard deviation) statistically significant conclusions cannot be drawn. In the group receiving only rosiglitazone, activation of PPAR-γ caused substantial inhibition of IL-6 expression both in serum and in intestinal homogenates. Analysis of effects of PPAR-γ antagonist on IL-6 expression revealed almost a double increase in its level in the group with induced inflammation. Therefore, it seems that BADGE is likely to block endogenous anti-inflammatory activity of PPAR-γ, which inhibits the expression of IL-6.

Our findings concerning the influence of PPAR-γ activation on IL-6 expression are less promising compared to Takaki *et al*. (34) who studied preventive and therapeutic effects of netoglitazone and pioglitazone on DSS-induced colitis. Immune-enzymatic analysis of IL-6 concentrations in intestinal homogenates and Western blot technique were applied to determine the expression of STAT3 and revealed considerable attenuation of colitis following therapeutic administration of pioglitazone at the dose of 150 mg/kg (34).

Yamamoto *et al* (37). demonstrated reduced IL-6 expression caused by PPAR-γ stimulation in the experimental model of DSS-induced colitis. Positive effects of 4-OHDHA acid, which is the agonist of PPAR-γ, on inhibition of colitis and IL-6 expression and increased synthetase of nitric oxide ($NOS_2$/iNOS) in macrophages activated by lipopolysaccharide (LPS) were reported. The anti-inflammatory activity of this agonist was found comparable to that of 5-aminosalicylic acid derivatives.

The results of immune-enzymatic tests regarding the effects activated PPAR-γ on expression of pro-inflammatory cytokines IL-1β and IL-6 remain ambiguous, although their decreased levels were observed (IL-1β during the course of inflammation, IL-6 in animals without induced inflammation).

Reduced activity of TNF-α, which is the crucial cytokine in the pathogenesis of IBD and particularly in Crohn's disease has very important role in effective reduction of colon inflamation. The action of biological remedy used in the treatment of inflammatory diseases is based on inhibition of pro-inflammatory TNF-α.

In the study by Desreumax *et al*. (31), macroscopic and histopathological examinations on intensification of inflammatory changes were supplemented with analysis of mRNA expression levels of TNF-α, IL-1β and MPO. These results were consistent with macro- and microscopic evaluation of our findings. Both rosiglitazone and troglitazone markedly reduced mRNA expression of TNF-α, IL-1β and MPO. Simultaneous administration of rosiglitazone and the agonist of RXR resulted in more intensive reduction in mRNA expression of those pro-inflammatory cytokines and MPO. These data confirmed observations reported by Sanchez-Hidalgo *et al*. (27, 29), *i.e*. the PPAR-γ agonist inhibits the signaling pathway of NF-κB and MAPK (31). Positive effects of rosiglitazone on intensification of TNF-α expression were also reported in other studies. (27, 29).

Concluding, rosiglitazone was found effective to reduce diarrhea and to maintain body weight in animals with induced colitis. Its efficacy was confirmed by histopathological examinations demonstrating decreased extent and activity of inflammation, limited edema and extent as well as depth of ulcerations of the large intestine. The immunoenzymatic analysis showed inhibitory effects of rosiglitazone on the expression of Il-1β and TNF-α evidenced by lower levels of these parameters in serum and intestinal homogenates. The effects of PPAR-γ antagonist on colitis is not explicit. The administration of BADGE together with the substance inducing colitis did not result in significant increase in the parameters of intestinal mucosa damage, although led to the highest loss in body weight. Moreover, immunoenzymatic results showed that PPAR-β antagonist significantly increased the serum levels of IL-6. BADGE administered to healthy animals did not cause significant morphological changes in the large intestine yet increased levels of IL-1β and IL-6. Simultaneous administration of BADGE and rosiglitazone in animals with experimentally induced colitis resulted in blockage of the receptor by the antagonist, which prevented the binding of the agonist with PPAR-γ and its activation. This led to abolition of beneficial effects of PPARs-γ, *i.e*. suppresion of the expression of proinflammatory cytokines. Our findings confirmed the preventive and therapeutic properties of rosiglitazone in acute colitis. In short-term observations, BADGE, the PPAR-γ antagonist, did not augment the inflammatory reaction yet abolished the beneficial anti-inflammatory influence of rosiglitazone. Long-term observations of BADGE effects on healthy animals were not performed; nevertheless, increased levels of proinflammatory cytokines in this group are likely to document the possible effects of BADGE on the pathogenesis of non-specific intestinal inflammations.

We conclude that rosiglitazone, the PPAR-γ agonist, decreases the severity of inflammatory changes in intravital, macroscopic and histological examinations of TNBS-induced colitis. Treatment with rosiglitazone down-regulates the levels of inflammatory cytokines IL-6 and TNF-α in experimentally induced colitis. Rosiglitazone used in animals without experimentally induced

colitis significantly reduces levels of IL-6 in intestinal homogenates. BADGE, the antagonist of PPAR-γ, failed to increase the levels of proinflammatory cytokines, however, its application together with rosiglitazone caused the inflammation similar to that developing in rats with experimentally induced colitis. Inhibition of PPAR-γ by administration of its antagonist (BADGE) applied before the treatment with agonist (rosiglitazone) abolished its positive anti-inflammatory effect, resulting in intensification of the inflammatory process.

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