Protective Effects of Pioglitazone on Dextran Sodium Sulphate-Induced Colitis in Rats

Sally El Awdan, Rasha E. Mostafa*

Department of Pharmacology, Medical Research Division, National Research Centre, Giza, Egypt

Abstract

The aim of the present study is to investigate the preventive effect of pioglitazone on colitis induced with dextran sulfate sodium (DSS) in rats. Twenty-four male Sprague-Dawley rats weighing 180-200 g were randomized into four groups. Rats of the 1st group received only saline and served as normal group. Colitis was induced in the remaining 3 groups by 1.5% DSS administered in drinking water for 8 days. Group 2 received only saline p.o. for 30 days and served as DSS control group. Groups 3 and 4 received pioglitazone (PIO; 10 mg/kg/day, p.o.) and sulfasalazine (SUL; 300 mg/kg) respectively for 30 days. All animals were sacrificed 24 h after the last treatment. Serum levels of interleukin-2 (IL-2), interleukin-6 (IL-6), and interleukin-17 (IL-17) were measured. Levels of tumor necrosis factor-α (TNF-α), reduced glutathione (GSH) and malondialdehyde (MDA), were measured in colon tissue. Significant elevation in GSH levels and reduction in MDA and TNF-α levels in colon tissue were observed in pioglitazone and sulfasalazine treated group when compared with the DSS control group. Significant elevation in serum IL-2 and reduction in serum IL-6 and IL-17 were observed when compared with the DSS control group. Pioglitazone reduced DSS-induced colitis possibly via reduction of MDA, TNF-α, IL-6, and IL-17 levels.

Keywords: Colitis, Pioglitazone, Dextran sodium sulphate, Rats, Interleukin-6, TNF-α.

1. Introduction

Ulcerative colitis and Crohn’s disease among other non-specific inflammatory bowel diseases (IBDs) can be defined as chronic recurrent noninfectious inflammatory diseases that present significant clinical problems in medical practice. The incidence of IBDs is increasing, particularly in the developed countries especially in rather young individuals [1]. However, the etiology and pathogenesis of IBDs and their underlying causes remain uncertain [2]. Although various treatments of IBD have been explored, unfortunately, none of them proved effective in maintaining full recovery and remission [3].
Many studies confirmed the involvement of immunological mechanisms in IBDs [4]. These mechanisms include significant increase in IgG-containing cells and abnormality of cellular immune and cytokine functions (anti-TNF antibody or anti-IL-10 antibody [5]. For these reasons, immunosuppressive drugs have been extensively used in the treatment of IBD [6]. On the other hand, oxidative stress caused by reactive oxygen species produced by neutrophils and macrophages also causes severe tissue damage [7]. And for this reason, the role of neutrophils in the pathogenesis of IBD is gaining huge attention [8]. Sulfasalazine is commonly used in IBD due to its antioxidant and metal chelating properties [9]. However, the patients treated with sulfasalazine are prone to severe adverse effects, including hypospermia, male infertility, pulmonary fibrosis, hepatotoxicity and ulcerogenic potential [10, 11]; the underlying mechanisms being not quite clear [12, 13].

Three subtypes of Peroxisome proliferator-activated receptors (PPARs) can be identified in humans; α, β, and γ. PPAR-γ agonists; also known as thiazolidinediones (glitazones); exert their actions mainly via specific and selective activation of PPAR-γ receptors [14, 15]. Glitazones have been clinically used to overcome insulin resistance in type 2 diabetes mellitus [16]. PPAR-γ agonists are used recently in treatment of atherosclerosis, rheumatoid arthritis, bronchial asthma, pancreatitis and IBDs [17]. The exact mechanism of PPAR-γ action has not yet been fully elucidated. However, the use of PPAR-γ agonists in treatment of IBD is controversial; where many studies confirmed their beneficial actions while some studies demonstrated their undesirable effects [2, 18]. PPAR-γ agonists’ anti-inflammatory actions can be attributed to the regulation of transcription of genes encoding cytokines and other inflammatory mediators [19]. Furthermore, PPAR-γ agonists blocks inducible nitric oxide synthase gene expression and transcription factors such as nuclear factor kappa B (NF-κB) [20].

Therefore, the current study aims at testing the anti-inflammatory potential of pioglitazone; a PPAR-γ agonist; against dextran sodium sulphate-induced colitis in rats and to compare these effects to those of sulfasalazine.

2. Materials And Methods

2.1. Animals

Twenty four adult male Sprague-Dawley rats weighing 180-200 g were utilized in the present study. Standard food pellets and tap water were supplied ad libitum. Animals and food pellets were obtained from the animal house colony of the National Research Centre (NRC, Egypt). The study was conducted in accordance with the National Research Centre, Medical Research Ethics Committee (NRC-MREC) for the use of animal subjects.

2.2. Preparation of Drugs

Colitis was induced using 1.5% dextran sodium sulphate (DSS) (Sigma Aldrich, St.
Louis, MS, USA) administered in drinking water for 8 days [2]. Pioglitazone (Actos®-Takeda Pharmaceuticals America, Inc.) was used in the current study in the dose of 10 mg/kg body weight, dissolved in 0.9% NaCl and administered orally for 30 days [21, 22]. Sulfasalazine (Sigma Aldrich, St. Louis, MS, USA) was used in the current study in a dose of 300 mg/kg body weight administered orally for 30 days [23, 24]. All other chemicals were of the highest available commercial grade.

2.3. Experimental Design

Animals were randomly allocated into four groups (6 rats each). Rats of the 1st group received only saline and served as normal group. Colitis was induced in the remaining 3 groups by 1.5% DSS administered in drinking water for 8 days. Group 2 received only saline p.o. for 30 days and served as DSS control group. Groups 3 and 4 received pioglitazone (PIO; 10 mg/kg/day, p.o.) and sulfasalazine (SUL; 300 mg/kg) respectively for 30 days. All animals were sacrificed 24 h after the last treatment.

2.4. Measured Parameters

Twenty-four hours after the last dose of treatment, rats were anaesthetized with diethyl ether and blood samples were withdrawn from the retro-orbital venous plexus. The collected blood samples were allowed to stand for 10 min at room temperature then centrifuged at 4°C using cooling centrifuge (Laborezentrifugen, 2k15, Sigma, Germany) at 3000 r.p.m for 10 min and sera were separated for the assessment of the levels of interleukin-2 (IL-2), interleukin-6 (IL-6) and interleukin-17 (IL-17) that were estimated using rat specific immunoassay kit (Biosource, USA) and expressed as pg/ml.

Directly after collecting the last blood sample in the experiment, the rats were sacrificed by cervical dislocation and the colon was excised. The colon was washed in normal saline and then divided into 2 parts: one part was homogenized (using MPW–120 homogenizer, Med instruments, Poland); the homogenate was centrifuged using a cooling centrifuge (Laborezentrifugen, 2k15, Sigma, Germany) at 3000 r.p.m for 10 min; the supernatant was taken for the determination of levels of lipid peroxides as malondialdehyde (MDA), reduced glutathione (GSH) and tumor necrosis factor alpha (TNF-α) according to the methods adopted by Ruiz-Larrea et al. (1994) [25], Beutler et al. (1963) [26] and Brouckaert (1993) [27] respectively using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cell Biolabs, Inc).

2.5. Histopathological Examination

The other parts of the intestines were fixed in 10% neutral buffered formalin and embedded in paraffin wax. 4µm thick sections were stained with Hematoxylin and Eosin (H&E) and examined using binocular Olympus CX31 microscope [28].

2.6. Statistical Analysis

All the values are presented as means ± standard error of the means (SE).
Comparisons between different groups were carried out using one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison post hoc test. Difference was considered significant when \( p < 0.05 \). GraphPad prism® software (version 6 for Windows, San Diego, California, USA) were used to carry out these statistical tests.

3. Results and Discussion

3.1. Effect of Pioglitazone (10 Mg/Kg/Day, P.O.) and Sulfasalazine (300 Mg/Kg/Day, P.O.) on Serum Cytokine Levels

Administration of 1.5% DSS in drinking water for 8 days resulted in acute colitis in rats as evidenced by the significant elevation of serum IL-6 and IL-17 to 345% and 390% and significant decrease of IL-2 to 28% as compared to the normal control group. Administration of pioglitazone (PIO; 10 mg/kg/day, p.o.) significantly decreased the elevated serum levels of IL-6 and IL-17 to 54% and 64% respectively and elevated serum levels of IL-2 to 217% as compared to the DSS control group. While Administration of sulfasalazine (SUL; 300 mg/kg/day, p.o.) significantly decreased the elevated serum levels of IL-6 and IL-17 to 63% and 71% respectively, and elevated serum levels of IL-2 to 153% as compared to the DSS control group (Figures 1-3).

![IL-2 Graph](image)

**Figure 1.** Effects of pioglitazone (PIO; 10 mg/kg/day, p.o.) and sulfasalazine (SUL; 300 mg/kg/day, p.o.) on serum levels of interleukin-2 (IL-2) in dextran sodium sulphate-induced colitis in rats.

Rats of the normal control group received only saline and served as normal control group. Colitis was induced in the remaining 3 groups by 1.5% DSS administered in drinking water for 8 days. Group 2 received only saline p.o. for 30 days and served as DSS control group. Groups 3 and 4 received pioglitazone (10 mg/kg/day, p.o.) and sulfasalazine (300 mg/ kg, p.o.) respectively for 30 days. All animals were sacrificed 24 h after the last treatment, blood samples were collected and sera were separated. Data is presented as mean ± SEM (n=6).

* Significantly different from Normal control group at \( p < 0.05 \) (Tukey’s post hoc test).

* Significantly different from DSS control group at \( p < 0.05 \) (Tukey’s post hoc test).
Effects of Pioglitazone on Dextran Sodium Sulphate-Induced Colitis

Figure 2. Effects of pioglitazone (PIO; 10 mg/kg/day, p.o.) and sulfasalazine (SUL; 300 mg/kg/day, p.o.) on serum level of interleukin-6 (IL-6) in dextran sodium sulphate-induced colitis in rats.

Rats of the normal control group received only saline and served as normal control group. Colitis was induced in the remaining 3 groups by 1.5% DSS administered in drinking water for 8 days. Group 2 received only saline p.o. for 30 days and served as DSS control group. Groups 3 and 4 received pioglitazone (10 mg/kg/day, p.o.) and sulfasalazine (300 mg/ kg, p.o.) respectively for 30 days. All animals were sacrificed 24 h after the last treatment, blood samples were collected and sera were separated.

Data is presented as mean ± SEM (n=6).

a Significantly different from normal control group at $p < 0.05$ (Tukey’s post hoc test).

b Significantly different from DSS control group at $p < 0.05$ (Tukey’s post hoc test).

Figure 3. Effects of pioglitazone (PIO; 10 mg/kg/day, p.o.) and sulfasalazine (SUL; 300 mg/kg/day, p.o.) on serum level of interleukin-17 (IL-17) in dextran sodium sulphate-induced colitis in rats.

Rats of the normal control group received only saline and served as normal control group. Colitis was induced in the remaining 3 groups by 1.5% DSS administered in drinking water for 8 days. Group 2 received only saline p.o. for 30 days and served as DSS control group. Groups 3 and 4 received pioglitazone (10 mg/kg/day, p.o.) and sulfasalazine (300 mg/ kg, p.o.) respectively for 30 days. All animals were sacrificed 24 h after the last treatment, blood samples were collected and sera were separated.

Data are presented as mean ± SEM (n=6).

a Significantly different from normal control group at $p < 0.05$ (Tukey’s post hoc test).

b Significantly different from DSS control group at $p < 0.05$ (Tukey’s post hoc test).
Results of Intestinal Tissue Tumor Necrosis Factor Alpha (TNF-α) And Oxidative Stress Parameters; MDA And GSH

Administration of 1.5% DSS in drinking water for 8 days resulted in significant elevation of intestinal tissue MDA and TNF-α concentrations to 134% and 381%, respectively and significant decrease in GSH concentration to 75% as compared to the normal control group. Administration of pioglitazone (PIO; 10 mg/kg/day, p.o.) significantly decreased the elevated intestinal tissue MDA and TNF-α concentrations to 85% and 58% respectively and elevated GSH concentration to 127% as compared to the DSS control group (Table 1).

3.3. Histopathological Examination of Intestinal Tissue

Histopathological examination of mucosal sections prepared from the colon of normal control rat showed normal histopathological picture. No edema or inflammatory cellular infiltration was observed. Examination of mucosal sections prepared from the colon of DSS-control rat revealed severe deterioration
Scattered ulcerations and inflammatory cellular infiltrates were detected in mucosa and submucosa. Examination of mucosal sections prepared from the colon of rat treated with DSS followed by sulfasalazine showed inflammatory cellular infiltrates in the submucosa while examination of mucosal sections prepared from the colon of rat treated with DSS followed by pioglitazone revealed huge improvement in the overall histopathological picture and restoration of normal histology of colonic epithelium (Figure 4).

3.4. Discussion

This study was designed to investigate whether pioglitazone can attenuate experimental colitis in rats. Our experiment was carried out in the animal model of colitis induced by 1.5% DSS administered in drinking water for 8 days. DSS-induced colitis model in rodents is a well-established model that reflects both the human ulcerative colitis clinical and histopathological features. DSS causes disruption of the intestinal barrier function of the epithelial cells [29]. Therefore, the luminal bacterial antigens can easily gain access to the lamina propria leading to
activation of an inflammatory cascade [30]. Some changes as weight loss and hemorrhagic diarrhea can occur upon administration of DSS in the drinking water in rats [31].

Celinski et al. (2009) found that the model of DDS-induced acute colitis resulted in severe inflammation resulting in higher body weight loss as well as severe inflammatory infiltration [17]. Celinski et al. (2011) reported that serum and colon homogenate concentrations of IL-1β, IL-6, IL-10, TNF-α and Myeloperoxidase (MPO) were also elevated when compared to normal rats. The study also showed that administration of 1.5% DSS in drinking water caused deterioration of the intestinal histopathological picture as demonstrated by severe intestines lesions, dispersed inflammatory changes, infiltration with neutrophils and profound edema [2].

Sanchez-Hidalgo et al. (2007) reported that colitis have been well proved to cause severe pathophysiological changes. Cytokines viz TNF-α, IL-1 and IL-8 are hyper secreted from the macrophages. Adhesion molecules; Eselectin and ICAM-1 were up regulated leading to adherence of neutrophils in endothelium and thus neutrophils’ penetration into the bowel wall. MPO activity, prostaglandin E2 production, cyclooxygenases (COX)-1 and -2, the nuclear factor kappa (NF-κB) and mitogen-activated protein kinase (MAPK) expressions were elevated. These overall events led to the characteristic features of inflammation signifying colitis [32]. Furthermore, DSS administration caused prominent changes in histopathological features of colorectal mucosal tissue which confirmed significant damage [33].

Our study revealed similar results where DSS administration resulted in elevation of serum IL-6 and IL-17 levels, elevation of intestinal TNF-α and MDA concentrations as well as reduction of serum IL-2 and intestinal GSH concentration when compared to normal control rats. DSS administration also resulted in severe histopathological changes of the intestinal mucosal tissue when compared to normal control rats.

Many studies examined the beneficial effects of PPAR-γ agonists on colitis [1, 2, 34, 35]. Saubermann et al. (2002) reported dose-related beneficial effects of PPAR-γ ligands; troglitazone, pioglitazone and rosiglitazone against DSS-induced experimental model of colitis in mice. Treatment with PPAR-γ agonists prior to the onset of colitis resulted in reduction of serum interferon-gamma and TNF-α levels as compared to mice with DSS-induced colitis. These results demonstrate protective effects of PPAR-γ ligands in IBD [36].

Sanchez-Hidalgo et al. (2007) reported that rosiglitazone managed to reduce the elevated serum TNF-α, IL-1, IL-8, MPO activity and prostaglandin E2 production along with immunohistochemical COX-1, COX-2, NF-κB and MAPK expressions. Rosiglitazone also improved the overall histopathological picture when compared to rats with trinitrobenzenesulphonic acid-induced colitis [32].
Celinski et al. (2011) found that rosiglitazone managed to reduce serum and colon homogenate concentrations of IL-1β, IL-6, IL-10, TNF-α and MPO along with improvement of the overall intestinal histopathological picture when compared to rats with DSS-induced colitis [2].

The current study focused on pioglitazone, a PPAR-γ receptor agonist that presents promising results in spite of the imperfection of any animal model in reproducing the real pathophysiological processes in IBDs. In our study, the rats were daily administered pioglitazone by oral gavage for 2 weeks. Pioglitazone, in the current work, was able to decrease the elevated serum IL-6 and IL-17 levels, decrease intestinal TNF-α and MDA concentrations; elevate serum IL-2, intestinal GSH concentration and modulate the intestinal histopathological picture when compared to DSS control rats.

Our study also revealed distortion of the histopathological pictures of colons of the rats treated with DSS when compared to normal rats. This data is in agreement with Yotsuya et al. (2001) who demonstrated sloughing of mucosal and submucosal tissue as well as edema following DSS administration [8]. Moreover, the study demonstrated that histopathological pictures of colons of rats treated with pioglitazone after DSS administration were hugely improved when compared to DSS control rats. Restoration of normal histology of colonic epithelium was clearly observed. Similar results were reported by with Celinski et al. (2011) who reported that DSS administration caused severe mucosal ulceration and inflammation while the rats receiving DSS followed by rosiglitazone showed moderate improvement of the overall pathological picture when compared to DSS control rats [2].

4. Conclusion

In conclusion, the current study revealed that pioglitazone; a PPAR-γ receptor agonist may exert beneficial effects in treatment of experimentally induced colitis in the rats. These effects are mainly due to downregulation of cytokines, inflammatory and oxidative stress markers along with improvement of the overall intestinal histopathological pictures. Therefore, pioglitazone exhibits promising effects in treatment of ulcerative colitis and other non-specific IBDs.

References


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