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Full Length Research Paper

Rosiglitazone protection against experimentally-induced intestinal ischemia/re-perfusion

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Several studies have shown that peroxisome proliferator-activated receptor-γ (PPARγ) agonists protect against ischemia/re-perfusion (I/R) damage in different organs. This study was carried out to assess the possible role of PPARγ on intestinal I/R-mediated tissue injury. This was achieved by evaluating the effects of the PPARγ agonist rosiglitazone and the PPARγ antagonist bisphenol A diglycidyl ether (BADGE) on experimentally-induced intestinal I/R. The possible underlying mechanisms, including changes in the release of NO and/or inflammatory cytokines (such as tumor necrosis factor-alpha, TNFα), infiltration of neutrophils, and apoptotic cell death were investigated. Rats were divided into sham-operated and I/R groups. The I/R group was further divided into sub-groups, and these rats were treated 30 min before induction of ischemia with rosiglitazone, BADGE, or a combination of BADGE + rosiglitazone; control rats received vehicle in place of the drugs. Intestinal I/R was then induced in each rat by occlusion of the superior mesenteric artery (SMA) for 45 min via non-traumatic clamp, followed by re-perfusion for 120 min before the rats were euthanized and intestinal tissues recovered for analyses. Rats in the control I/R group showed a significant increase in intestinal malondialdehyde (MDA), NO, and TNFα contents, as well as increases in myeloperoxidase (MPO) enzyme activity, diffuse histological damage, and strong levels of Fas staining. Pre-treatment of rats with rosiglitazone resulted in a significant reduction in the intestinal MDA, NO, and TNFα contents and MPO enzyme activity associated with I/R; these rats also evidenced only mild histological damage and Fas staining. The protective effects of rosiglitazone were completely abolished by BADGE administration. Therefore, based on the results of this study, we conclude that the PPARγ system plays an important role in intestinal I/R injury. We also note that free radicals (including NO), neutrophil infiltration, as well as select cytokines, are important mediators in I/R injuries.

Key words: Ischemia/re-perfusion, peroxisome proliferator-activated receptor-γ (PPARγ), rosiglitazone, bisphenol A diglycidyl ether (BADGE), cytokines, nitric oxide.

INTRODUCTION

I/R injury of the intestine is a significant problem in abdominal aortic aneurysm surgery, small bowel transplantation, cardiopulmonary bypass, and strangulated hernias (Collard and Gelman, 2001). It occurs as a consequence of collapse of the systemic circulation, as in hypovolemic and septic shock (Swank and Deithch, 1996). Occlusion and re-perfusion of the splanchnic arteries can precipitate circulatory shock mainly through an increase in vascular permeability, causing the activation and adhesion of polymorpho-nuclear neutrophils (PMN), release of pro-inflammatory

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substances and formation of free radicals (Cuzzocrea et al., 2002). Activated neutrophils contributed to tissue damage through release of free radicals, proteolytic enzymes, stimulation of cytokine release from local cells, thus promoting further neutrophil recruitment and, plugging of capillaries causing no-flow phenomenon (Jordan et al., 1999; Vermeiren et al., 2000).

Previous studies have shown an important role of tumor necrosis factor-α (TNFα) for re-perfusion-induced tissue injury and lethality (Granger et al., 2001; Souza et al., 2001, 2002). In contrast, interleukin (IL)-10 modulated pro-inflammatory cytokine production and tissue injury following I/R injury (Zingarelli et al., 2001; Souza et al., 2003). It was found that the small amount of nitric oxide (NO) produced via constitutive nitric oxide synthase (NOS; as neuronal [nNOS] or endothelial [eNOS] forms) is beneficial during intestinal I/R injury (Kubes, 1993; Chan et al., 1999), however, high levels of NO associated with the induction of inducible nitric oxide synthase (iNOS) were found to be detrimental to intestinal integrity (Suzuki et al., 2000).

The peroxisome proliferator-activated receptor-γ (PPARγ) is a member of the nuclear hormone receptor superfamily that is involved in several physiological and pathological states including atherosclerosis, inflammation, cancer, infertility, and nerve demyelination (Alarcón de la Lastra et al., 2004). Thiazolidinediones like rosiglitazone are synthetic PPARγ ligands used effectively in treatment of Type 2 diabetes (Young et al., 1998). PPARγ agonists were found to reduce the lesions associated with I/R of the kidney (Sivarajah et al., 2003), heart (Khandoudi et al., 2002; Wayman et al., 2002), and lung (Ito et al., 2004).

This study was carried out to assess the possible role of PPARγ on intestinal I/R-mediated tissue injury. This was achieved by evaluating effects of the PPARγ agonist rosiglita-zone and the PPARγ antagonist bisphenol A diglycidyl ether (BADGE) on experimentally-induced intestinal I/R in rats. The possible underlying mechanisms that may be involved in this process, like changes in the production of free radicals, nitric oxide (NO), and pro-inflammatory cytokines (such as TNFα), activation and infiltration of neutrophils, and increases in the levels of apoptotic cell death were also investigated.

MATERIALS AND METHODS

Animals

Adult male albino rats (14-weeks-old, 150 to 200 g) obtained from the animal house of the National Research Center (NRC, Cairo, Egypt) were used in these studies. Rats were acclimated for 1 week prior to the experiment. All rats were housed under specific pathogen-free conditions with a 12-h light/12-h dark cycle, at constant temperature (25 ± 2°C) and relative humidity (55 ± 5%), and had access to food and water *ad libitum*. All animal studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

After acclimatization, the rats were weighed and then randomly allocated into several experimental groups: Sham-operated (superior mesenteric artery was exposed but no clamping was carried out; n = 8) and I/R groups wherein a total of 40 rats were randomly distributed into five equal subgroups (n = 8 /group) and injected with different drugs (or control vehicle) 30 min before induction of the ischemia, as follows: (A) 0.3 mg rosiglitazone/kg (supplied in saline vehicle; SmithKline Beecham, UK) intravenously (IV; Cuzzocrea et al., 2003); (B) 1 mg BADGE/kg (supplied in dimethyl sulfoxide (DMSO) vehicle and diluted on site with saline (final DMSO concentration in saline was 2.3 mg/ml); Sigma, St. Louis, MO), IV (Cuzzocrea et al., 2003); (C) BADGE + rosiglitazone (BADGE 30 min before rosiglitazone (Cuzzocrea et al., 2003); or, (D) a DMSO-saline vehicle (IV) at same volume as delivered to rats in other groups; (E) DMSO vehicle (IV) at same volume as delivered to rats in other groups.

Induction of intestinal I/R

After overnight fasting, rats were anaesthetized via an intraperitoneal injection of 1.3 g urethane/kg BW (Squadrito et al., 2000). A 3 cm midline abdominal incision was performed and the area was then cleansed with sterile alcohol. The abdomen was explored and intestinal ischemia induced by clamping the superior mesenteric artery (SMA) near its aortic origin for 45 min using non-traumatic clamp (Megison et al., 1990). Intestinal ischemia was confirmed by obvious lack of pulse in the SMA and paleness of the jejunum and ileum. Re-perfusion was performed by removal of the clamp, and confirmed by the return of pulses and re-establishment of pink color to the intestine. Rats were sacrificed by cervical dislocation 120 min after re-perfusion. Body temperature was maintained throughout the procedures at 37ºC by the use of a heating lamp.

Sample collection

After I/R, the entire small intestine was carefully removed and placed on ice. The oral 10 cm segment (duodenum) was removed, and the remainder of the intestine divided into two equal segments representing the proximal (jejunum) and distal (ileum) segments. These segments were rinsed thoroughly with saline, weighed, and then placed at -20°C for later biochemical studies. Portions of each small intestinal sample were placed in 10% neutral buffered formalin (pH 7.4) for later histopathologic and immunohistochemical examination.

Assessment of intestinal myeloperoxidase enzyme activity (MPO)

MPO enzyme activity, an indicator of PMN accumulation, was determined according to the method described by Grisham et al. (1990). Intestinal tissues were homogenized in ice-cold potassium phosphate buffer (pH 7.4). The homogenate was then centrifuged at 20,000 x g for 20 min at 4°C, and the pellet then homogenized in 10 vol ice-cold 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyl-trimethyl ammonium bromide (HETAB) and 10 mM EDTA. An aliquot (200 µl) of the homogenate was removed and added to a 2 ml reaction volume containing 80 mM potassium phosphate buffer (pH 5.4), 0.5% HETAB, and 1.6 mM tetramethyl benzidine. The mixture was warmed to 37ºC and then 200 µl of 0.3 mM H_2O_2 added. The rate of change in absorbance was then measured at 655 nm in a double beam spectrophotometer (Shimadzu UV-PC 1601, Kyoto, Japan). One unit (U) of activity was

Table 1. Grading based on the feature profile in the stained intestinal samples.

Grade	Features
0	Normal; villus to crypt ratio 5 or 6:1 Minimal number of lymphocytes and plasma cells Tall columnar surface epithelial cells
1	Epithelial cell degenerative changes (cuboidal, vacuolated) but intact Mild increase of lymphocytes and plasma cells in lamina propria Decreased villus height, yielding villus to crypt ratio =1 or less
2	Epithelial cell necrosis, erosions More chronic inflammation in lamina propria ± neutrophils Glandular dilatation
3	Villi effaced (flat surface) Epithelial cell necrosis, erosions May be pseudomembrane on surface Glandular destruction, inflammation extending deep to muscle layer
4	Transmural changes (all of above plus change in muscle layer)

defined as the amount of enzyme present that produced a change in 1.0 absorbance unit/min at 37ºC. Protein content of the homogenate was determined by biuret reagent according to the method of Fleury and Eberhard (1951), using a Biodiagnostic kit (Epico, Egyptian Int. Pharmaceutical Industries Co., Cairo, Egypt). MPO activity was ultimately calculated as U/mg protein.

Determination of intestinal tumor necrosis factor-α (TNFα) levels

Intestinal tissues were homogenized in ice cold phosphate buffered saline containing protease inhibitor cocktail and 0.05% Tween 20. Samples were centrifuged at 3000 rpm for 10 min; the resultant supernatant was used for analyzing TNFα levels in an ELISA assay using a TNFα kit (Biosource Rt, Belgium).

Determination of intestinal nitric oxide (NO) contents (measured as nitrate/ nitrite)

Intestinal NO levels were determined via the method of Miranda et al. (2001). Briefly, isolated tissues were homogenized in 10 ml of ice-cold saline solution and then absolute ethanol was added to precipitate proteins. After allowing for the materials to separate over a 15 min period (at 25°C), the supernatant was recovered. To 0.5 ml of supernatant, 0.5 ml vanadium (III) chloride (8 mgVCl_3/ml) was added, rapidly followed by the addition of 0.5 ml freshly prepared Griess reagent. The mixture was then vortexed and incubated at 37°C for 30 min before its absorbance was measured at 540 nm in the double-beam spectrophotometer.

Determination of intestinal lipid peroxide levels

Intestinal malondialdehyde (MDA) content was determined as an indicator of *in situ* lipid peroxidation (Yoshioka et al., 1979). Briefly, intestinal tissues were homogenized in 10 ml of ice-cold 1.15% (w/v) potassium chloride solution. To 0.5 ml of the homogenate, 3 ml of 0.5% (w/v) trichloroacetic acid and 1 ml 0.6% (w/v) thiobarbituric acid were added; the entire solution was then mixed and heated for 45 min in a boiling water bath. After cooling, 4 ml of *n*-butanol was added and the sample vigorously shaken. After allowing for phase separation, the butanol layer was isolated and the absorbance of the pink colored product in the layer measured at 535 nm in the double-beam spectrophotometer.

Histopathological examination of intestine sections

Paraffin-embedded middle intestinal segments (4 to 7-µm thickness) were stained with hema-toxylin and eosin (H&E) to assess the intestinal damage by light microscopy (Stallion et al., 2005). All samples were graded based on the feature profile outlined in Table 1.

Immunohistochemical determination of Fas in intestine sections

Fas expression was detected by the immunostaining of tissue sections prepared from formalin-fixed, paraffin-embedded middle intestinal segments. An immunoperoxidase (PAP, peroxidase/anti-peroxidase) technique was materials supplied in a kit obtained from Lab Vision (Fremont, CA). In this manner, the cytoplasm of each Fas^+ cell was stained brown.

Statistical analysis

Results were expressed as the mean (± SD). Comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by Tukey-Kramer test using Minitab computer software (Version 13; Minitab Inc., State College, PA). Statistical significance was accepted at $p < 0.05$.

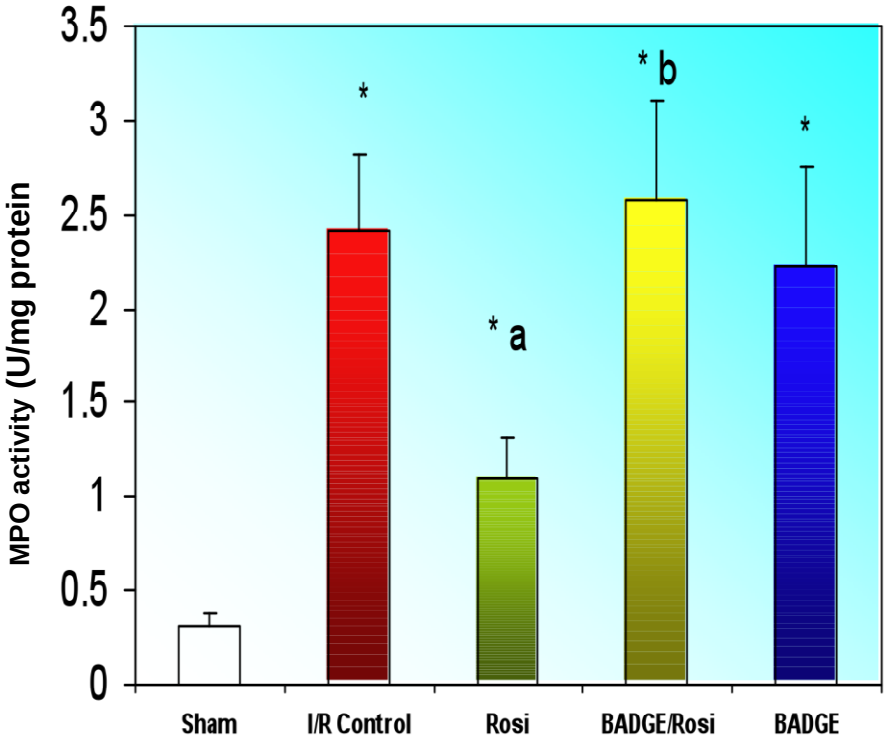

Figure 1. Effect of PPAR-γ-modulating agents on intestinal myeloperoxidase (MPO) enzyme activity. Rats were subjected to either sham operation or intestinal ischemia followed by re-perfusion. I/R-operated rats were pre-treated 30 min before induction of ischemia with control vehicles, rosiglitazone (0.3 mg/kg, IV), BADGE (1 mg/kg, IV), or BADGE 30 min prior to rosiglitazone. Intestinal MPO activity was determined at the end of re-perfusion period. Data are presented as the mean (± SD; n = 8 /group) MPO enzyme activity (U/mg protein). Statistical analysis was ANOVA followed by Tukey-Kramer test. *, Significant difference from sham-operated group ($p < 0.05$); [a], significantly different from I/R group ($p < 0.05$), [b], significantly different from rosiglitazone group ($p < 0.05$).

RESULTS

Effect of PPARγ-modulating agents on intestinal myeloperoxidase enzyme activity

Rats subjected to intestinal I/R displayed a significant increase (663.41%) in intestinal MPO enzyme activity as compared to that in the organs from sham-operated rats. Pre-treatment of rats with 0.3 mg rosiglitazone/kg led to a significant reduction (54.46%) in intestinal MPO activity as compared to levels in the tissues from the control I/R saline rats. Treatment with 1 mg BADGE/kg blocked any rosiglitazone-induced reduction in intestinal MPO activity (Figure 1).

Effect of PPARγ-modulating agents on intestinal TNFα content

Rats subjected to intestinal I/R showed a significant increase (4111.45%) in intestinal TNFα content as compared to levels in tissues from sham-operated hosts. Pre-treatment of the rats with 0.3 mg rosiglitazone/kg resulted in a significant reduction (69.37%) in the levels of intestinal TNFα as compared to those in control I/R saline animals. Treatment with 1 mg BADGE/kg abolished any rosiglitazone-induced reduction in intestinal TNFα levels (Figure 2).

Effect of PPARγ-modulating agents on intestinal nitric oxide (NO) levels

Rats subjected to intestinal I/R showed a significant increase (75.44%) in the intestinal NO content as compared to that in the samples from sham-operated rats. Pre-treatment of the rats with 0.3 mg rosiglitazone/kg gave rise to a significant reduction (39.49%) in intestinal NO as compared to the levels seen in the tissues from the control I/R hosts. Treatment with 1 mg BADGE/kg abrogated any rosiglitazone-induced reduction in intestinal NO levels (Figure 3).

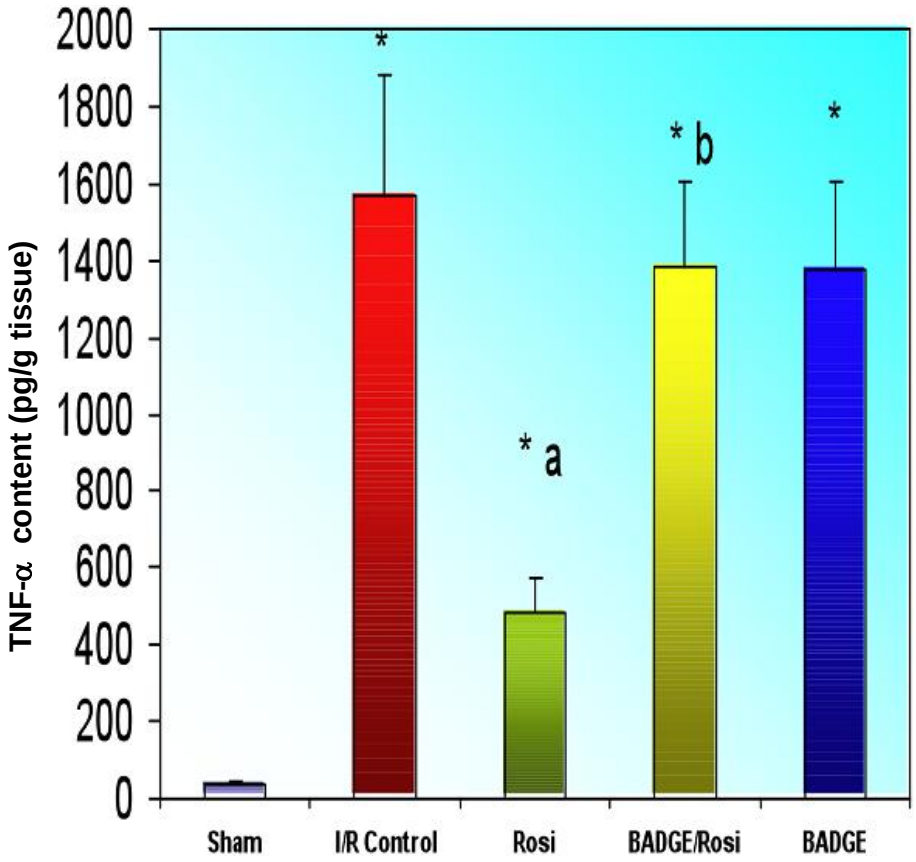

Figure 2. Effect of PPAR-γ-modulating agents on intestinal TNFα levels. Rats were subjected to either sham operation or intestinal ischemia followed by re-perfusion. I/R-operated rats were pre-treated 30 min before induction of ischemia with control vehicles, rosiglitazone (0.3 mg/kg, IV), BADGE (1 mg/kg, IV), or BADGE 30 min prior to rosiglitazone. Intestinal TNFα contents were determined at the end of reperfusion period. Data are presented as the mean (± SD; n = 8 /group) TNFα content (pg/g tissue). Statistical analysis was ANOVA followed by Tukey-Kramer test. *, Significant difference from sham-operated group ($p < 0.05$); [a], significantly different from I/R group ($p < 0.05$), [b], significantly different from rosiglitazone group ($p < 0.05$).

Effect of PPARγ-modulating agents on intestinal lipid peroxide content

Rats subjected to intestinal I/R showed a significant increase (115.1%) in intestinal MDA content as compared to levels noted in tissues from the sham-operated hosts. Pre-treatment of the rats with 0.3 mg rosiglitazone/kg led to a significant reduction (42.48%) in intestinal MDA levels as compared to those found in the tissues from control I/R rats. Treatment with 1 mg BADGE/kg abolished rosiglitazone-induced reduction in intestinal MDA content and produced even higher levels of MDA compared to control I/R group (Figure 4).

Effect of PPARγ-modulating agents on small intestine histology

Histopathological examination of mid-intestine segments from the sham-operated rats revealed an apparently normal architecture of the intestinal epithelium and wall, with the sites being infiltrated by a minimal number of lymphocytes and PMN (Grade 0 to 1) (Figure 5A). In contrast, intestinal sections from I/R-operated rats revealed diffuse transmural inflammatory infiltrates of mononuclear cells and PMN. The epithelial surfaces in these samples displayed evidence of a complete sloughing along with the formation of focal pseudo-membranes (Grade 4) (Figure 5B). In the intestinal sections of rats pre-treated with 0.3 mg rosiglitazone/kg, the segments were found to contain intact vacuolated epithelial cells (vacuolar degeneration) and a mild mononuclear cellular infiltrate; in addition, there were some PMN present in the lamina propria (Grade 1) (Figure 5C). Rats that received BADGE prior to rosiglitazone administration had a diffuse transmural inflammatory infiltrate in the form of mononuclear cells and PMN in these sections. In addition, the epithelial

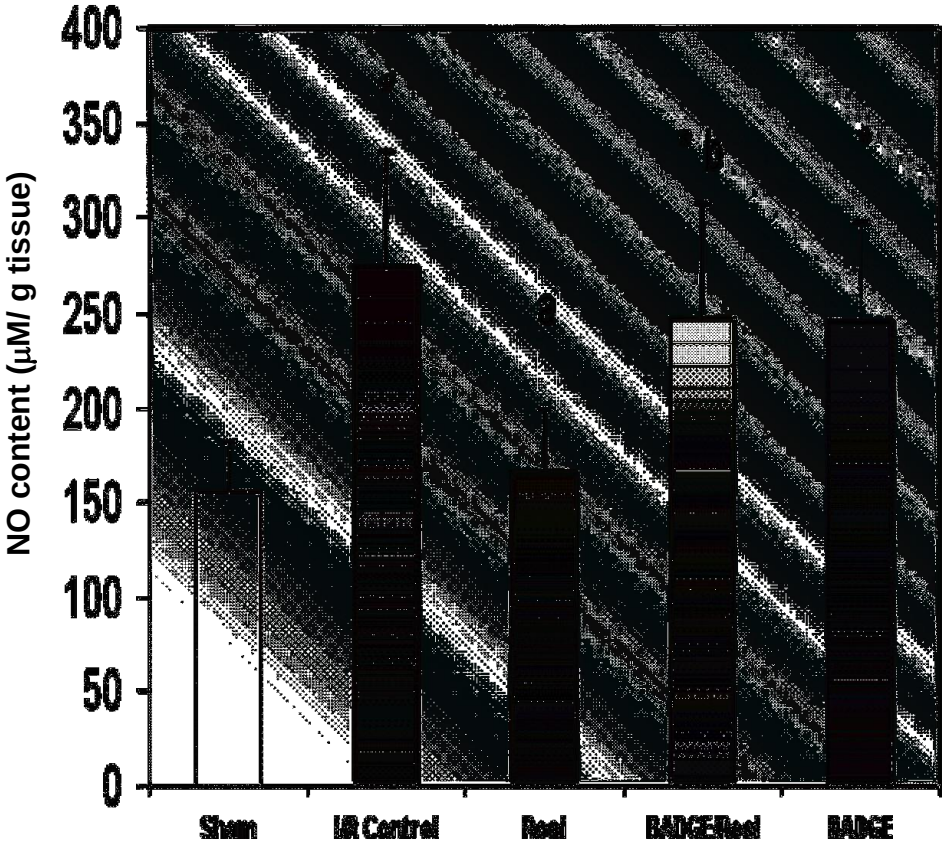

Figure 3. Effect of PPAR-γ-modulating agents on intestinal nitric oxide (NO) levels**.** Rats were subjected to either sham operation or intestinal ischemia followed by re-perfusion. I/R-operated rats were pre-treated 30 min before induction of ischemia with control vehicles, rosiglitazone (0.3 mg/kg, IV), BADGE (1 mg/kg, IV), or BADGE 30 min prior to rosiglitazone. Intestinal NO (measured as nitrate/nitrite) was measured at the end of re-perfusion period. Data are presented as the mean (± SD; n = 8 /group) NO content (µM/g tissue). Statistical analysis was ANOVA followed by Tukey-Kramer test. *, Significant difference from sham-operated group ($p < 0.05$); [a], significantly different from I/R group ($p < 0.05$), [b], significantly different from rosiglitazone group ($p < 0.05$).

surface in these samples showed evidence of complete sloughing, with focal pseudo-membrane formation (Grade 4) (Figure 5D).

Effects of PPARγ-modulating agents on Fas expression in the small intestine

Microscopic examination of mid-intestine sections obtained from the sham-operated rats revealed weak non-specific staining for Fas (±) (Figure 6A). In comparison, sections from I/R-operated rats displayed strong cytoplasmic staining for Fas (+++); in addition, there was apical glandular cytoplasmic staining in superficial epithelial cells and a moderate number of Fas^+ lymphocytes in the lamina propria that were associated with Fas^+ crypt cells (Figure 6B). Sections from rats that received 0.3 mg rosiglitazone/kg showed mild cytoplasmic staining for Fas (+) (Figure 6C). Sections from rats that had received BADGE prior to rosiglitazone evidenced a strong cytoplasmic staining for Fas (+++); further, there was apical glandular cytoplasmic staining in superficial epithelial cells and a moderate number of Fas^+ lymphocytes in the lamina propria that were associated with Fas^+ crypt cells (Figure 6D).

DISCUSSION

In this study, intestinal I/R injury induced by occlusion of the SMA for 45 min was followed by de-clamping to allow re-perfusion. I/R-induced intestinal injury was confirmed biochemically by an increase in myelo-peroxidase (MPO) activity as a measure of neutrophil infiltration, and histopathologically by the presence of a diffuse transmural inflammatory infiltrate (in the form of mononuclear cells and PMN) and complete sloughing of the epithelial surface and focal pseudo-membrane

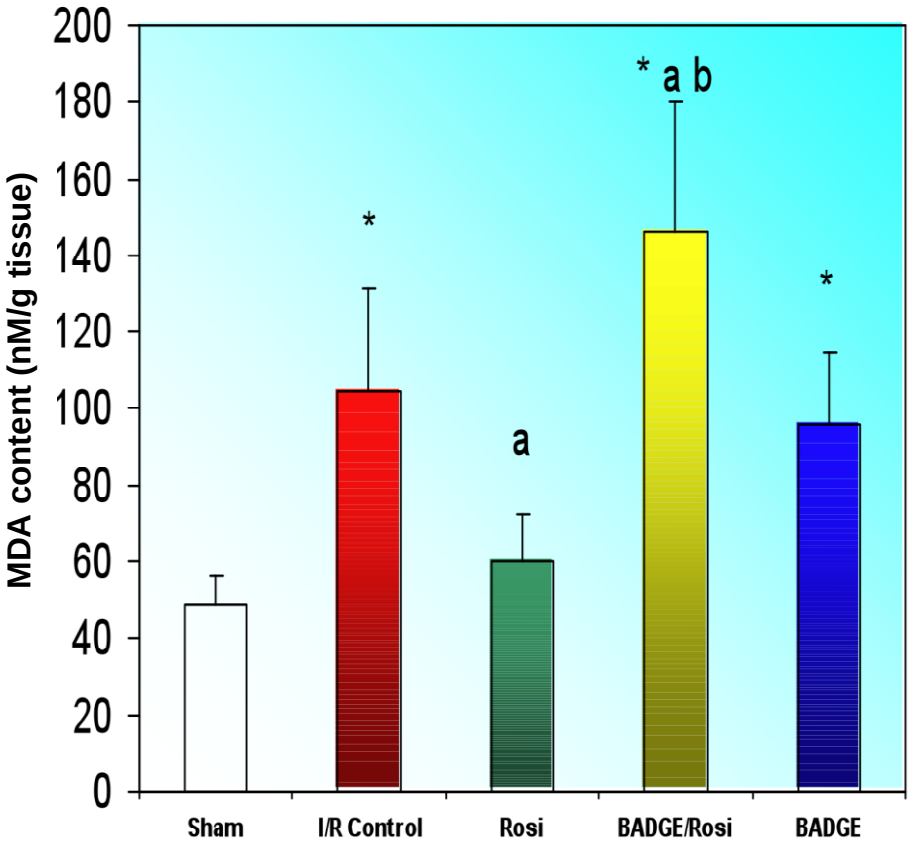

Figure 4. Effect of PPAR-γ modulating agents on intestinal lipid peroxide contents. Rats were subjected to either sham operation or intestinal ischemia followed by re-perfusion. I/R-operated rats were pre-treated 30 min before induction of ischemia with control vehicles, rosiglitazone (0.3 mg/kg, IV), BADGE (1 mg/kg, IV), or BADGE 30 min prior to rosiglitazone. Intestinal lipid peroxides contents (measured as MDA) were determined at end of re-perfusion period. Data are presented as the mean (± SD; n = 8 /group) MDA content (nM/g tissue). Statistical analysis was ANOVA followed by Tukey-Kramer test. *Significant difference from sham-operated group ($p < 0.05$); [a], significantly different from I/R group ($p < 0.05$), [b], Significantly different from rosiglitazone group ($p < 0.05$).

formation. These results were in agreement with many previous studies (Grisham et al., 1986; Souza et al., 2000). Activated neutrophils produced superoxide anion ($\bullet O_2^-$) through the activity of NADPH oxidase, which reduced molecular oxygen to the $\bullet O_2^-$ radical, and through MPO enzyme that catalyzed the formation of such potent cytotoxic oxidants as hypochlorous acid from the reaction of hydrogen peroxide (H_2O_2), chloride ion, and *N*-chloramines. In a gastric oxidative stress injury model, one study showed that neutrophils also released proteases, lactoferrin, and lipid mediators that contributed to gastric injury (Villegas et al., 2002).

Pre-treatment of rats with rosiglitazone (0.3 mg/kg, intravenously [IV]) protected against I/R injury as evidenced by the significant reduction in MPO enzyme activity and confirmed by the histopathological findings. This finding was in agreement with many previous studies that showed that different peroxisome proliferator-activated receptor-γ (PPARγ) agonists could protect against I/R in different organs (Nakajima et al., 2001; Naito et al., 2002; Cuzzocrea et al., 2003; Akahori et al., 2007). Pre-treatment of rats with BADGE that selectively blocked PPARγ receptors significantly abolished the beneficial protective effects of rosiglitazone against intestinal I/R. This result suggested that the beneficial effects of rosiglitazone on both biochemical and histological parameters could be attributed to its binding and activation of PPARγ receptors in the small intestine (Cuzzocrea et al., 2003). PPARγ expression in small intestine was found to be significantly higher than in other organs susceptible to I/R injury (Braissant and Wahli, 1998; Nakajima et al., 2001).

Activation of PPARγ by rosiglitazone resulted in down-regulation of intercellular adhesion molecule 1 (ICAM-1) expression by intestinal endothelium, and thus neutrophil infiltration (Nakajima et al., 2001; Cuzzocrea et al., 2003).

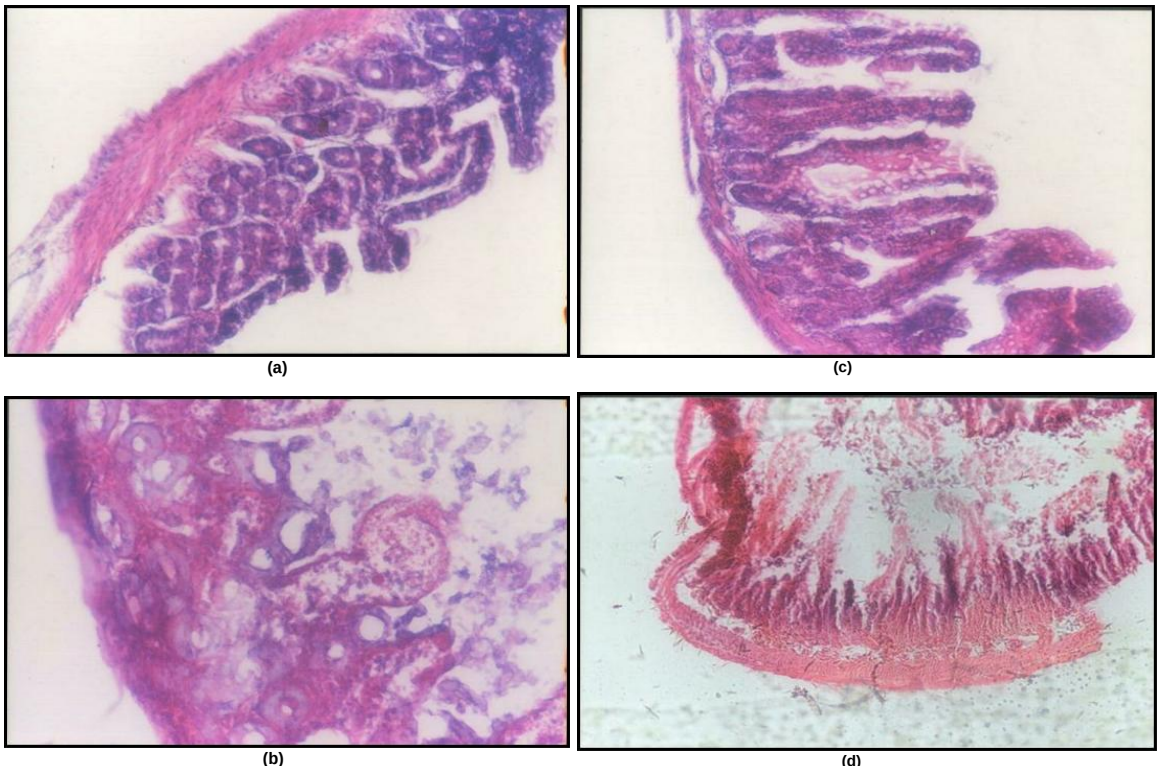

Figure 5. Histology of intestinal sections recovered from rats in the various treatment groups**.** All images are at 250X magnification of H&E-stained tissues and are representative of samples from rats in each group. Section from: **(a)** Sham-operated rat showing apparently normal architecture of the intestinal epithelium and wall, with minimal number of lymphocytes and PMN; **(b)** I/R-operated rat (control) showing diffuse transmural inflammatory infiltrate in the form of mononuclear cells and PMN - epithelial surface shows complete sloughing with focal pseudo-membrane formation; **(c)** Rosiglitazone-pre-treated rat showing intact vacuolated epithelial cells with mild mononuclear cellular infiltrate and some PMN; and, **(d)** Rat that received BADGE prior to rosiglitazone, showing diffuse transmural inflammatory infiltrate in the form of mononuclear cells and PMN - epithelial surface shows complete sloughing with focal pseudo-membrane formation.

The decrease in neutrophil infiltration may also be secondary to reduction in TNF-α-dependent ICAM-1 expression. It was demonstrated that PPARγ activation by BRL-49653 protected against I/R-induced injury through inhibition of nuclear factor–κB (NF-κB)-mediated transcription (Nakajima et al., 2001). Treatment with the PPARγ ligand caused a decrease in the mRNA levels of TNF-α and ICAM-1 in the I/R-injured intestine; both TNF–α and ICAM-1 are down-stream targets of NF-κB (Barnes and Karin, 1997).

In the present study, intestinal TNF-α content was significantly elevated in rats in the I/R group as compared to among the sham-operated rats. Previous studies have shown an important role of TNFα for re-perfusion-induced tissue injury and lethality (Wellborn et al., 1996; Carden and Granger, 2000; Granger et al., 2001; Souza et al., 2001, 2002). Wang et al. (2008) explored the role of TNF–α in the pathogenesis of peripheral nerve I/R injury that was established in wild-type and TNF–α knockout (KO) mice. Electrophysiology, behavioral score and morphological indices (that is, edema and ischemic fiber degeneration) were examined to determine the influence of TNF-α on peripheral nerve structure and function following I/R. TNF-α KO mice had marked improvement in nerve pathology as well as a significant improvement in electrophysiological and (some) behavioral indices. In the studies here, administration of 0.3 mg rosiglitazone/kg resulted in a significant reduction in TNF-α content as compared to that in the intestinal samples from the control I/R rats. BADGE completely abolished this effect on TNF–α content. It was also seen here that up-regulation of TNF-α production in the gastric mucosa correlated with the development of re-perfusion injury. This outcome would be in keeping with results of earlier studies that showed that pre-treatment of rats with rosiglitazone attenuated the production of cytokines (Villegas et al., 2004).

Recently, it has been demonstrated that ciglitazone reduced myocardial damage induced by I/R, neutrophil infiltration, blunted creatine kinase levels, and TNF–α

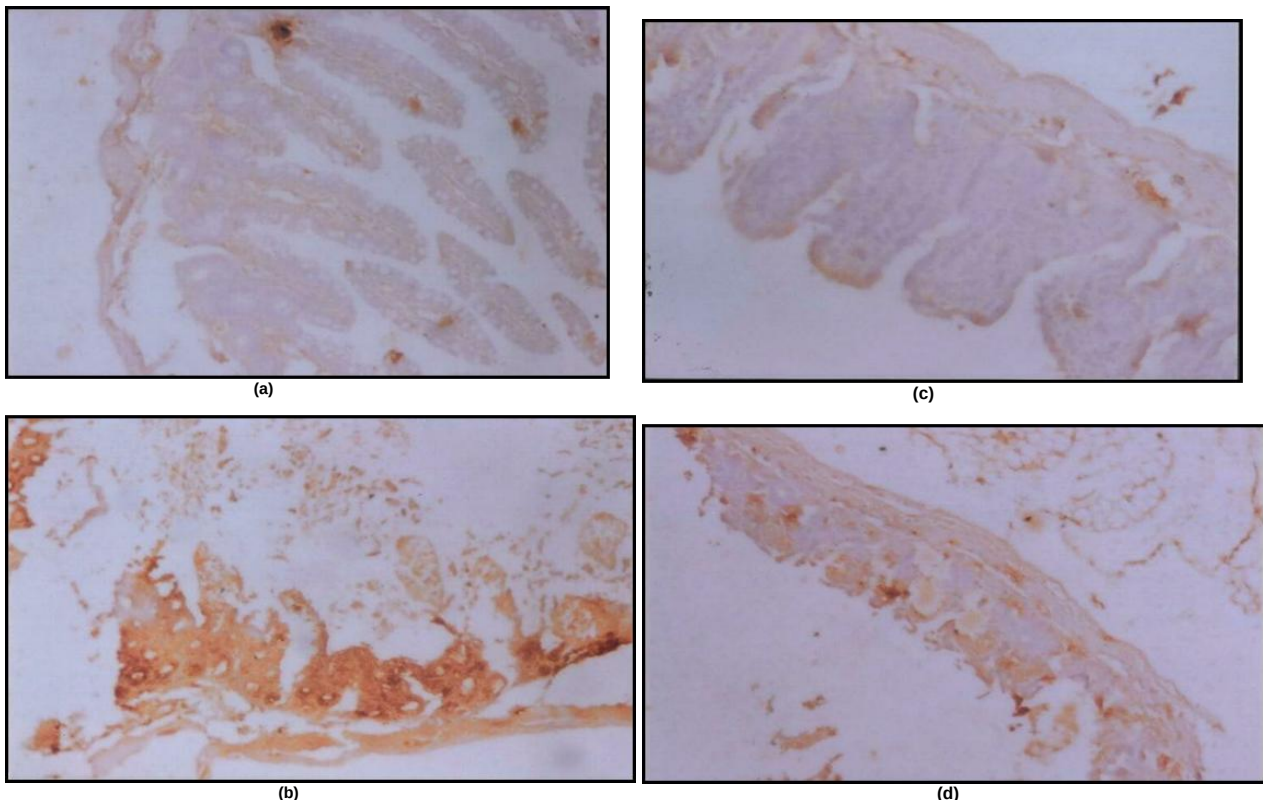

Figure 6. Histology of intestinal sections recovered from rats in the various treatment groups. All images are at 250X magnification of PAP-stained tissues and are representative of samples from rats in each group. Section from: **(A)** sham-operated rat showing weak (±) staining for Fas^+ cells; **(B)** I/R-operated rat (control) showing strong (+++) staining for Fas^+ cells; **(C)** rosiglitazone-pre-treated rat showing mild (+) staining for Fas^+ cells; and, **(D)** rat that received BADGE prior to rosiglitazone, showing strong (+++) staining for Fas^+ cells.

production (Zingarelli et al., 2007). These beneficial effects were associated with an enhancement of PPARγ DNA binding and reduction in NF-κB activation. Interestingly, the cardioprotection afforded by ciglitazone was attenuated by the GW-9662 PPARγ antagonist.

Nitric oxide (NO) produced by endothelial constitutive nitric oxide synthase (ecNOS) was found to act as a protective molecule at the onset of the I/R of the small bowel. NO has also been previously shown to play a significant role in maintenance of mucosal integrity (Salzman, 1995; Lefer and Lefer, 1999). In the mesenteric endothelium, low-level continuous release of NO by eNOS is thought to be a major determinant of vascular tone and regulation of blood flow to the mucosa (Salzman, 1995). In addition to suppressing neutrophil activation and scavenging reactive oxygen species (ROS), NO has also been shown to inhibit enzymes responsible for the release of superoxide (Clancy et al., 1992; Cote et al., 1996). Excess NO production has been attributed to the inducible NOS (iNOS) that is induced in response to systemic inflammatory states, including I/R. The iNOS has been implicated in the pathogenesis of I/R, as the inhibition of iNOS activity and NO production attenuated the intestinal I/R injury (Takada et al., 1998; Turnage et al., 1998; Suzuki et al., 2000; Xia et al., 2001). In the current study, rats subjected to intestinal I/R showed significant increase in intestinal NO content as compared to the sham-operated group. Administration of rosiglitazone resulted in a significant reduction in intestinal NO content as compared to the control I/R group. This effect was antagonized by pre-treatment of rats with BADGE, confirming that the protective effect of rosiglitazone is mainly mediated through activation of PPARγ system.

It has been reported that the PPARγ agonists troglitazone and J series prostaglandins are potent anti-inflammatory agents that prevent cytokine- and endotoxin-stimulated activation of peripheral and resident tissue macrophages and cytokine-induced NOS expression by β–cells (Maggi et al., 2000). Those authors indicated that these outcomes likely arose by an inhibition of transcriptional activation and induction of heat shock responses. Rosiglitazone also exerted potent anti-inflammatory effects, in terms of inhibition of paw edema, pleural exudate formation, mononuclear cell infiltration, and histological injury, in acute inflammation models

(Cuzzocrea et al., 2004). Furthermore, rosiglitazone reduced the increase in the staining for nitrotyrosine and PARP, the expression of iNOS, COX-2, ICAM-1 and P-selectin in the lung of carrageenan-treated rats. Administration of the PPARγ antagonist BADGE significantly abolished the anti-inflammatory effects of rosiglitazone.

It has been reported that prolonged ischemia alone resulted in injury due to oxygen deprivation. However, cellular changes during shorter periods of ischemia initiated the production of ROS when the tissue is re-oxygenated (Alarcón de la Lastra et al., 1997, 1999). The fundamental mechanism of re-perfusion injury is the xanthine oxidase-based free radical generating system that is operative within the endothelial cell alone, even in the absence of neutrophils. The small intestine is a rich source of xanthine oxidase (Zimmerman and Granger, 1994; Kacmaz et al., 1999). Therefore, ischemia and re-perfusion caused an oxidative stress that was characterized by an imbalance between ROS and the anti-oxidative defense system. Re-perfusion of ischemic tissue, although necessary for a reparative mechanism, has been shown to worsen acute ischemic injury via the release of ROS (Carden and Granger, 2000).

In the current study, rats subjected to intestinal I/R showed significant increase in intestinal MDA content as compared to the sham-operated group. These results are in agreement with many previous reports (Otamiri, 1988; Lehmann et al., 1995; Kacmaz et al., 1999). The increase in neutrophil infiltration to intestinal mucosa after I/R paralleled the increase in lipid peroxides, suggesting that neutrophil is an important producer of these reactive oxygen and nitrogen species. Administration of rosiglitazone resulted in a significant reduction in intestinal MDA content as compared to that found in the control I/R group. Treatment with BADGE completely antagonized this rosiglitazone-induced effect. Cuzzocrea et al. (2003) reported that rosiglitazone and 15Δ-PGJ_2 reduced oxidative and nitrosative stress (that is, reduced the degree of immuno-staining for nitrotyrosine) caused by I/R in a rat intestine; this effect was also abolished after BADGE administration. Therefore, based on the results here, in addition to its anti-inflammatory potential, it can be suggested that rosiglitazone possesses antioxidant properties (as evidenced by the decrease in lipid peroxide content). Mechanistically, this outcome could be explained by an inhibitory effect on infiltration of PMN, a key source of ROS. Rosiglitazone also inhibited NO production, and in consequence NO-derived reactive species.

The Fas antigen (CD95, APO-1) is a trans-membrane cell surface receptor that mediates apoptosis of many cell types when bound by Fas ligand or cross-linked by an agonist antibody (Suda et al., 1993). In the current study, immunohistochemical examination of sections from I/R-operated rats revealed a strong cytoplasmic staining for Fas (expression). These results were in agreement with many previous reports (Wu et al., 2002, 2003; Fujise et al., 2006). Rosiglitazone-pre-treated rats showed mild cytoplasmic staining for Fas expression in their intestinal segments. Administration of BADGE prior to rosiglitazone significantly increased Fas expression in the intestines. It has been shown previously that rosiglitazone markedly reduced post-ischemic myocardial apoptosis in hypercholesterolemic rabbits, via inhibition of nitrosative stress and subsequent pro-apoptotic MAPK activation (Liu et al., 2004). It has also been demonstrated that troglitazone decreased the extent of apoptotic cell death as a result of renal I/R injury due to an induction of hepatocyte growth factor (Doi et al., 2007). Furthermore, the numbers of cleaved caspase-3 and single-stranded DNA^+ cells were decreased in rats treated with troglitazone.

In conclusion, this study has demonstrated that PPARγ agonists such as rosiglitazone protected against experimentally induced intestinal I/R injury. This protective effect is mediated through activation of PPARγ system, as administration of BADGE (a selective PPARγ antagonist) completely abolished rosiglitazone protective effects. Activation of PPARγ by rosiglitazone caused a reduction in MPO activity as a measure of neutrophil infiltration, intestinal TNF-α, NO, and MDA contents as well as Fas expression as a marker for apoptotic cell death. All of these effects may be secondary to reduction in NF-κB activation by PPARγ agonists.

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