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European Journal of Pharmacology 505 (2004) 195–203

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Rosiglitazone, an agonist of peroxisome proliferator-activated receptor gamma, protects against gastric ischemia–reperfusion damage in rats: role of oxygen free radicals generation

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Received 10 June 2004; received in revised form 24 September 2004; accepted 4 October 2004
Available online 28 October 2004

Abstract

Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a nuclear hormone receptor super family that has recently been implicated in atherosclerosis, inflammation, cancer, infertility, and demyelination. Oxidative stress, neutrophil infiltration, proinflammatory cytokines, and the exhibition of luminal acid play a role in the pathogenesis of gastric injury induced by ischemia–reperfusion. Rosiglitazone, a specific PPAR-γ ligand, has been shown to have antiinflammatory activity, but its effects on experimental ischemia–reperfusion gastric injury remain unknown. We have investigated the effects of the rosiglitazone on gastric injury caused by ischemia following reperfusion in rats. Tumour necrosis factor-alpha (TNF-α) levels and changes in enzymatic activities of myeloperoxidase, as a marker of neutrophils infiltration, xanthine oxidase, superoxide dismutase, and glutathione peroxidase, were determined. Histological analysis of the lesions was also carried out. Pretreatment with 1 or 4 mg/kg of rosiglitazone ameliorated the gastric damage induced by clamping the celiac artery for 30 min followed by 60 min of reperfusion. It significantly ($P<0.05$) reduced the index of neutrophil infiltration and the levels of the cytokine. Rosiglitazone did not revert the reduced glutathione peroxidase activity but enhanced significantly ($P<0.01$) the decreased xanthine oxidase and superoxide dismutase activities in gastric mucosa of ischemic rats. In conclusion, rosiglitazone reduces the damage in ischemia–reperfusion gastric injury and alleviates the inflammatory response and the oxidative events.

Keywords: PPAR-γ; Rosiglitazone; Ischemia–reperfusion; Reactive oxygen specie; Neutrophil; Cyclooxygenase

1. Introduction

Reperfusion following ischemia gastric injury associated with haemorrhage and other shock states is characterized by a number of microvascular and mucosal alterations, including endothelial cell swelling, capillary plugging, a prolonged reduction in gastric blood flow, and mucosal barrier dysfunction. Adhesion neutrophils to endothelial cells, various inflammatory mediators, the exhibition of luminal acid, and excessive formation of reactive oxygen species play a role in the pathogenesis of gastric injury induced by ischemia–reperfusion (Alarcón De La Lastra et al., 1997, 1999; Kwiecien et al., 2002; Brzozowski et al., 2003).

The main chemoattractant for neutrophils is tumour necrosis factor-alpha (TNF-α), a proinflammatory cytokine that, in association with interleukin-1β, induces enzyme activities (nitric oxide synthase, phospholipase A_2, cyclooxygenases, proteases) that stimulate adhesion molecules on endothelial cells and promote neutrophil adherence (Appleyard et al., 1996). Against these injurious agents exist endogenous antioxidant systems capable of inhibiting oxidation without requiring much energy. Between them, glutathione peroxidase is an endogenous enzyme that protects cells from these harmful effects by catalysing the

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doi:10.1016/j.ejphar.2004.10.020

glutathione redox cycle. Also, the superoxide dismutase is an important antioxidant enzyme which catalyses the dismutation of O_2^- into less noxious H_2O_2 that is further degraded by catalase or glutathione peroxidase (Halliwell and Gutteridge, 1989).

The peroxisome proliferator-activated receptor gamma (PPAR-γ) is a member of the nuclear hormone receptor super family that is involved in several physiological processes, such as glucose homeostasis, cellular differentiation, regulation of lipid, and lipoprotein metabolism, as well as in pathological states including atherosclerosis, inflammation, cancer, infertility, and demyelination (Alarcón de la Lastra et al., in press). PPAR-s regulated gene expression by binding, as heterodimers, with retinoid X receptors to specific PPAR-response elements in the promoter regions of specific target genes (Devergne and Wahli, 1999). Thiazolidinediones are synthetic PPAR-γ ligands used in the type 2 diabetes control, being rosiglitazone the most potent and selective agent in this class, which binds the receptor with a higher affinity than other thiazolidinediones, such as pioglitazone or ciglitazone (Young et al., 1998; Nosjean and Boutin, 2002).

Recent studies indicate that PPAR-γ agonists have a protecting role against ischemia–reperfusion damage. In fact, Konturek et al. (2003) confirmed that pioglitazone reduced gastric mucosal injury induced by ischemia–reperfusion, and other authors demonstrated that rosiglitazone ameliorated the lesions associated with ischemia–reperfusion of the kidney, heart, and gut possibly due to the antiinflammatory properties (Khandoudi et al., 2002; Wayman et al., 2002; Cuzzocrea et al., 2003; Sivarajah et al., 2003). However, the effects of PPAR-γ agonists on reactive oxygen species generation in conditions associated with ischemia–reperfusion injury have not yet been investigated. This study investigates the effects of the PPAR-γ agonist, rosiglitazone, on oxidative stress production in a rodent model of gastric ischemia–reperfusion injury. Accordingly, changes in the activities of oxidative stress-related enzymes, such as xanthine oxidase, superoxide dismutase, and glutathione peroxidase, were studied.

As mentioned above, neutrophils and proinflammatory cytokines are clearly involved in the pathogenesis of ischemia–reperfusion injury. Therefore, inflammation response was assessed by histology and myeloperoxidase activity as an index of quantitative inflammation and neutrophil infiltration in the mucosa. Mucosal TNF-α production and histological analysis of the lesions were also performed.

2. Material and methods

2.1. Animals groups and drug preparation

Male Wistar rats supplied by the Animal Services, Faculty of Medicine, University of Seville, Spain, and with 180–250 g body weight were placed singled in cages with wire-net floors in a controlled room (temperature 22–24 °C, humidity 70–75%, and lighting regimen of 12 L/12 D) and were fed a normal laboratory diet. Rats were deprived of food for 24 h before experimentation but allowed free access to tap water throughout. They were randomly assigned to groups of 6–10 animals. Experiments followed a protocol approved by the local animal Ethics Committee and the Local Government. All experiments were in accordance with the recommendations of the European Union regarding animal experimentation (Directive of the European Counsel 86/609/EC).

Rosiglitazone (GlaxoSmithKline, Madrid, Spain) at the doses of 1 and 4 mg/kg body weight was suspended in 0.9% saline solution and administered by gavage for 30 min before surgical procedure. Control groups received vehicle in a comparable volume (10 ml/kg body weight) also by the same route. The animals were randomly divided into four groups: sham (nonulcerated), ischemia–reperfusion ulcerated control, and rosiglitazone (1 and 4) groups.

2.2. Production of ischemia–reperfusion lesions

Ischemia–reperfusion damage was produced in rats by method proposed by Ueda et al. (1989). Rats were anaesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 50 mg kg^{-1} body weight. The left side of the abdomen was shaved, and a 3-cm incision was made from the midline to below the ribcage using a diathermy. Briefly, the celiac artery was dissected free of excess fat and clamped for 30 min (ischemia phase) approximately 0.5 cm from its origin from the aorta using an atraumatic microvascular clamp. Reoxygenation was allowed by removal of the clamp for 60 min (reperfusion phase). At the end of the experimental period, the animals were killed by exsanguinations via the abdominal aorta. The stomach of each rat was removed and opened along the greater curvature, and any lesions were examined macroscopically; the number and area of gastric lesions were determined using a planimetry (Morphomat, Carl Zeiss, Berlin, Germany) by one investigator who was unaware of the treatment given. The length and width of the ulcers were measured on a cold stand, and the sum of the damaged areas was calculated. Results were expressed in terms of ulcer index (UI; mm^2 and score: 0—absence of lesions; 1—petechiaes; 2—from one to five pointed lesions <3 mm; 3—more than five pointed lesions <3 mm or one pointed lesion >3 mm; 4—mainly lesions >3 mm; Martín et al., 1997). Then, the mucosa was scrapped off by means of two glass slides on ice, weighed and frozen at −70 °C until biochemical determinations.

2.3. Histological examination

In six rats of each group, samples of macroscopically normal and ulcerated stomachs were processed by routine methods for subsequent histological evaluation.

For examination with the light microscope, we used tissue samples from the gastric mucosa of each animal fixed in 4% buffered paraformaldehyde, dehydrated in grade ethanol, and embedded in paraffin. Thereafter, sections of tissue were cut at 5 μm on a rotary microtome (Leica Ultracut), mounted on clean glass slides, and dried overnight at 37 °C. Sections were cleared, hydrated, and stained with Giemsa and haematoxylin and eosin for histological evaluation of gastric damage and cell infiltration according to standard protocols, and the slides were coded to prevent observer bias during evaluation. All tissue sections were examined in an Olympus BH-2 microscope for characterization of histopathological changes.

Photographs taken from gastric samples were digitised using Kodak D290 Zoom camera Eastman Kodak, USA, and Motic® Images 2000 release 1.1 (MicroOptic Industrial Group; B1 Series System Microscopes). Analysis of the figures was carried out by Adobe®Photoshop® Version 5.0 (Adobe Systems) image analysis program.

2.4. Immunohistochemical study

Gastric tissues were fixed in 4% buffered paraformaldehyde, dehydrated through graded concentrations of ethanol, embedded in paraffin, and sectioned. Sections (5 μm thick) were mounted on slides, cleared, and hydrated. All of them were treated with a buffered blocking solution (3% bovine serum albumin) for 15 min. Then, sections were coincubated with primary antibodies for COX-1 and COX-2 (goat polyclonal, M-19 and M-20 of Santa Cruz Biotechnologies) at a dilution of 1:400 at room temperature for 1 and 24 h, respectively. Sections were washed with PBS and coincubated with secondary antibody (antisheep IgG, peroxidasic-conjugated, Sigma, Spain; 1:500 in PBS, v/v) at room temperature for 1 h. Thereafter, sections were washed as before and with Tris–HCl 0.05 M, pH 7.66, and then coincubated with a 3,3′ -diaminobencidine solution in the dark at room temperature for 10 min. Sections were washed with Tris–HCl, stained with haematoxylin according to standard protocols, and observed under an Olympus BH-2 microscope.

2.5. Assessment of leukocyte involvement

Myeloperoxidase activity was assessed as a marker of neutrophil infiltration (Grisham et al., 1990). In all animals, one sample from the body of the stomach (gastric corpus) was obtained. Samples were excised from each animal and rapidly rinsed with ice-cold saline, blotted dry, and frozen at −70 °C. The tissue was thawed, weighed, and homogenized in 10 volumes 50 mM phosphate-buffered saline (PBS), pH 7.4. The homogenate was centrifuged at 20.000×g, 20 min, 4 °C. The pellet was again homogenized in 10 volumes 50 mM PBS, pH 6.0, containing 0.5% hexadecyl-trimethylammonium bromide (HETAB) and 10 mM EDTA. This homogenate was subjected to one cycle of freezing/thawing and a brief period of sonication. A sample of homogenate (0.5 μl) was added to a 0.5 ml reaction volume containing 80 mM PBS, pH 5.4, 0.5% HETAB, and 1.6 mM 3,3′ ,5,5′ -tetramethylbenzidine (TMB). The mixture was incubated at 37 °C for 5 min, and the reaction started by the addition of 0.3 mM H_2O_2.

Each tube containing the complete reaction mixture was incubated for exactly 3 min at 37 °C. The reaction was terminated by the sequential addition of catalase (20 μg/ml) and 2 ml 0.2 M sodium acetate, pH 3.0. The changes in absorbance at 655 nm were measured with a spectrophotometer. One unit of myeloperoxidase activity was defined as the amount of enzyme present that produced a change in absorbance of 1.0 U/min at 37 °C in the final reaction volume containing the acetate. Results were expressed as unit per milligram protein.

2.6. Measurement of tumour necrosis factor-alpha (TNF-α) levels

Gastric mucosal samples were weighed and homogenized, after thawing, in 100 mg simple/0.9 ml phosphate buffer saline solution (PBS pH 7.2) at 4 °C. They were centrifuged at 12000 rpm for 20 min. Mucosal TNF-α level was assayed by using a commercially available TNF-α enzyme-linked immunosorbent assay (ELISA) kit (Genzyme Diagnostics, Cambridge). The TNF-α values were expressed as picogram per milligram protein.

2.7. Assessment of xanthine oxidase activity

The tissue was homogenized in buffer consisting of Tris(+)–HCl, EDTA, phenylmethylsulfonyl fluoride (PMSF), dithiothreitionin, and leupeptine, pH 8.1. The homogenate was centrifuged, and the supernatant was separated by Sephadex (G-25) column. Xanthine was used as substrate for xanthine oxidase activity studies. Xanthine oxidase activity was assayed as uric acid production by the increase in absorbance at 294 nm in the absence of NAD^+. One unit of xanthine oxidase activity corresponds to the formation of 1 μM of uric acid per minute (Devenyi et al., 1987). Results were expressed as unit per milligram protein per minute.

2.8. Determination of glutathione peroxidase activity

Glutathione peroxidase activity was determined according to the method of Lawrence and Burk (1976). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM NaN_3, 0.2 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 1 EU/ml oxidized glutathione (GSSG)-reductase, 1 mM GSH, and 0.25 mM H_2O_2.. Samples were added to 0.8 ml of the above mixture and incubated for 5 min at 25 °C before initiating the reaction with the addition of peroxide solution. A sample of supernatant fluid with 10%

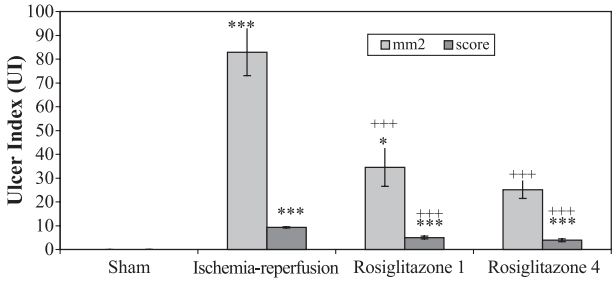

Fig. 1. Gastric ulcer index (UI, mm^2 and score) in rats after pretreatment with rosiglitazone (1 or 4 mg/kg of body weight) 30 min before ischemia–reperfusion procedure. The presence of lesions was established at postmortem by visual inspection of the stomach. Vehicle or different doses of drugs were administered p.o., n=8–10 per group. The control groups were evaluated at the same periods that as respective treated groups. The data are shown as means±S.E.M. (*P<0.05 and ***P<0.001 vs. sham; ^{+++}P<0.001 vs. ischemia–reperfusion control).

homogenate solution and 1.15% KCl was prepared by centrifugation at 4000×g for 10 min at 4 °C. The absorbance at 340 nm was recorded for 5 min. The activity was the slope of the lines as nanomole of NADPH oxidized per minute. The blank datum (the enzyme was replaced with distilled water) was subtracted from each value. Results were expressed as nanomole per milligram protein per minute.

2.9. Superoxide dismutase activity

The enzymatic activity of superoxide dismutase is based on the inhibition of the reduction of cytochrome C according to the method of McCord and Fridovich (1969). Samples of gastric mucosa were homogenized in a mixture of PBS and EDTA. The homogenate was supplemented with

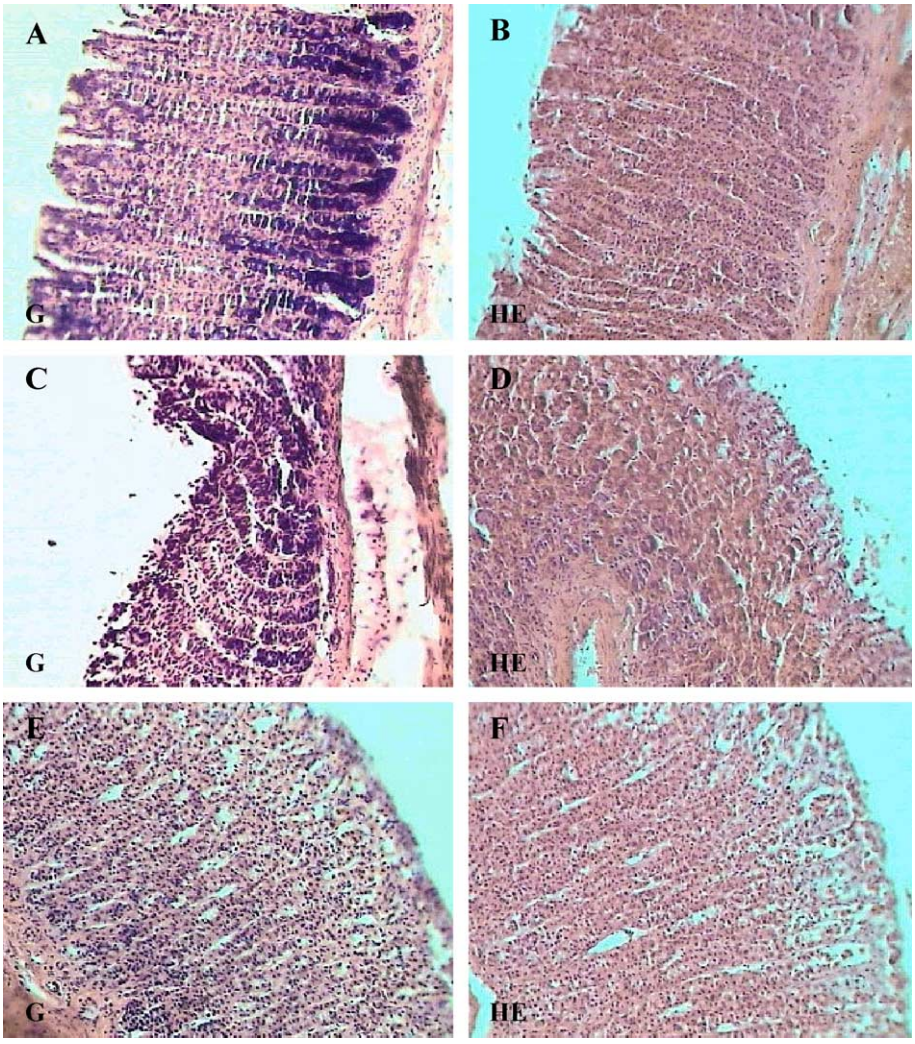

Fig. 2. Effects of rosiglitazone on histological findings of gastric damage induced by ischemia–reperfusion in rats after Giemsa stain (G) or haematoxylin and eosin stain (H-E): sham (A and B), ischemia–reperfusion control (C and D), and rosiglitazone 4 mg/kg body weight (E and F). Histopathological features of the stomach: (A) and (B) no histological modification was present in the sham animals. (C) and (D) After ischemia following reperfusion, histopathological features included superficial exfoliation, zones with haemorrhages and blood cell infiltration. (E) and (F) Pretreatment with rosiglitazone produced a reduction of exfoliation of superficial cells, haemorrhage, and blood cell infiltration. The recuperation of the typical structure and alignment of the gastric gland mucosa also was observable. Original magnification 20×.

0.1% Triton. The assay method used cytochrome C, xanthine, and sufficient milk xanthine oxidase to give a rate of increase in absorbance of 0.025/min at pH 7.8 and 25 °C. The reaction kinetic was measured in a spectrophotometer at 550 nm at a rate of 0–80 s. Results were expressed as unit per milligram protein per minute. One unit of superoxide dismutase is defined as the amount of enzyme that causes 50% inhibition of cytochrome C reduction.

2.10. Statistical analysis

All values in the figures and text are expressed as arithmetic means ± standard error of the mean (S.E.M.). The data were evaluated with Graph Pad Prism® Version 2.01 software. The statistical significance of differences for each parameter among the groups was evaluated by one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. P values of <0.05 were considered statistically significant. In the experiment involving histology or immunohistochemistry, the figures shown are representative of at least six experiments performed on different days.

3. Results

Our results show that gastric injury was significantly increased after 60 min reperfusion following 30 min of ischemia by clamping the celiac artery. The lesions were oedematous and haemorrhagic and occurred in a linear fashion. The total area of lesions reached a value of 82.9±9.8 mm^2. Treatment with either 1 or 4 mg/kg of rosiglitazone clearly diminished the number and severity of ulcers. The mean ulcer indexes (UI) were significantly lower (P<0.001) than that of ischemia–reperfusion control rats (Fig. 1).

No damage was observed histologically in the gastric mucosa of normal rats (Fig. 2A–B). Nevertheless, after ischemia following reperfusion, exfoliation and necrosis of superficial cells, structural alterations on the 2/3 parts of the glandular pits, and bleeding erosions developed in all stomachs. The architecture of the crypts was distorted, and an infiltrate consisting of polymorphonuclear leukocytes, lymphocytes, and eosinophils was observed (Fig. 2C–D). Pretreatment with rosiglitazone produced a reduction of exfoliation of superficial cells, haemorrhage, and blood cell infiltration. The recuperation of the typical structure and alignment of the gastric gland mucosa was also observable (Fig. 2E–F).

Table 1
Effects of rosiglitazone (1 or 4 mg/kg of body weight) on myeloperoxidase (MPO) activity and tumour necrosis factor-alpha (TNF)-α levels after gastric ischemia/reperfusion

Group	N	MPO (U/mg protein)	(TNF)-α (pg/mg protein)
Sham	7	2.97±0.37	39.53±8.20
Ischemia–reperfusion	9	7.07±0.72[a]	142.37±20.90[a]
Rosiglitazone 1	6	5.02±0.65	95.03±4.25
Rosiglitazone 4	7	4.58±0.53[b]	86.52±10.76[b]

Gastric mucosal myeloperoxidase activity and tumour necrosis factor-alpha levels were quantified in the absence of treatment but with administration of the vehicle saline solution (sham and ischemia–reperfusion groups) or in the presence of rosiglitazone (1 or 4 mg/kg body weight) administered p.o. for 30 min before the experimentation. Data are shown as means±S.E.M.
[a] P<0.001 significantly different from sham.
[b] P<0.05 significantly different from ischemia–reperfusion control.

Table 2
Effect of rosiglitazone on xanthine oxidase (XO), glutathione peroxidase (GSH-px), and superoxide dismutase (SOD) activities after gastric ischemia–reperfusion injury

Group	XO × 10^{-5} (U/mg protein/min)	GSH-px (nmol/mg protein/min)	SOD (U/mg protein/min)
Sham	5.12±0.56	5.21±0.31	5.22±0.35
Ischemia–reperfusion	9.00±1.89[a]	3.89±0.67	3.26±0.25[a]
Rosiglitazone 1	4.42±0.28[b]	3.28±0.17	6.75±0.88[b]
Rosiglitazone 4	4.37±0.15[b]	2.95±0.27	6.27±0.45[b]

Gastric mucosal xanthine oxidase and glutathione peroxidase activities were quantified in the absence of treatment but with administration of the vehicle saline solution (sham and ischemia–reperfusion groups) or in the presence of rosiglitazone 1 or 4 mg/kg body weight) administered p.o. for 30 min before the experimentation. Data are shown as means±S.E.M.
[a] P<0.05 significantly different from sham.
[b] P<0.01 significantly different from ischemia–reperfusion control.

As shown in Table 1, myeloperoxidase activity was significantly (P<0.001) increased in ulcerated control animal compared with sham animals (7.07±0.72 U/mg protein). These data were consistent with the histological findings. However, pretreatment with the highest dose of the PPAR-γ agonist rosiglitazone significantly (P<0.05) reduced the degree of neutrophils infiltration into the gastric mucosa. Table 1 also presents the production of the proinflammatory cytokine, TNF-α, in gastric mucosa. After ischemia–reperfusion, the content of TNF-α experimented a significant increase up to 142.37±20.90 pg/mg protein (P<0.001). This increase was significantly reduced (P<0.05) after rosiglitazone administration.

The activity of xanthine oxidase in gastric mucosa of vehicle-treated rats was (5.12±0.56) ×10^{-5} U/mg protein/min (Table 2). Ischemia–reperfusion procedure resulted in a significant ascent of this enzymatic activity to (9.00±1.89) 10^{-5} U/mg protein/min (P<0.05). By contrast, rosiglitazone pretreatment (1 and 4 mg/kg) induced a significant reduction (P<0.01) of xanthine oxidase activity versus ischemia–reperfusion control group and displayed a xanthine oxidase activity level next to sham animals.

Changes in superoxide dismutase and glutathione peroxidase activities are also shown in Table 2. Our data showed that superoxide dismutase activity was significantly decreased in gastric mucosa following gastric injury from a basal level of 5.22±0.35 to 3.26±0.25 U/mg protein/min (P<0.05), but treatment with rosiglitazone at doses of 1 or 4 mg/kg resulted in a significant (P<0.01)

increase of the superoxide dismutase activity up to the ischemic control. Animals subjected to ischemia–reperfusion procedure presented a significant ($P<0.05$) decrease in the activity of the glutathione peroxidase (3.89±0.67 nmol/mg protein/min) than in the sham group, which activity was of 5.21±0.31 nmol/mg protein/min. In addition, no significant changes with regard to the control values in the activity of glutathione peroxidase were observed after pretreatment with the PPAR-γ agonist at doses of 1 and 4 mg/kg (Table 2).

Immunohistochemical studies revealed that cyclooxygenase-1 and -2 proteins are located in different sites (Figs. 3B and 4B). No significant quantitative differences among the individual groups were observed in cyclooxygenase-1 assessment, although the cellular distribution of the isoenzime was different: cyclooxygenase-1 was expressed mainly in mucous neck cells and in the base of the gastric glands of sham animals (Fig. 3B), while after ischemia–reperfusion procedure, specific immunosignals for cyclooxygenase-1 were obtained in cells of the surface epithelium (Fig. 3C). Pretreatment with rosiglitazone produced a reduction of cyclooxygenase-1 expression distortion provoked by ischemia–reperfusion, showing a similar location that in the sham controls (Fig. 3D). On the contrary, cyclooxygenase-2 was scarcely found in mucosa of the sham group (Fig. 4B), although it was clearly present in the ulcerated control after ischemia–reperfusion (Fig. 4C and D) where COX-2 was detected in mucous surface cells and mucous cells of the foveoles adjacent to the ulcer crater (Fig. 4D). Nevertheless, oral administration of rosiglitazone diminished the induced up-regulation of cyclooxygenase-2 (Fig. 4E).

4. Discussion

The results of this study indicate that rosiglitazone dose-dependently reduced development of the gastric mucosal lesions induced by ischemia–reperfusion in rats. A similar profile was observed when the beneficial effects of this PPAR-γ ligand against myocardial (Yue Tl et al., 2001), renal (Sivarajah et al., 2003), and intestinal (Cuzzocrea et al., 2003) ischemia–reperfusion injuries were explored.

We have previously reported that prolonged ischemia alone results in injury due to oxygen deprivation. However, cellular changes during shorter periods of ischemia initiate the production of reactive oxygen species when the tissue is reoxygenated (Alarcón De La Lastra et al., 1997, 1999). The fundamental mechanism of reperfusion injury is the xanthine oxidase-based free radical generating system which is operative within the endothelial cell alone, even in the absence of neutrophils. During ischemia, ATP is degraded to hypoxanthine, and xanthine dehydrogenase is converted to xanthine oxidase. In the reperfusion state, xanthine oxidase catalyses the conversion of molecular

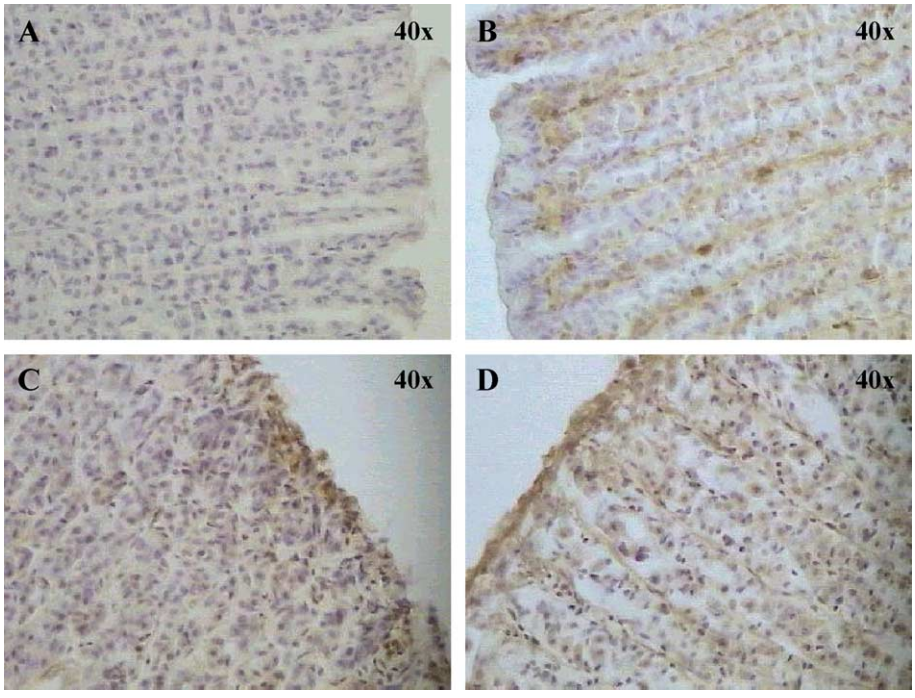

Fig. 3. Immunohistochemical localization of cyclooxygenase-1 isoenzyme in sections of the stomach. To assess the specificity of the immunoreactions, control sections were incubated without the primary antibody [negative control (A)]. Cyclooxygenase-1 expression in mucous neck cells is particularly evident in this image of normal gastric mucosa (B). Cyclooxygenase-1 expression in the stomach of ischemia–reperfusion control rats (C). Cyclooxygenase-1 expression in the stomach of rats after pretreatment with rosiglitazone (4 mg/kg of body weight) 30 min before ischemia–reperfusion procedure (D). Original magnification 40×.

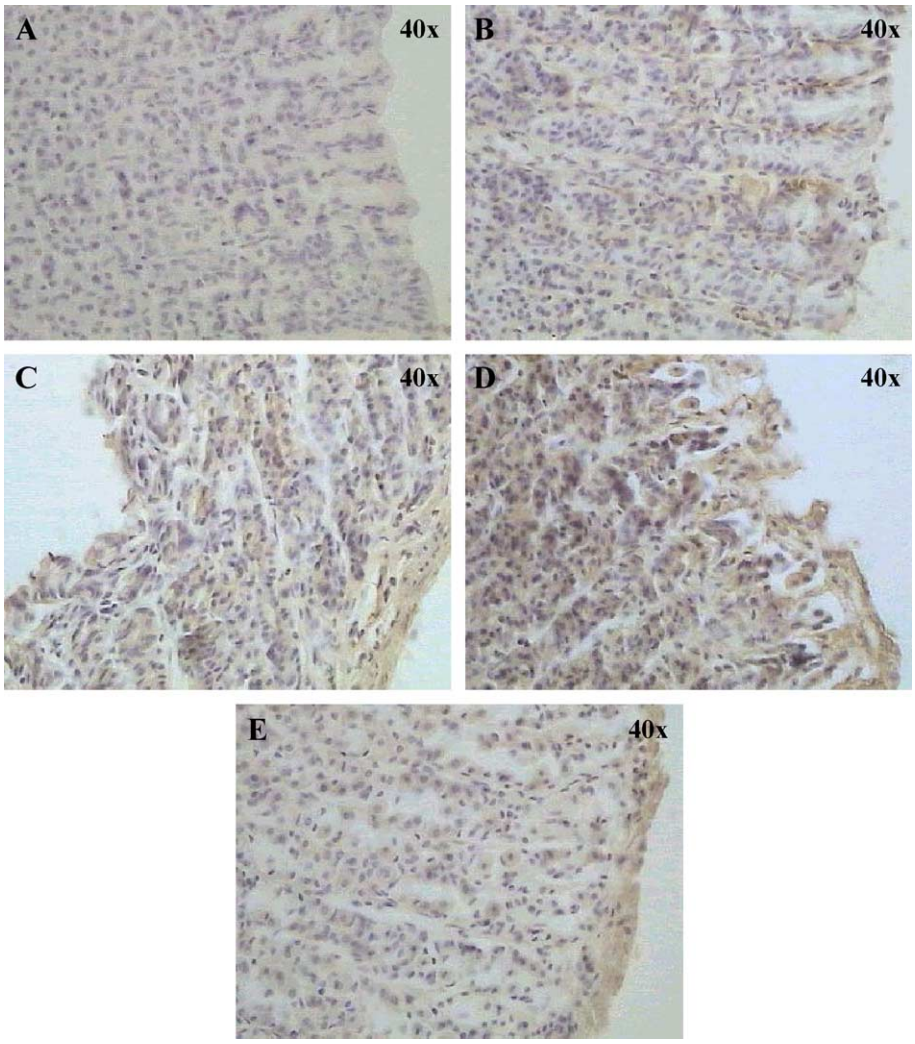

Fig. 4. Immunohistochemical localization of cyclooxygenase-2 isoenzyme in sections of stomach. Negative control (A); cyclooxygenase-2 expression in normal gastric mucosa (B); cyclooxygenase-2 expression in the stomach of ischemia–reperfusion control rats (C and D). Cyclooxygenase-2 is strongly expressed in the tissue adjacent to the ulcer crater (D). Cyclooxygenase-2 expression in the stomach of rats after pretreatment with rosiglitazone (4 mg/kg of body weight) 30 min before ischemia–reperfusion procedure (E). Original magnification 40×.

oxygen to O_2^-, which rapidly react with the free radical nitric oxide and peroxynitrite anion and other reactive species. This initiates the process of lipid peroxidation and release of substances that recruit and activate polymorphonuclear leukocytes (Cabeza et al., 2001).

The results of this study clearly show that the protective effect of the PPAR-γ ligand against ischemia–reperfusion injury was linked to its ability to reduce the activity of the xanthine oxidase system and in consequence to a reduction of O_2^- generation. Neither rosiglitazone nor other PPAR-γ agonists have been reported to have antioxidant activities in vitro, particularly the inhibition of the generation of reactive oxygen species via the xanthine oxidase. In a recent report, Naito et al. (2001) showed a beneficial effect of the PPAR-γ, ligand pioglitazone on aspirin-induced gastric mucosal injury and significantly inhibited the increased in thiobarbituric acid-reactive substance production, as index of lipid peroxidation.

This study also shows that myeloperoxidase activity, an index of tissue-associated neutrophil accumulation and the production of the proinflammatory cytokine TNF-α, was increased in the gastric mucosa after clamping the celiac artery for 30 min, followed thereafter by reperfusion. In this experimental model, neutrophils act as a secondary amplifier rather than an initial trigger of reperfusion injury (Alarcón De La Lastra et al., 1997, 1999; Kwiecien et al., 2002). Activated neutrophils produce O_2^- through NADPH oxidase, which reduces molecular oxygen to the O_2^- radical, and through the enzyme myeloperoxidase that catalyzes the formation of such potent cytotoxic oxidants as hypochlorous acid from H_2O_2 and chloride ions and *N*-chloramines. In addition, neutrophils can also release proteases, lactoferrin, and lipid mediators that can contribute to gastric injury (Villegas et al., 2002). We found that pretreatment with the highest dose of rosiglitazone significantly reduced the myeloperoxidase activity. These

data are in agreement with previous reports which suggest that rosiglitazone and other PPAR-ã agonists can regulate inflammatory responses thorough several mechanisms, including reduction of the expression of proinflammatory mediators and neutrophil infiltration (Cuzzocrea et al., 2003).

It has been suggested that the main chemoattractants for neutrophil are proinflammatory cytokines, such as interleukin-1β, interferon-γ, and TNF-α, that regulate endothelial adhesion molecules expression on vascular endothelial cells and promotes neutrophil adherence to these cells. In our study, in agreement with reports from other studies (Kwiecien et al., 2002; Ichikawa et al., 2002; Konturek et al., 2003), up-regulation of TNF-α production in gastric mucosa was correlated with the development of reperfusion injury. Besides, the pretreatment of rats with the PPAR-γ agonist attenuated the production of the cytokine. Although the exact mechanism of action by which PPAR-γ agonists exert their antiinflammatory effects in the gastric mucosa is currently unknown, some authors (Naito et al., 2001; Brzozowski et al., 2001; Cabrero et al., 2002; Cuzzocrea et al., 2003) have suggested that this activity may be mediated through the repression of transcription factor nuclear (NF)-κB signalling and TNF-α production. Thus, we propose that one mechanism underlying the protective effects of rosiglitazone involves a reduction of neutrophils infiltration into the gastric mucosa, possibly via inhibition of TNF-α production.

To protect tissues against the deleterious effects of reactive oxygen species, all cells possess numerous antioxidant enzymes and free radical scavengers. Primary defences include the enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Glutathione peroxidase is an important enzyme which plays a role in the elimination of H_2O_2 and lipid hydroperoxides in the gastric mucosal cell. The antioxidant activity of glutathione peroxidase is coupled to the oxidation of reduced glutathione, an important intracellular antioxidant which can subsequently be reduced by glutathione reductase with NADPH as the reducing agent. Thus, inhibition of this enzyme may result in the accumulation of H_2O_2 with subsequent oxidation of lipids. Our results revealed that glutathione peroxidase activity significantly decreased in gastric mucosa during ischemia–reperfusion. These findings are in agreement with other studies on gastric injury induced by the same surgical procedure (Tanaka and Yuda, 1993; Kim and Kim, 2001). However, there were nonsignificant changes in glutathione peroxidase activity in rosiglitazone-treated rats.

A role for superoxide anion in the present model of gastric injury is supported by the observation that superoxide dismutase activity was significantly reduced in control ulcerated stomach. These findings are in agreement with other studies on gastric injury induced by ischemia–reperfusion (Cabeza et al., 2001; Kwiecien et al., 2002). In our study, clearly, rosiglitazone administration resulted in a significant increase of the superoxide dismutase activity nearly up to the nonulcerated control values.

In conclusion, in addition to antiinflammatory mechanisms, specially to the reduction of TNF-α production, our results suggest that the inhibitory effects of rosiglitazone on reactive oxygen generation mainly, superoxide anion, probably derived via xanthine oxidase and/or neutrophils activation, and the increase of superoxide dismutase activity seem to contribute significantly to the gastroprotection afforded by the PPAR-ã ligand in this experimental model.

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