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Rosiglitazone and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, ligands of the peroxisome proliferator-activated receptor-γ (PPAR-γ), reduce ischaemia/reperfusion injury of the gut

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1 The peroxisome proliferator-activated receptor-γ (PPAR-γ) is a member of the nuclear receptor superfamily of ligand-dependent transcription factors related to retinoid, steroid and thyroid hormone receptors. The thiazolidinedione rosiglitazone and the endogenous cyclopentenone prostaglandin (PG)D_2 metabolite, 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2), are two PPAR-γ ligands, which modulate the transcription of target genes.

2 The aim of this study was to investigate the effect of rosiglitazone and 15d-PGJ_2 on the tissue injury caused by ischaemia/reperfusion (I/R) of the gut.

3 I/R injury of the intestine was caused by clamping both the superior mesenteric artery and the coeliac trunk for 45 min, followed by release of the clamp allowing reperfusion for 2 or 4 h. This procedure results in splanchnic artery occlusion (SAO) shock.

4 Rats subjected to SAO developed a significant fall in mean arterial blood pressure, and only 10% of the animals survived for the entire 4 h reperfusion period. Surviving animals were killed for histological examination and biochemical studies. Rats subjected to SAO displayed a significant increase in tissue myeloperoxidase (MPO) activity and malondialdehyde (MDA) levels, significant increases in plasma tumour necrosis factor (TNF)-α and interleukin (IL)-1β levels and marked injury to the distal ileum.

5 Increased immunoreactivity to nitrotyrosine was observed in the ileum of rats subjected to SAO. Staining of sections of the ileum obtained from SAO rats with anti-intercellular adhesion molecule (ICAM-1) antibody resulted in diffuse staining.

6 Administration at 30 min prior to the onset of gut ischaemia of the two PPAR-γ agonists (rosiglitazone (0.3 mg kg^{-1} i.v.) and 15d-PGJ_2 (0.3 mg kg^{-1} i.v.)) significantly reduced the (i) fall in mean arterial blood pressure, (ii) mortality rate, (iii) infiltration of the reperfused intestine with polymorphonuclear neutrophils (MPO activity), (iv) lipid peroxidation (MDA levels), (v) production of proinflammatory cytokines (TNF-α and IL-1β) and (vi) histological evidence of gut injury. Administration of rosiglitazone and 15d-PGJ_2 also markedly reduced the nitrotyrosine formation and the upregulation of ICAM-1 during reperfusion.

7 In order to elucidate whether the protective effects of rosiglitazone and 15d-PGJ2 are related to the activation of the PPAR-γ receptor, we also investigated the effect of a PPAR-γ antagonist, bisphenol A diglycidyl ether (BADGE), on the protective effects of rosiglitazone and 15d-PGJ_2. BADGE (1 mg kg^{-1} administered i.v. 30 min prior to the treatment of rosiglitazone or 15d-PGJ_2) significantly antagonised the effect of the two PPAR-γ agonists and thus abolished the protective effect against gut I/R.

8 These results demonstrate that the two PPAR-γ agonists, rosiglitazone and 15d-PGJ_2, significantly reduce I/R injury of the intestine.
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Abbreviations: I/R, Ischaemia/reperfusion; MABP, Mean arterial blood pressure; PMNs, polymorphonuclear leucocytes; ICAM, intercellular adhesion molecules; IL, Interleukin; TNF, Tumour necrosis factor; PPARs, Peroxisome proliferator-activated receptors; RXRs, Retinoid X receptors; PPREs, PPAR response element; TZDs, Thiazolidinediones; PG, Prostaglandin; 15d-PGJ_2, 15-deoxy-$^{12,14}PGJ_2$; SAO, splanchnic artery occlusion; MPO, Mycloperoxidase; MDA, Malondialdehyde; BADGE, Bisphenol A diglycidyl ether; NF, Nuclear factor; IKK, IK kinase

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Introduction

Ischaemia leads to hypoxia, which initiates a series of events primarily related to activation of platelets and release of their vasoconstrictor mediators (e.g. thromboxane A_2 and 5-hydroxytryptamine), which further restrict blood flow to the ischaemic area (Lefer, 1987).

It is important to realise that reperfusion of an ischaemic organ is not only associated with local changes and that, in some situations, reperfusion is also associated with systemic changes. For example, in a model of ischaemia/reperfusion (I/R) of the intestine, local functional alterations include a progressive fall in the mean arterial blood pressure (MABP), release of proinflammatory mediators from the reperfused tissues into the systemic circulation and ultimately reduced survival (Bittermann & Lefer, 1988; Bittermann *et al*., 1991; Zingarelli *et al*., 1992).

One of the earliest phenomena occurring in I/R is the endothelial dysfunction, which is considered the 'trigger' of reperfusion injury and is amplified by the subsequent recruitment of polymorphonuclear leucocytes (PMNs) to the reperfusion site (Bulkley, 1989; Lefer & Lefer, 1993). Leucocyte–endothelial interaction involves a complex system of adhesion molecules (selectins, β_2 integrins and the immunoglobulin superfamily) (Geng *et al*., 1990; von Andrian *et al*., 1991; Butcher, 1992), and begins with PMN rolling, followed by adherence and transendothelial migration. To allow PMN emigration from the vessel, a firmer adherence of the PMN to the endothelial surface is required. This firm adherence involves the interaction between β_2 integrins on the PMN surface and intercellular adhesion molecule (ICAM)-1 on the endothelial cell surface (Lawrence & Springer, 1991; Butcher, 1992). ICAM-1 is normally expressed at a low basal level, but its expression can be enhanced by various inflammatory mediators such as interleukin (IL)-1 and tumour necrosis factor (TNF)-α (Wetheimer *et al*., 1992).

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors that are related to retinoid, steroid and thyroid hormone receptors (Evans, 1988). PPARs regulate gene expression by binding, as heterodimers, with retinoid X receptors (RXRs) to specific PPAR response elements (PPREs) in the promoter regions of specific target genes. Binding of the activated heterodimer to the promoter region results in either the activation or suppression of the target gene (Desvergne & Wahli, 1999).

The recent development of a novel class of insulin-sensitising drugs, the thiazolidinediones (TZDs), represents a significant advance in antidiabetic therapy. There is now good evidence that the beneficial effects of TZDs are due to the activation of PPAR-γ (Spiegelman, 1998). The insulin-sensitiser rosiglitazone is the most potent and selective PPAR-γ agonist (Lehmann *et al*., 1997; Young *et al*., 1998; Murphy & Holder, 2000), and there is a good correlation between the potency of the TZDs as PPAR-γ agonists *in vitro* and their efficacy at lowering glucose levels *in vivo* (Willson *et al*., 2000). There is less information regarding endogenous ligand(s) for PPAR-γ. However, the cyclopentenone prostaglandin (PG) 15-deoxy-$\Delta^{12,14}$ PGJ_2 (15d-PGJ_2), which is a metabolite of PGD_2, has been suggested to function as an endogenous ligand for PPAR-γ (Ricote *et al*., 1998). There is recent evidence that PPAR-γ agonists may also have a therapeutic role in conditions associated with inflammation (Wada *et al*., 2001; Cuzzocrea *et al*., 2002), but the effects of PPAR-γ agonists in conditions associated with I/R of the gut have, however, not yet been investigated. This study investigates the effects of two PPAR-γ agonists in a rodent model of gut I/R. Specifically, we have investigated the effects of the PPAR-γ ligands rosiglitazone (a TZD) and 15d-PGJ_2 on the gut injury caused by splanchnic artery occlusion (SAO). In particular, we have investigated the effect of the two PPAR-γ agonists (rosiglitazone and 15d-PGJ_2) on the I/R-induced (i) fall in MAP, (ii) PMN infiltration (myeloperoxidase (MPO) activity), (iii) lipid peroxidation (malondialdehyde (MDA) levels), (iv) upregulation of ICAM-1 (by immunohistochemistry), (v) the nitration of tyrosine residues (an indicator of the formation of peroxynitrite) (by immunohistochemistry) and (vi) gut damage (histology). Finally, in order to elucidate whether the protective effects of rosiglitazone and 15d-PGJ_2 are related to the activation of the PPAR-γ receptor, we have also investigated whether bisphenol A diglycidyl ether (BADGE), which functional studies have indicated to be an antagonist of PPAR-γ (Wright *et al*., 2000), attenuates the protective effects of rosiglitazone and 15d-PGJ_2.

Methods

Animals

Male Sprague–Dawley rats (250–300 g; Harlan Nossan, Milan, Italy) were housed in a controlled environment and allowed access to food and water *ad libitum*. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purpose (D.M. 116192), as well as with the EEC regulations (O.J. of E.C.L 358/1 12/18/1986).

Surgical procedures

Rats were anaesthetised with sodium pentobarbital (45 mg kg^{-1}, i.p.). Following anaesthesia, catheters were placed in the carotid artery and jugular vein, as described previously (Caputi *et al*., 1980). Blood pressure was monitored continuously by a Maclab A/D converter (Ugo Basile, Varese, Italy), and stored and displayed on a Macintosh personal computer. After midline laparotomy, the celiac and superior mesenteric arteries were isolated near their aortic origins. During this procedure, the intestinal tract was maintained at 37°C by placing it between gauze pads soaked with warmed saline (0.9% (w v^{-1}) NaCl) solution. Rats were observed for a 30 min stabilisation period before either splanchnic ischaemia or sham ischaemia. SAO shock was induced by clamping both the superior mesenteric artery and the celiac trunk, resulting in a total occlusion of these arteries for 45 min. After this period of occlusion, the clamps were removed and the splanchnic circulation was allowed to reperfuse for 2 h, at which time the experiment was terminated. After 2 h reperfusion, animals were killed and an 8–10 cm length of ileum 30 cm distal to the stomach was isolated, washed in saline and used for histological examination of the bowel and for biochemical studies, as previously described by Hayward & Lefer (1998).

Experimental groups

Rats were randomly allocated into the following groups: (i) *I/R + saline group*: rats were subjected to SAO shock ($N=10$), (ii) *15d-PGJ$_2$ group*: identical to the *I/R + saline group*, but were administered 15d-PGJ$_2$ (0.3 mg kg^{-1} i.v. bolus) 30 min prior to ischaemia ($N=10$), (iii) *BADGE + 15d-PGJ$_2$ group*: identical to the *15d-PGJ$_2$ group*, but BADGE was administered (1 mg kg^{-1} i.v. bolus) 30 min prior to 15d-PGJ$_2$ ($N=10$), (iv) *rosiglitazone group*: identical to the *I/R + saline group*, but were administered rosiglitazone (0.3 mg kg^{-1} i.v. bolus) 30 min prior to ischaemia ($N=10$), (v) *BADGE + rosiglitazone group*: same as the *rosiglitazone group*, but BADGE was administered (1 mg kg^{-1} i.v. bolus) 30 min prior to rosiglitazone ($N=10$), (vi) *I/R + vehicle group*: identical to the *I/R + saline group*, but animals received 10% (v v^{-1}) dimethylsulphoxide (DMSO, vehicle for 15d-PGJ$_2$, 2 ml kg^{-1} i.v. bolus) 30 min prior ischaemia ($N=10$), (vii) *sham + saline group*: rats were subjected to identical surgical procedures as the above groups, except that the blood vessels were not occluded and the rats were maintained under anaesthesia for the duration of the experiment ($N=10$), (viii) *sham + 15d-PGJ$_2$ group*: identical to *sham + saline group*, except for the administration of 15d-PGJ$_2$ (0.3 mg kg^{-1} i.v. bolus) 30 min prior to identical surgical procedures ($N=10$), (ix) *sham + rosiglitazone group*: identical to *sham + saline group*, except for the administration of rosiglitazone (0.3 mg kg^{-1} i.v. bolus) 30 min prior to identical surgical procedures ($N=10$) and (x) *sham + BADGE group*: identical to *sham + saline group*, except for the administration of BADGE (1 mg kg^{-1} i.v. bolus) 30 min prior to identical surgical procedures ($N=10$). The doses of rosiglitazone and 15d-PGJ$_2$ used here to reduce ischaemia reperfusion injury in the gut have previously been reported by us to reduce the tissue injury caused by ischaemia reperfusion in the heart (dose–response curve study) (Wayman *et al*., 2002).

Light microscopy

After fixation for 1 week at room temperature in Dietrich solution (14.25% (v v^{-1}) ethanol, 1.85% (v v^{-1}) formaldehyde, 1% (v v^{-1}) acetic acid), tissues were dehydrated in graded ethanol and embedded in Paraplast (Sherwood Medical, Mahwah, New Jersey, U.S.A.). Thereafter, 7-μm sections were deparaffinised with xylene, stained with haematoxylin-eosin and observed in a Dialux 22 Leitz (Wetziar, Germany) microscope.

MPO activity

MPO activity, an indicator of PMN accumulation, was determined as previously described (Mullane *et al*., 1985). Intestinal tissues, collected at the end of the reperfusion period, were homogenised in a solution containing 0.5% (w v^{-1}) hexadecyl-trimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at 20,000 × *g* at 4°C. An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl-benzidine (1.6 mM) and 0.1 mM H_2O_2. The rate of change in absorbance was measured spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 μmol of peroxide min^{-1} at 37°C and was expressed in units per gram weight of wet tissue.

MDA measurement

The levels of MDA in the intestinal tissues were determined as an indicator of lipid peroxidation (Ohkawa *et al*., 1979). After 120 min of reperfusion, intestinal tissues were removed, weighed and homogenised in 1.15% (w v^{-1}) KCl solution. An aliquot (100 μl) of the homogenate was added to a reaction mixture containing 200 μl of 8.1% (w v^{-1}) sodium dodecyl sulphate, 1500 μl of 20% (w v^{-1}) acetic acid (pH 3.5), 1500 μl of 0.8% (w v^{-1}) thiobarbituric acid and 700 μl distilled water. Samples were then boiled for 1 h at 95°C and centrifuged at 3000 × *g* for 10 min. The absorbance of the supernatant was measured by spectrophotometry at 515–553 nm.

Measurement of cytokines

The levels of TNF-α and IL-1β were evaluated in the plasma collected at the end of the reperfusion period by using a colorimetric, commercial kit (Calbiochem-Novabiochem Corporation, La Jolla, CA, U.S.A.).

Localisation of nitrotyrosine and ICAM-1 by immunohistochemistry

At the end of the experiment, the tissues were fixed in 10% (w v^{-1}) formaldchyde in phosphate-buffered saline (PBS; 0.01 M; pH 7.4) and 8 μm sections were prepared from paraffin-embedded tissues. After deparaffinisation, endogenous peroxidase was quenched with 0.3% (v v^{-1}) hydrogen peroxide in 60% (v v^{-1}) methanol for 30 min. The sections were permeabilised with 0.1% (w v^{-1}) Triton X-100 in PBS for 20 min. Nonspecific adsorption was minimised by incubating the section in 2% (v v^{-1}) normal goat serum in PBS for 20 min. Endogenous biotin and avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin, respectively (DBA, Milan, Italy). Sections were incubated overnight with antinitrotyrosine rabbit polyclonal antibody (1 : 500 in PBS, v v^{-1}) or with mouse anti-rat antibody directed at ICAM-1 (CD54) (1 : 500 in PBS, v v^{-1}) (DBA, Milan, Italy). Specific labelling was detected with a biotin-conjugated goat anti-rabbit or goat anti-mouse IgG and avidin–biotin peroxidase complex (DBA, Milan, Italy). To verify the binding specificity for ICAM-1, some sections were also incubated with primary antibody only (no secondary antibody) or with secondary antibody only (no primary antibody). In these situations, no positive staining was found in the sections indicating that the immunoreactions were positive in all the experiments carried out. In order to confirm that the immunoreactions for the nitrotyrosine were specific, some sections were also incubated with the primary antibody (antinitrotyrosine) in the presence of excess nitrotyrosine (10 mM) to verify the binding specificity.

Immunocytochemistry photographs ($N=5$) were assessed by densitometry as previously described (Cuzzocrea *et al*., 2000) by using Optilab Graftek software on a Macintosh personal computer.

Evaluation of animal survival

The various groups of rats were monitored for 240 min after SAO and reperfusion, and survival rates and survival times were evaluated.

Materials

Unless otherwise stated, all compounds were obtained from Sigma-Aldrich Company Ltd (Milan, Italy). 15d-PGJ_2 was obtained from Cayman Chemicals (Milan, Italy). Rosiglitazone was obtained from Alexis Biochemicals (Bingham, Nottingham, U.K.). Biotin blocking kit, biotin-conjugated goat anti-rabbit IgG and avidin–biotin peroxidase complex were obtained from Vector Laboratories (DBA, Milan, Italy). Primary antinitrotyrosine antibody was purchased from Upstate Biotech (Milan, Italy). Primary ICAM-1 (CD54) was purchased from Santa Cruz Biotechnologies (Milan, Italy). All other chemicals were of the highest commercial grade available.

Data analysis

All values in the figures and text are expressed as mean ± standard error of the mean (s.e.m.) of N observations. For the *in vivo* studies, N represents the number of animals studied. In the experiments involving histology or immunohistochemistry, the figures shown are representative of at least three experiments performed on different experimental days on the tissues section collected from all the animals in each group. The results were analysed by two-way ANOVA followed by a Bonferroni *post hoc* test for multiple comparisons. A P-value of less than 0.05 was considered significant.

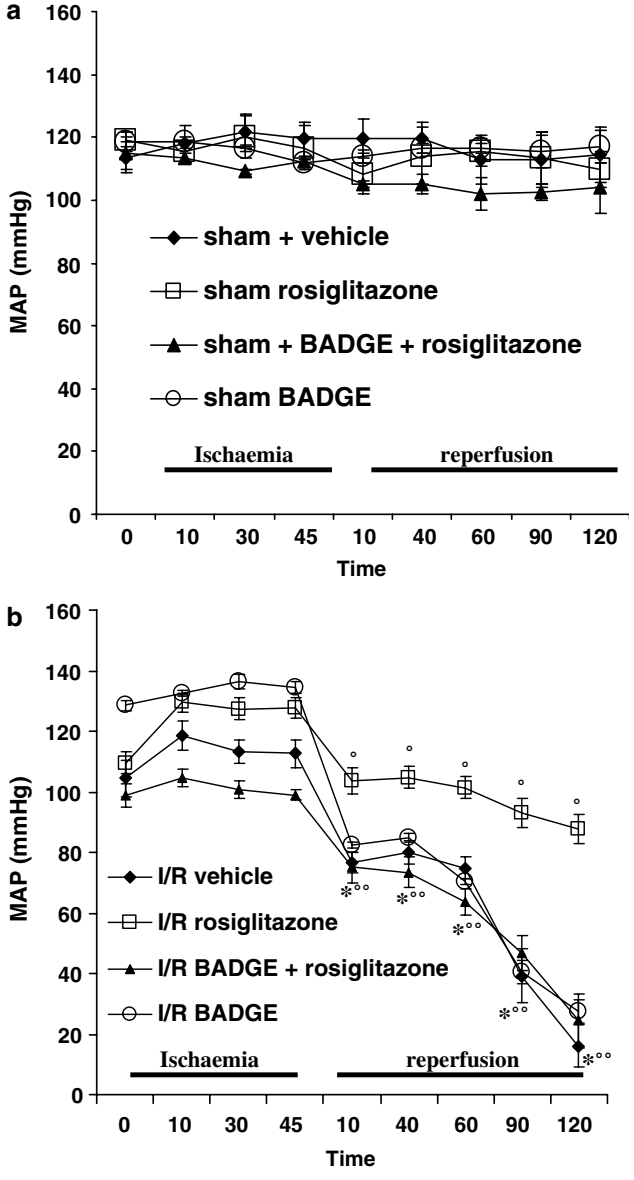

Figure 1 No significant alteration of MAP was observed in sham-operated rats (a). Fall in MAP in SAO rats ($N=10$) is blocked by rosiglitazone ($0.3\,mg\,kg^{-1}$) but not by BADGE ($1\,mg\,kg^{-1}$) (b). Coadministration of BADGE and rosiglitazone significantly blocked the effect of the rosiglitazone (b). $^{*}P<0.01$ *versus* sham, $^{\circ}P<0.01$ *versus* I/R, $^{\circ\circ}P<0.01$ *versus* rosiglitazone.

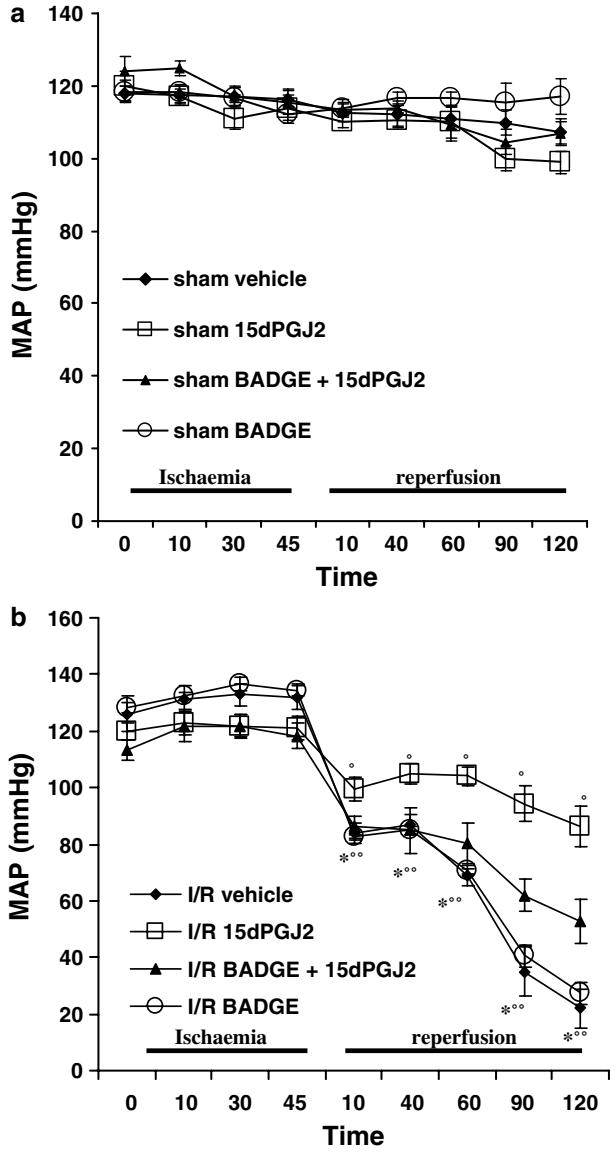

Figure 2 No significant alteration of MAP was observed in sham-operated rats (a). Fall in MAP in SAO rats ($N=10$) is blocked by 15d-PGJ_2 ($0.3\,mg\,kg^{-1}$) but not by BADGE ($1\,mg\,kg^{-1}$) (b). Coadministration of BADGE and 15d-PGJ_2 significantly blocked the effect of the 15d-PGJ_2 (b). $^{*}P<0.01$ *versus* sham, $^{\circ}P<0.01$ *versus* I/R, $^{\circ\circ}P<0.01$ *versus* 15d-PGJ_2.

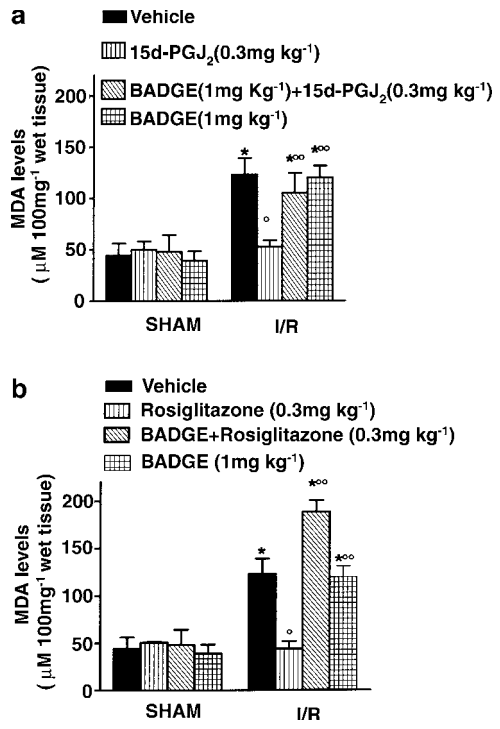

Figure 3 Reperfusion of the ischaemic splanchnic circulation leads to profound increase in MDA levels in ileum tissues, which is inhibited by PPAR-γ agonists 15d-PGJ_2 ($0.3\,mg\,kg^{-1}$ (a)) or by rosiglitazone ($0.3\,mg\,kg^{-1}$ (b)) but not by BADGE ($1\,mg\,kg^{-1}$). Coadministration of BADGE and rosiglitazone or 15d-PGJ_2 significantly blocked the effect of the two PPAR-γ agonists. $^{*}P<0.01$ *versus* sham, $^{\circ}P<0.01$ *versus* I/R, $^{\circ\circ}P<0.01$ *versus* rosiglitazone or 15d-PGJ_2.

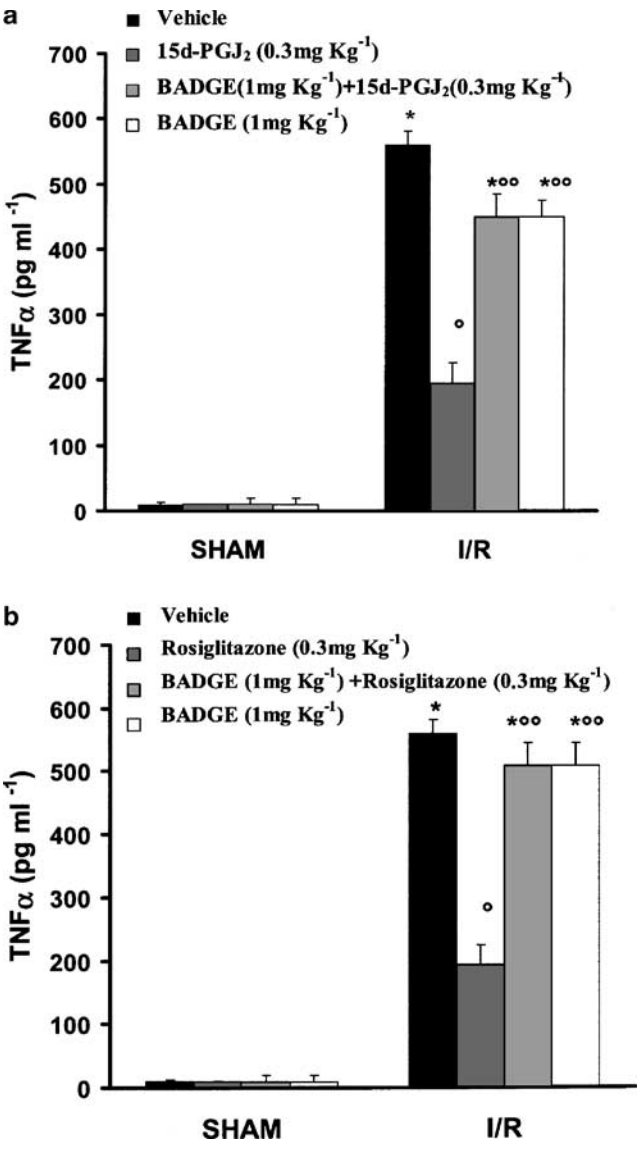

Figure 4 Reperfusion of the ischaemic splanchnic circulation leads to profound increase in plasma TNFα production and this is inhibited by PPAR-γ agonists 15d-PGJ_2 ($0.3\,mg\,kg^{-1}$ (a)) or by rosiglitazone ($0.3\,mg\,kg^{-1}$ (b)) but not by BADGE ($1\,mg\,kg^{-1}$). Coadministration of BADGE and rosiglitazone or 15d-PGJ_2 significantly blocked the effect of the two PPAR-γ agonists. $^{*}P<0.01$ *versus* sham, $^{\circ}P<0.01$ *versus* I/R, $^{\circ\circ}P<0.01$ *versus* rosiglitazone or 15d-PGJ_2.

Results

Effects of rosiglitazone and 15d-PGJ_2 in SAO shock

Occlusion of the splanchnic arteries produced an increase in MABP which then significantly decreased during reperfusion (Figures 1, 2). Animals were killed after 120 min reperfusion in order to collect blood and tissues for biochemical analysis. Reperfusion of the ischaemic splanchnic circulation led to the following events: a substantial increase in plasma lipid peroxidation as indicated by increased MDA levels (Figure 3), increased plasma levels of TNF-α (Figure 4) and IL-1β (Figure 5) and profound PMN infiltration into intestinal tissues (Figure 6). These inflammatory events were triggered during the reperfusion phase since no changes were observed when blood or tissues were removed after the period of ischaemia alone (data not shown).

The two PPAR-γ agonists rosiglitazone ($0.3\,mg\,kg^{-1}$; Figure 1) or 15d-PGJ_2 ($0.3\,mg\,kg^{-1}$; Figure 2), but not BADGE ($1\,mg\,kg^{-1}$; Figures 1, 2), prevented the fall in MABP observed during reperfusion. Coadministration of BADGE and rosiglitazone or 15d-PGJ_2 significantly blocked the effect of the two PPAR-γ agonists (Figures 1, 2).

Rosiglitazone ($0.3\,mg\,kg^{-1}$) or 15d-PGJ_2 ($0.3\,mg\,kg^{-1}$), but not the PPAR-γ antagonist BADGE ($1\,mg\,kg^{-1}$, given i.v. 30 min prior to ischaemia), significantly inhibited the increased levels of MDA in gut tissue (Figures 3a, b), reduced the plasma levels of TNF-α (Figures 4a, b) and IL-1β (Figures 5a, b) as well as the reduction of PMN infiltration into the ileum (Figures 6a, b). Coadministration of BADGE and rosiglitazone or 15d-PGJ_2 significantly blocked the effect of the two PPAR-γ agonists (Figures 3–5).

Immunohistochemical localisation of nitrotyrosine and ICAM-1 expression in reperfused intestine

Ileum tissue sections obtained from SAO-shocked rats undergoing 45 min of ischaemia followed by 2 h of reperfusion showed positive staining for nitrotyrosine, which was localised to the submucosa vessels (Figures 7b, 8). No staining was observed in *sham + vehicle group* (Figures 7a, 8). Administration of rosiglitazone or 15d-PGJ_2 reduced the degree of immunostaining for nitrotyrosine in the reperfused intestine

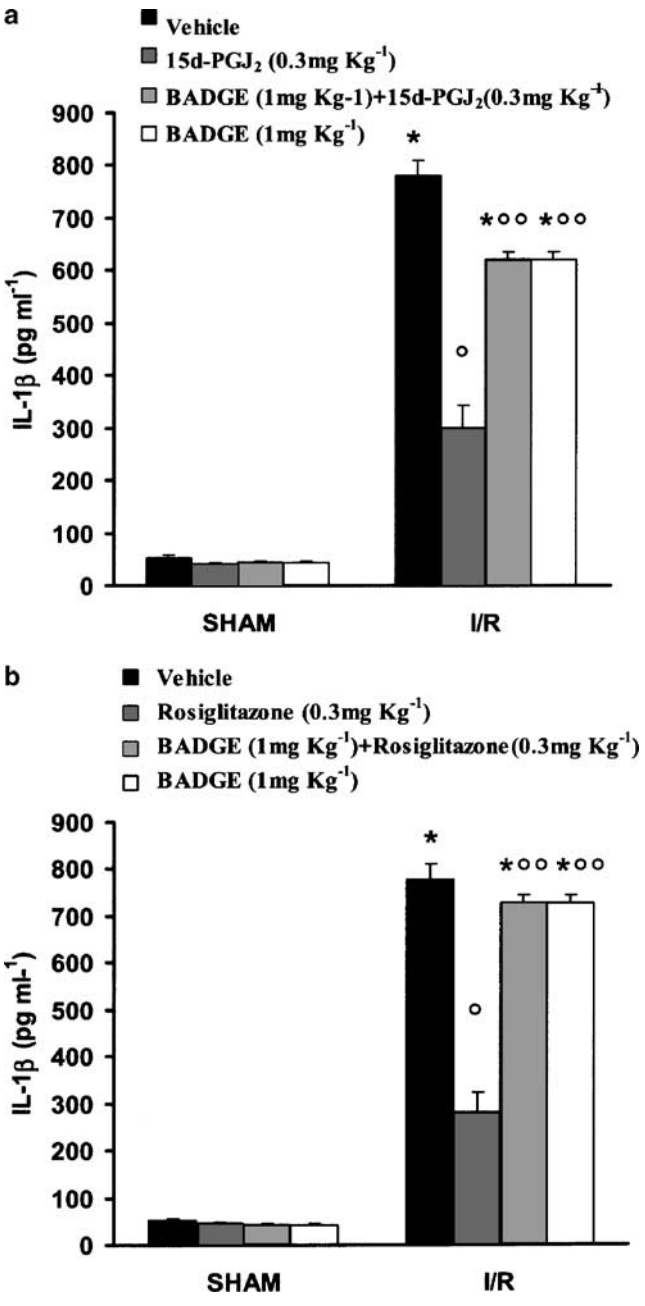

Figure 5 Reperfusion of the ischaemic splanchnic circulation leads to profound increase in plasma IL-1β production and this is inhibited by PPAR-γ agonists 15d-PGJ_2 (0.3 mg kg^{-1} (a)) or by rosiglitazone (0.3 mg kg^{-1} (b)) but not by BADGE (1 mg kg^{-1}). Coadministration of BADGE and rosiglitazone or 15d-PGJ_2 significantly blocked the effect of the two PPAR-γ agonists. $^{*}P<0.01$ *versus* sham, $^{\circ}P<0.01$ *versus* I/R, $^{\circ\circ}P<0.01$ *versus* rosiglitazone or 15d-PGJ_2.

(Figures 7c, d, 8). Staining with anti-ICAM-1 antibody of sections of ileum obtained from *sham + vehicle group* showed a specific staining along vessels and demonstrated a constitutive expression of ICAM-1 (1.6 ± 0.13% of total tissue area) in endothelial cells (Figures 8, 9a). After 2 h of reperfusion, the staining intensity substantially increased along vessels and in the necrotic tissue (Figures 8, 9b). Sections from rosiglitazone-treated and 15d-PGJ_2-treated rats did not reveal any upregulation of constitutively expressed ICAM-1, which was normally

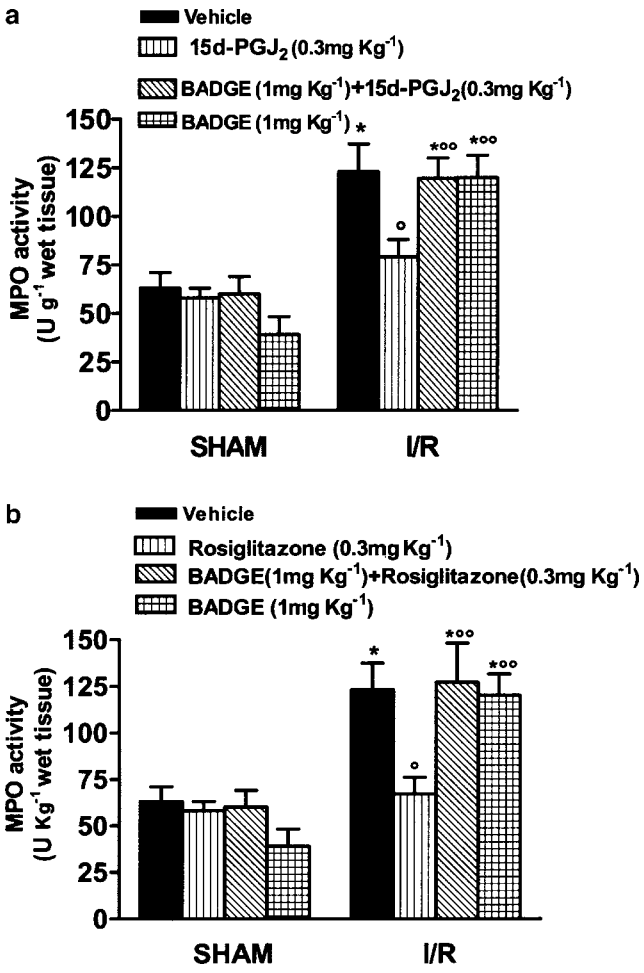

Figure 6 Reperfusion of the ischaemic splanchnic circulation leads to the infiltration of PMNs into ileum tissues, which is inhibited by PPAR-γ agonists 15d-PGJ_2 (0.3 mg kg^{-1} (a)) or by rosiglitazone (0.3 mg kg^{-1} (b)) but not by BADGE (1 mg kg^{-1}). Coadministration of BADGE and rosiglitazone or 15d-PGJ_2 significantly blocked the effect of the two PPAR-γ agonists. $^{*}P<0.01$ *versus* sham, $^{\circ}P<0.01$ *versus* I/R, $^{\circ\circ}P<0.01$ *versus* rosiglitazone or 15d-PGJ_2.

expressed in the endothelium along the vascular wall (Figures 8, 9c, d). Coadministration of BADGE and rosiglitazone or 15d-PGJ_2 significantly blocked the effect of the two PPAR-γ agonists (data not shown). BADGE treatment alone did not effect adhesion molecule expression (data not shown).

Effects of rosiglitazone and 15d-PGJ_2 on histological changes caused by gut I/R

Histological examinations of the small intestine after 120 min of reperfusion revealed expected and characteristic pathological changes (see representative sections in Figure 10). Histological features of the normal gut tissue were observed in gut tissues prepared from sham-operated rats (Figure 10a). Ileum sections revealed PMN infiltration through the gut wall and concentrated below the epithelial layer (Figure 10b). Administration of rosiglitazone or 15d-PGJ_2 significantly prevented ileum damage induced by I/R (Figures 10c, d). Coadministration of BADGE and rosiglitazone or 15d-PGJ_2 significantly blocked the effect of the two PPAR-γ agonists (data not

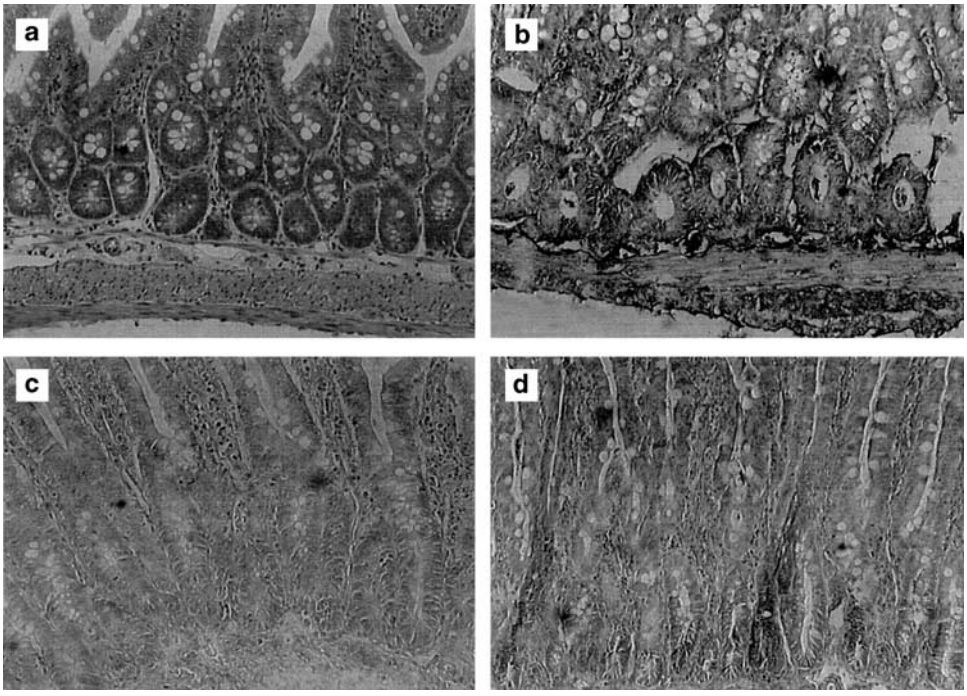

Figure 7 Immunohistochemical staining of nitrotyrosine was absent in the ileum section from sham-operated rats (a). After reperfusion nitrotyrosine staining was localised in the injured area from an SAO-shocked rat (b). There was no detectable immunostaining in the ileum from rosiglitazone (c) or from 15d-PGJ_2- (d) treated rats. Original magnification, $\times 500$. The figure is representative of at least three experiments performed on different experimental days.

shown). BADGE treatment did not affect the organ injury induced by SAO shock (data not shown).

Effects of rosiglitazone and 15d-PGJ_2 on survival rate

All sham-operated rats survived the entire 2 h observation period (Figure 11). In contrast, SAO produced a profound shock state characterised by a 90% lethality at the end of the 4 h reperfusion period (Figure 11). Administration of either rosiglitazone or 15d-PGJ_2 significantly prevented the mortality induced by I/R (Figure 11). Coadministration of BADGE and rosiglitazone or 15d-PGJ_2 significantly blocked the beneficial effects of the two PPAR-γ agonists (Figure 11). BADGE treatment did not affect the mortality induced by SAO shock (Figure 11).

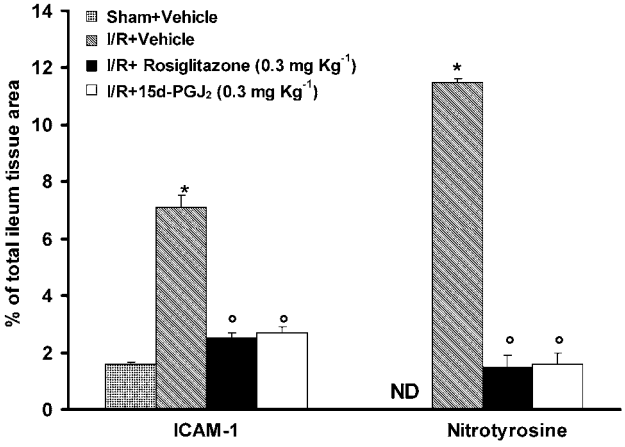

Figure 8 Typical densitometry evaluation. Densitometry analysis of immunocytochemistry photographs ($n=5$) for ICAM-1 and nitrotyrosine from ileum was assessed. The assay was carried out by using Optilab Graftek software on a Macintosh personal computer (CPU G3-266). Data are expressed as percentage of total tissue area. $^*P<0.01$ *versus* sham, $^\circ P<0.01$ *versus* IR. ND, not determined.

Discussion

Recent evidence suggests that PPAR-γ agonists might have therapeutic potential in the treatment of diabetes, inflammation and cancer (Spiegelman, 1998; Desvergne & Wahli, 1999; Murphy & Holder, 2000). There are some reports of the beneficial effects of PPAR-γ agonists such as rosiglitazone in I/R injury of the heart (Yue *et al*., 2001; Wayman *et al*., 2002). Nakajima *et al*. (2001) investigated the potential beneficial actions of the PPAR-γ agonist rosiglitazone (BRL-49653) against gut I/R and reported that rosiglitazone attenuated the expression of ICAM-1 and TNF-α messenger RNA levels. We report here that pretreatment of rats (at 30 min prior to onset of ischaemia) with the TZD rosiglitazone caused a substantial reduction of the ileal injury and the mortality caused by ischaemia and reperfusion of the intestine. In order to confirm whether this effect of rosiglitazone is due to activation of PPAR-γ, we have investigated whether the PPAR-γ antagonist BADGE attenuates the observed protective effect of rosiglitazone. We demonstrate here that BADGE does, indeed, attenuate the anti-ischaemic effects of rosiglitazone in rats subjected to splanchnic artery occlusion and reperfusion. Thus, we propose that the protective effects of rosiglitazone are secondary to the activation of the PPAR-γ receptor.

There is good evidence that the PGD_2 metabolite 15d-PGJ_2 is a potent PPAR-γ agonist *in vitro*, and that 15d-PGJ_2 may

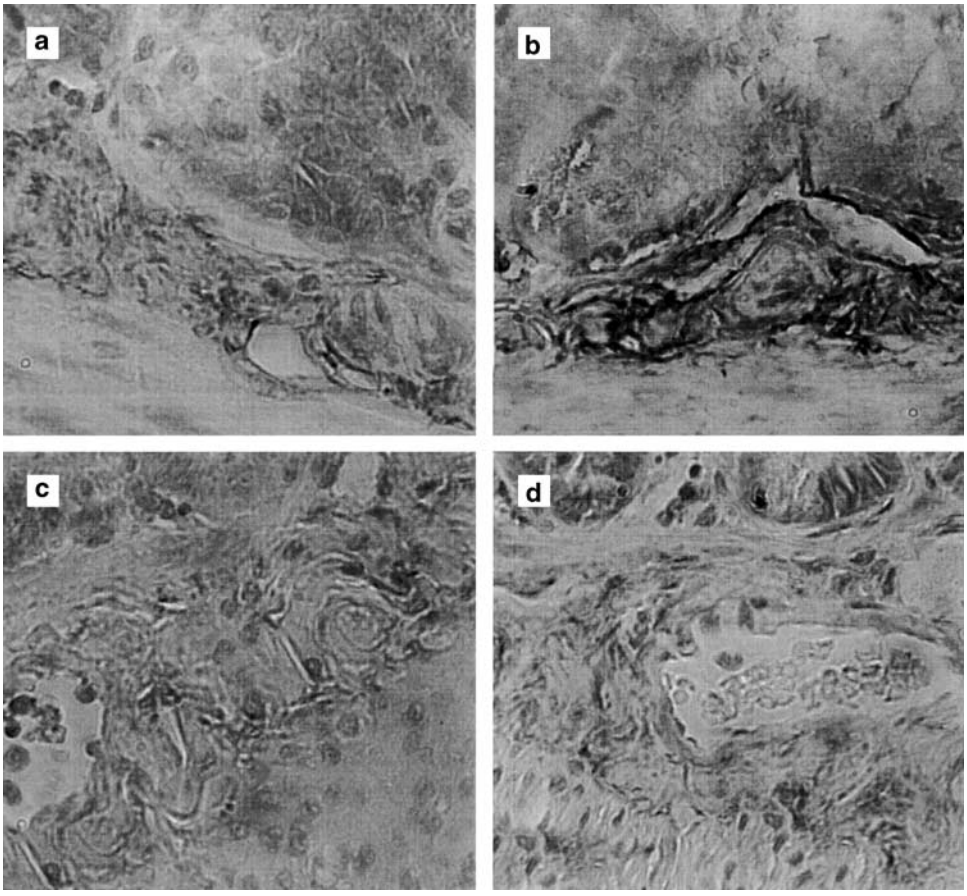

Figure 9 Control tissue from sham-operated rats (a) showed a dark brown staining of endothelium of blood vessels, indicating the presence of constitutive ICAM-1 protein. I/R induced an increase of the positive staining for ICAM-1 along the endothelium wall (b). In rosiglitazone (c) or from 15d-PGJ_2 (d) treated rats subjected to SAO shock, there was no increase of immunostaining for ICAM-1, which was present only along the endothelium wall. Original magnification, $\times$ 500. The figure is representative of at least three experiments performed on different experimental days.

serve as an endogenous PPAR-γ ligand (Drew & Chavis, 2001). In the present study, we have clearly demonstrated that pretreatment of rats with 15d-PGJ_2 (0.3 mg kg^{-1} i.v.) caused a very substantial reduction in the ileal injury and the associated mortality caused by splanchnic ischaemia and reperfusion. We have also demonstrated that these beneficial effects of 15d-PGJ_2 were reduced, but not abolished, by the PPAR-γ antagonist BADGE. Taken together, these findings support the view that the activation of PPAR-γ contributes to the protective effects of rosiglitazone and 15d-PGJ_2 in rats subjected to intestinal ischaemia and reperfusion.

In addition to being a potent agonist of PPAR-γ, 15d-PGJ_2 is also an agonist of PPAR-γ and clearly exerts effects, which are independent of the activation of PPAR receptors (Fajas *et al*., 2001; Reilly *et al*., 2001; Wilmer *et al*., 2001; Clarkr, 2002). As the protective effects of 15d-PGJ_2 were very substantial and the beneficial effects of this cyclopentanone PG were only partially antagonised by BADGE, it is possible that other non-PPAR-related effects of this cyclopentenone PG contribute to the observed protective effects. For instance, there is good evidence that 15d-PGJ_2 inhibits the activation of the transcription factor nuclear factor (NF)-κB by preventing the phosphorylation of IK kinase (IKK) and, hence, preventing the degradation of Iκ-B (Rossi *et al*., 2000). Recent studies have demonstrated that pretreatment of rats with 15d-PGJ_2 attenuates the activation of NF-κB caused by regional myocardial I/R (Wayman *et al*., 2002). NF-κB activation induces the transcription of many proinflammatory genes, including TNF-α, IL-1β and ICAM-1, to name but a few (Manning *et al*., 1995; Izumi *et al*., 2001; Telek *et al*., 2001). We report in the present study here that gut I/R in the rat results in the production of the proinflammatory cytokines TNF-α and IL-β, as well as the protein expression of ICAM-1. We found that pretreatment of rats with rosiglitazone and 15d-PGJ_2 attenuated the production of TNF-α and IL-β, as well as the upregulation of ICAM-1. We have also demonstrated that the coadministration of BADGE and rosiglitazone or 15d-PGJ_2 significantly blocked the effect of the two PPAR-γ agonists. These findings, therefore, suggest (i) that gut I/R results in the activation and the subsequent expression of proinflammatory genes and (ii) that rosiglitazone and 15d-PGJ_2 attenuate the activation of the PPAR-γ receptor and the expression of downstream target genes. The adhesion molecules ICAM-1, vascular cell adhesion molecule (VCAM-1) and P-selectin play an important role in the recruitment of PMNs into the previously ischaemic tissue (Hayward & Lefer, 1998; Kubes, 1999). Infiltrated PMNs release reactive oxygen species (ROS), leading to tissue injury *via* excessive lipid peroxidation, generation of DNA strand breaks and activation of PARP (Cuzzocrea *et al*., 1997; Liaudet *et al*., 2000; Mazzon *et al*., 2002). In this study, we demonstrate that the reduced expression of ICAM-1 will also result in reduced ROS

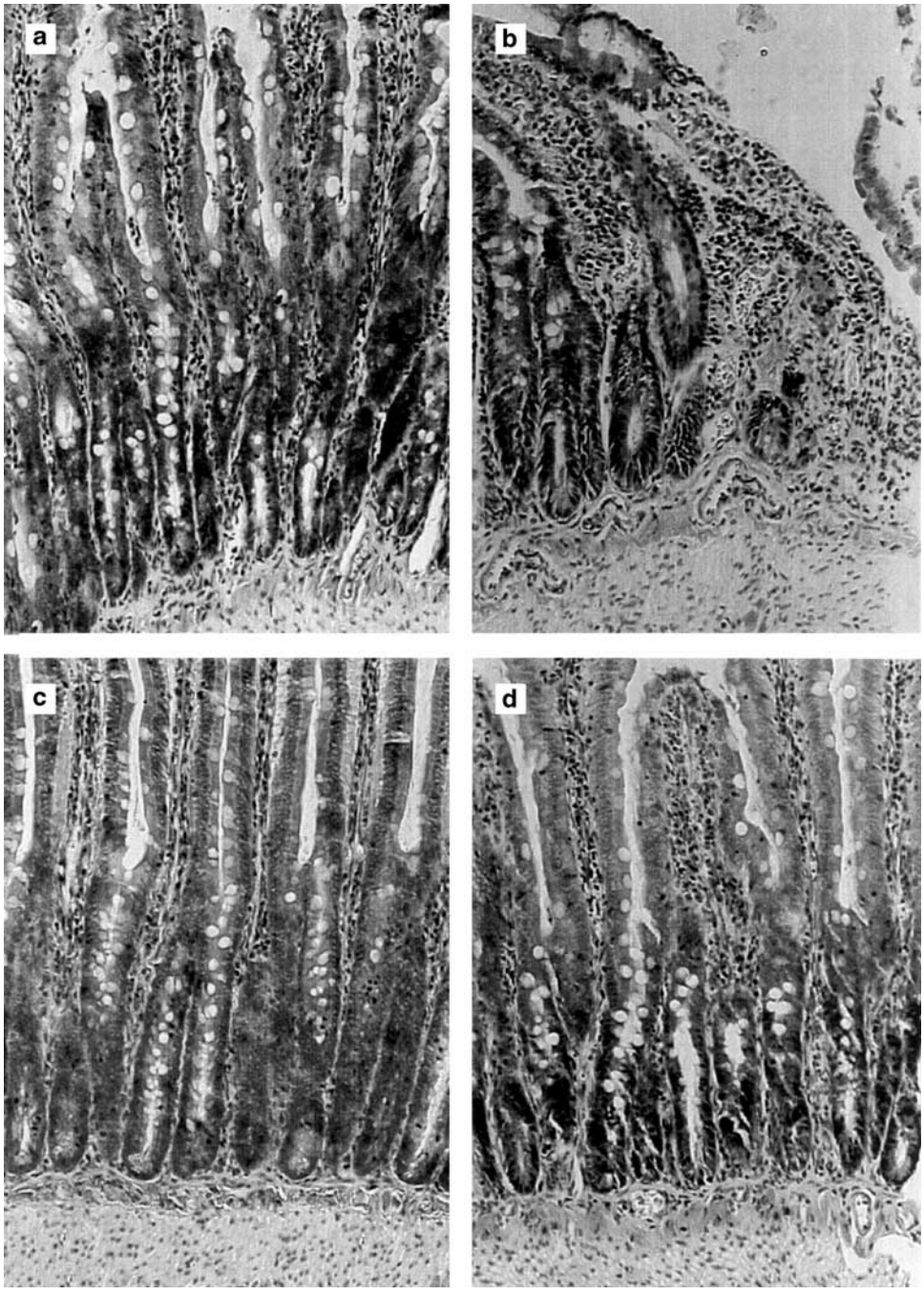

Figure 10 Distal ileum section from a sham rat, demonstrating the normal architecture of the intestinal epithelium and wall (a). Distal ileum section from SAO-shocked rats showed inflammatory infiltration by PMNs and lymphocytes extending through the wall and concentrated below the epithelial layer and demonstrating oedema of the distal portion of the villi (b). Distal ileum from rosiglitazone (c) or from 15d-PGJ_2-(d) treated rats shows reduced SAO-induced organ injury. Original magnification, $\times 125$. The figure is representative of at least three experiments performed on different experimental days.

formation associated with reduced infiltration of PMNs into the ileum. Together, reduction of ROS production will reduce the formation of peroxynitrite, which causes gut injury *via* direct oxidant injury and protein tyrosine nitration (Beckman, 1996; Cuzzocrea *et al*., 2000). Thus, administration of rosiglitazone and 15d-PGJ_2 reduces the oxidative and nitrosative stress caused by I/R of the rat intestine.

In conclusion, this study provides evidence that chemically distinct ligands of PPAR-γ (including the TZD rosiglitazone, as well as the cyclopentenone PG 15d-PGJ_2) cause a substantial reduction of intestinal I/R injury in the rat. In addition, we also demonstrate, for the first time *in vivo*, that a PPAR-γ antagonist, BADGE, significantly attenuates the protective effect of rosiglitazone and 15d-PGJ_2. Thus, we demonstrate here that the mechanisms underlying the protective effects of rosiglitazone and 15d-PGJ_2 are dependent on the activation of PPAR-γ. The activation of PPAR-γ by rosiglitazone and 15d-PGJ_2, in turn, results in

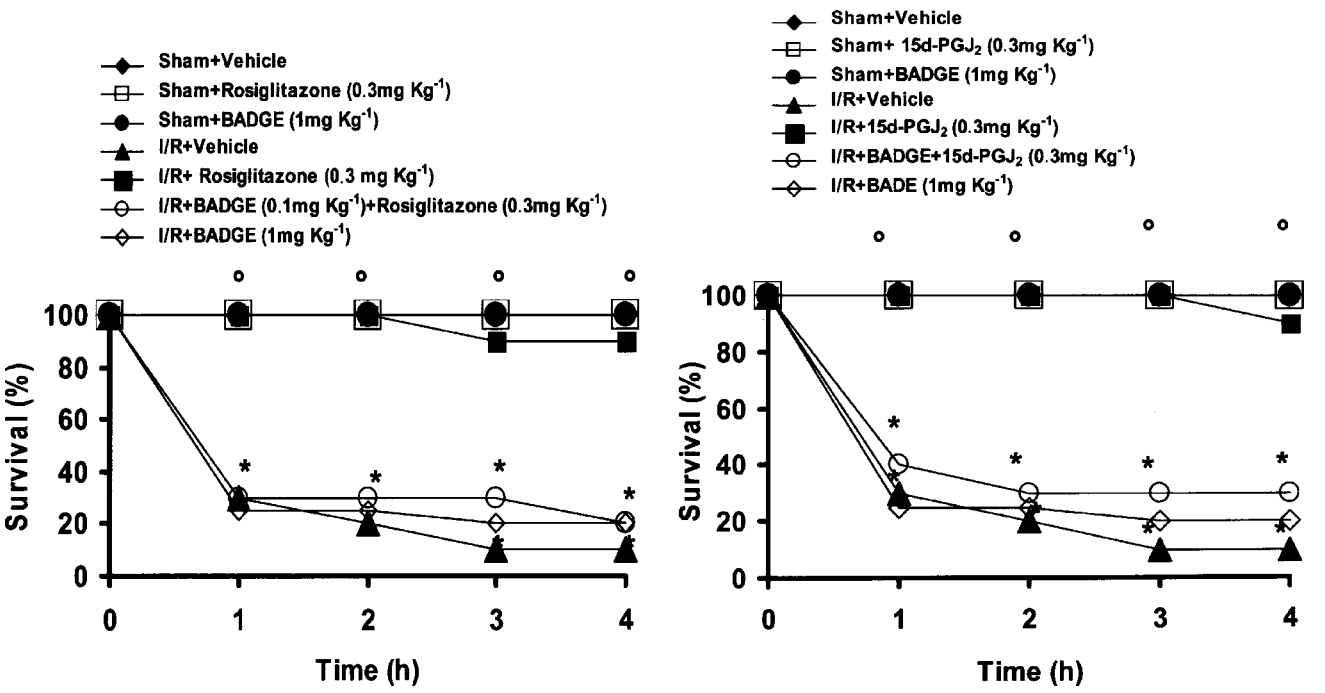

Figure 11 SAO shock-induced mortality. Survival was monitored for 4 h after SAO shock. $^{*}P<0.01$ *versus* sham, $^{\circ}P<0.01$ *versus* I/R.

a reduction of (i) the nitration of proteins by peroxynitrite, (ii) the formation of the proinflammatory cytokines and (iii) the expression of the adhesion molecule ICAM-1. We speculate that ligands of PPAR-γ may be useful in the therapy of conditions associated with I/R of the gut, among other organs such as the heart (e.g. against myocardial infarction and in heart transplantation and bypass surgery) and other organs.

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