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The cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$- PGJ_2 attenuates the development of colon injury caused by dinitrobenzene sulphonic acid in the rat

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1 Inflammatory bowel disease (IBD) is characterized by oxidative and nitrosative stress, leukocyte infiltration, and increased expression of the adhesion molecules intercellular adhesion molecule 1 (ICAM-1) in the colon. Recent evidence also suggests that the cyclopentenone prostaglandin (PG) 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d- PGJ_2) functions as an early anti-inflammatory signal.

2 The aim of the present paper is to investigate the effects of 15d-PGJ_2 in rats subjected to experimental colitis.

3 Colitis was induced in rats by intra-colonic instillation of dinitrobenzene sulphonic acid (DNBS). 15d-PGJ_2 was administered daily as intraperitoneal injection (20 or 40 $\mu g\ kg^{-1}$). On day 4, animals were sacrificed and tissues were taken for histological and biochemical analysis.

4 15d-PGJ_2 significantly reduced the degree of haemorrhagic diarrhoea and weight loss caused by administration of DNBS. 15d-PGJ_2 also caused a substantial reduction of (i) the degree of colonic injury, (ii) the rise in myeloperoxidase (MPO) activity (mucosa), (iii) the increase in the tissue levels of malondialdehyde (MDA) and (iv) of the pro-inflammatory cytokines tumour necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β).

5 Furthermore, 15d-PGJ_2 reduced the increase in immunohistochemical staining for (i) inducible nitric oxide synthase (iNOS), (ii) nitrotyrosine and (iii) poly (ADP-ribose) polymerase (PARP), as well as (iv) the increased expression of ICAM-1 caused by DNBS in the colon.

6 Electrophoresis mobility shift assay (EMSA) of inflamed colon revealed that 15d- PGJ_2 also caused a substantial reduction of the activation of nuclear factor-kappaB (NF-κB). Furthermore, 15d-PGJ_2 stimulates the activation of heat shock protein 72 (*hsp72*) in the inflamed colon, as assessed by Western blot analysis.

7 In conclusion, 15d-PGJ_2 reduces the development of experimental colitis.
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Keywords: 15-deoxy-$\Delta^{12,14}$-PGJ_2; PPAR; inflammation; colitis

Abbreviations: 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; DNBS, dinitrobenzene sulphonic acid; EMSA, electrophoresis mobility shift assay; *hsp*, heat shock protein; IBD, inflammatory bowel disease; ICAM, intercellular cell adhesion molecule; iNOS, inducible nitric oxide synthase; IL, interleukin; MDA, malondialdehyde; MPO, myeloperoxidase; NF-κB, nuclear factor-κB; NO, nitric oxide; PARP, poly (ADP-ribose) polymerase; PBS, phosphate buffered saline; PMN, polymorphonuclear leukocyte; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; RXR, retinoid X receptor; TNF, tumour necrosis factor

Introduction

The cyclopentenone prostaglandin (PG) J_2 is formed by dehydration within the cyclopentenone ring of the endogenous prostaglandin PGD_2. PGJ_2 is metabolized further to yield Δ^{12}-PGJ_2 and 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2). Several members of the cyclopentenone family of prostaglandins possess anti-neoplastic, anti-viral activity and anti-inflammatory properties (Straus & Glass, 2001).

Many actions of the cyclopentenone prostaglandins do not appear to be mediated by binding to G-protein coupled prostanoid receptors, but secondary to their interaction with other cellular target proteins. For instance, 15d-PGJ_2 is a high affinity ligand for the peroxisome proliferator-activated receptor-γ (PPARγ). PPARγ is a nuclear hormone receptor, which regulates gene expression by heterodimerizing with the retinoid X receptor (RXR). Binding of the activated heterodimer to promotor region of specific target genes results in either the activation or suppression of the target gene. Various PPARγ ligands have been reported to possess anti-inflammatory properties *in vitro* (Jiang *et al*., 1998) and

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in vivo (see below). Therefore recently it has been documented that PPARγ ligands decreased colonic inflammation and injury in human and experimental inflammatory bowel diseased (Su *et al*., 1999; Lewis *et al*., 2001). It is possible that PPARγ trans-represses the expression of pro-inflammatory mediators at the transcriptional level by inhibiting NF-κB, STAT-1 and activator protein-1 (AP-1) signalling (Ricote *et al*., 1998).

Other activities of the cyclopentenone prostaglandins are mediated by the reactive α,β- unsaturated carbonyl group located in the cyclopentenone ring. For instance, 15d-PGJ_2 attenuates the activation of the transcription factor NF-κB by preventing the phosphorylation of its inhibitor protein by IK kinase (Rossi *et al*., 1997). It is now widely accepted that 15d-PGJ_2 attenuates the NF-κB-mediated transcriptional activation of many pro-inflammatory genes by PPARγ-dependent and PPARγ-independent mechanisms (Straus & Glass, 2001). For instance, 15d-PGJ_2 attenuates the formation of the cytokines TNFα and IL-12 (Drew & Chavis, 2001), the expression of the adhesion molecules VCAM-1 and ICAM-1 (Pasceri *et al*., 2000) and the expression of the inducible, pro-inflammatory proteins, cyclo-oxygenase-2 (COX-2), cytosolic phospholipase A_2, (Tsubouchi *et al*., 2001) and inducible nitric oxide (NO) synthase (iNOS) (Ricote *et al*., 1998; Colville-Nash *et al*., 1998). There is, however, also evidence that 15d-PGJ_2 may enhance the formation of the pro-inflammatory chemokine IL-8 in human macrophages/monocytes stimulated with endotoxin in a PPARγ-dependent fashion (Zhang *et al*., 2001). It is well known that 15d-PGJ_2 is also able to induce the expression of cytoprotective heat shock proteins (*hsp*) (Santoro, 2000) and *hsp72* has recently been shown to play a key role in the resolution of inflammatory process (Ianaro *et al*., 2001).

There are recent reports which document that 15d-PGJ_2 and the PPARγ ligands troglitazone or rosiglitazone reduce the degree of inflammation (i.e. suppression of polymorphonuclear leukocyte (PMN) infiltration and colon damage) associated with experimental colitis (Ajuebor *et al*., 2000; Desreumaux *et al*., 2001). The present study was designed to gain a better understanding of the effects of 15d-PGJ_2 in rodent models of dinitrobenzene sulphonic acid (DNBS)-induced colitis. In order to gain a better insight into the mechanism(s) of action of the observed anti-inflammatory effects of 15d-PGJ_2, we have also investigated the effects of 15d-PGJ_2 on (i) the activation of NF-κB (EMSA of inflamed colon), (ii) the degree of colonic injury, (iii) the rise in MPO activity (mucosa), (iv) the expression of *hsp72* (immunoblot of inflamed colon), (v) the increase in the tissue levels of malondialdehyde, (vi) the increase in staining (immunohistochemistry) for iNOS, nitrotyrosine and PARP, as well as (vii) the increased expression of ICAM-1 caused by DNBS in the colon.

Methods

Animals

Male Sprague–Dawley rats (300–350 g; Charles River; Milan; Italy) were housed in a controlled environment and provided with standard rodent chow and water. 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).

Experimental groups

In the treated group of animals, 15d-PGJ_2 given daily as an intraperitoneal (i.p.) injection starting 24 h (day 1) after the administration of DNBS (20 or 40 μg kg^{-1}) (DNBS + 15d-PGJ_2 group, $n=20$). In a vehicle-treated group of rats, vehicle (10% dimethyl sulphoxide (DMSO)) was given instead of 15d-PGJ_2 I (DNBS group, $n=10$). In separate groups of rats, surgery was performed in its every aspect identical to the one in the DNBS group, except that the saline was injected instead of DNBS (SHAM group, $n=10$). In an additional group of animals, sham-surgery was combined with the administration of 15d- PGJ_2 (doses as above) (Sham + 15d-PGJ_2).

Induction of experimental colitis

Colitis was induced by using a technique of acid-induced colon inflammation as described previously (Morris *et al*., 1989). In fasted rats lightly anaesthetized with isoflurane, a 3.5 F catheter was inserted into the colon *via* the anus until the splenic flexure was reached (approximately 8 cm from the anus). 2,4-dinitrobenzenesulphonic acid (DNBS; 25 mg rat^{-1}), dissolved in 50% ethanol (total volume, 0.8 ml) was administered as an enema (DNBS group) whereas other animals received an enema consisting of DNBS vehicle alone (50% ethanol, 0.8 ml, SHAM group). Thereafter, the animals were kept for 15 min in a Trendelenburg position to avoid reflux. After colitis and sham-colitis induction, the animals were observed for 3 days. On day 4, the animals were weighed and anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.), and the abdomen was opened by a midline incision. The colon was removed, freed from surrounding tissues, opened along the antimesenteric border, rinsed, weighed, and processed for histology and immunohistochemistry. Colon damage (macroscopic damage score) was evaluated and scored by two independent observers as described previously (Wallace *et al*., 1992; Miller *et al*., 1995; Zingarelli *et al*., 1993), according to the following criteria: 0, no damage; 1, localized hyperaemia without ulcers; 2, linear ulcers with no significant inflammation; 3, linear ulcers with inflammation at one site; 4, two or more major sites of inflammation and ulceration extending >1 cm along the length of the colon; and 5–8, one point is added for each centimetre of ulceration beyond an initial 2 cm.

Light microscopy

After fixation for 1 week at room temperature in Dietrich solution (14.25% ethanol, 1.85% formaldehyde, 1% acetic acid), samples were dehydrated in graded ethanol and embedded in Paraplast (Sherwood Medical, Mahwah, New Jersey). Thereafter, 7-μm sections were deparaffinized with xylene, stained with haematoxylin-eosin and trichromic van Giesson's stain, and observed in a Dialux 22 Leitz (Wetziar, Germany) microscope. In order to have a quantitative estimation of colon damage, section ($n=6$ for each animals) was scored by two independent observers blinded to the

experimental protocol. The following morphological criteria were considered: score 0, no damage; score 1 (mild), focal epithelial oedema and necrosis; score 2 (moderate), diffuse swelling and necrosis of the villi; score 3 (severe), necrosis with presence of neutrophil infiltrate in the submucosa; score 4 (highly severe), widespread necrosis with massive neutrophil infiltrate and haemorrhage.

Myeloperoxidase activity

Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte (PMN) accumulation, was determined as previously described (Mullane *et al*., 1985). At 4 days after intra-colonic injection of DNBS, the colon was removed and weighed. The colon was homogenized in a solution containing 0.5% hexa-decyl-trimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at $20{,}000 \times g$ at 4°C. An aliquot of the supernatant was then allowed to react with a solution of tetramethyl-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 per min at 37°C and was expressed in milliunits per gram weight of wet tissue.

Malondialdehyde measurement

The levels of malondialdehyde (MDA) in the colon were determined as an indicator of lipid peroxidation (Ohkawa *et al*., 1979). At 4 days after intra-colonic injection, the colon was removed, weighed and homogenized in 1.15% KCl solution. An aliquot (100 μl) of the homogenate was added to a reaction mixture containing 200 μl of 8.1% SDS, 1500 μl of 20% acetic acid (pH 3.5), 1500 μl of 0.8% thiobarbituric acid and 700 μl distilled water. Samples were then boiled for 1 h at 95°C and centrifuged at $3000 \times g$ for 10 min. The absorbance of the supernatant was measured by spectrophotometer at 515–553 nm.

Measurement of cytokines

The levels of TNFα and IL-1β were evaluated in the colon 4 days after intra-colonic injection of DNBS. The assay was carried out by using a colorimetric, commercial kit (Calbiochem-Novabiochem Corporation, U.S.A.).

Localization of nitrotyrosine, PARP, ICAM-1 and iNOS by immunohistochemistry

At the end of the experiment, the tissues were fixed in 10% PBS-buffered formaldehyde and 8 μm sections were prepared from paraffin embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% H_2O_2 in 60% methanol for 30 min. The sections were permeabilized with 0.1% Triton X-100 in PBS for 20 min. Non-specific adsorption was minimized by incubating the section in 2% normal goat serum in PBS for 20 min. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin (DBA, Milan, Italy). Sections were incubated overnight with (1) anti-nitrotyrosine rabbit polyclonal antibody (1 : 500 in PBS) or with anti-poly (ADP-ribose) goat polyclonal antibody (1 : 500 in PBS) or (2) with primary anti-iNOS 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, donkey anti-goat or goat anti-mouse IgG and avidin-biotin peroxidase complex (DBA, Milan, Italy).

Preparation of whole extracts

All the extraction procedures were performed on ice using ice-cold reagents. Tissues from each rat were suspended in 6 ml of a high-salt extraction buffer (mM: pH 7.9 HEPES 20, NaCl 420, $MgCl_2$ 1.5, EDTA 25% glycerol 0.2, phenylmethylsulphonylfluoride 0.5, soybean trypsin inhibitor 1.5 μg ml^{-1}, pepstatin A 7 μg ml^{-1}, leupeptin 5 μg ml^{-1}, benzamidine 0.1, dithiothreitol 0.5) and homogenized at the highest setting for 2 min in a Polytron PT 3000 tissue homogeniser. The homogenates were chilled on ice for 15 min and then vigorously shaken for few minutes in the presence of 20 μl of 10% Nonidet P-40. After centrifugation at $13{,}000 \times g$ at 4°C for 5 min, the protein concentration in the supernatant was determined by the Bio-Rad (Bio-Rad) protein assay kit and then it was aliquoted and stored at −80°C.

Electrophoretic mobility-shift assay (EMSA)

Double-stranded oligonucleotides containing the NF-κB recognition sequence (5′-GAT CGA GGG GAC TTT CCC TAG-3′) (Tacchini *et al*., 1997) were end labelled with γ-[^{32}P]ATP (ICN Biomedicals). Aliquots of whole extracts (20 μg of protein for each sample) were incubated for 30 min with radiolabelled oligonucleotides ($2.5-5.0 \times 10^4$ c.p.m.) in 20 μl reaction buffer containing 2 μg poly dI-dC, 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM ethylenediaminotetraacetic acid, 1 mM DL-dithiothreitol, 1 g/ml bovine serum albumin, 10% glycerol. The specificity of the DNA/protein binding was determined for NF-κB by competition reaction in which a 50 fold molar excess of unlabelled wild-type, mutant or Sp-1 oligonucleotide was added to the binding reaction 10 min before addition of radiolabelled probe. Protein-nucleic acid complexes were resolved by electrophoresis on 4% nondenaturing polyacrylamide gel in 0.5 × Tris borate ethylenediaminotetraacetic acid buffer at 150 V for 2 h at 4°C. The gel was dried and autoradiographed with intensifying screen at −80°C for 20 h. Subsequently, the relative bands were quantified by densitometric scanning of the X-ray films with GS- 700 Imaging Densitometer (Bio-Rad) and a computer program (Molecular Analyst, IBM).

Immunoprecipitation and Western blot analysis

The levels of *hsp72* were quantified in whole extracts, by immunoprecipitation followed by Western blot analysis according to the manufacturer's instruction (Santa Cruz Biotechnology). Briefly, equivalent amounts of whole extracts (100 μg for each sample) were mixed with 40 μl of protein A-sepharose and 2 μl of anti-*hsp72* (SPA-812, StressGen), which specifically recognizes the inducible, but not the constitutive member of the hsp70 family polyclonal antibodies, and left overnight at 4°C with continuous shaking. Precipitated

immunocomplexes were electrophoresed, transferred onto nitrocellulose membranes and then incubated with anti-*hsp72* antibodies for 1 h at room temperature. Recombinant human *hsp72* protein (StressGen) was used as positive control. The membranes were then incubated with anti-rabbit immunoglobulins coupled to peroxidase. The immunocomplexes were visualized by the ECL chemiluminescence method (Amersham). Subsequently, the relative expression of the proteins was quantified by densitometric scanning of the X-ray films with GS-700 Imaging Densitometer (Bio-Rad) and a computer program (Molecular Analyst, IBM). β-actin (Sigma) Western blot analysis was performed to ensure equal sample loading.

Materials

Unless otherwise stated, all compounds were obtained from Sigma-Aldrich Company Ltd. (Rome, Italy). 15d-PGJ_2 was obtained from Cayman (Milan, Italy). Biotin blocking kit, biotin-conjugated goat anti-rabbit IgG and avidin-biotin peroxidase complex were obtained from Vector Laboratories (Milan, Italy). Primary anti-nitrotyrosine antibody was purchased from Upstate Biotech (Milan, Italy). Primary ICAM-1 (CD54) antibody was purchased from Pharmingen (Milan, Italy). All other chemicals were of the highest commercial grade available.

Statistical analysis

All values in the figures and text are expressed as mean ± standard error of the mean (s.e.mean) of the mean for 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. The results were analysed by one-way ANOVA followed by a Bonferroni *ad-hoc* test for multiple comparisons. A P-value less of than 0.05 was considered significant.

Results

Effects of 15d-PGJ_2 on the degree of colitis (histology)

In sham-treated rats, the histological features of the colon were typical of a normal architecture. Four days after intracolonic administration of DNBS, at a macroscopic observation the colon appeared flaccid and filled with liquid stool and showed evidence of mucosal congestion, erosion and hemorrhagic ulcerations (see Figure 1A for macroscopic damage score). On histological examination of the colon from DNBS-treated rats, the histopathological features included transmural necrosis and oedema and diffuse PMN infiltration into the submucosa (Figure 2 for representative section). The average degree of damage (on a scale of 0–4) amounted to 3.42 ± 0.2 (Figure 1B). Treatment of rats with 15d-PGJ_2 significantly attenuated the extent and severity of the colon injury caused by DNBS (Figures 1A,B and 2C). The inflammatory changes of the intestinal tract were associated with an increase in the weight of the colon (Table 1). A significant increase in the weight of the spleen, an indicator of inflammation, was also noted in vehicle-treated

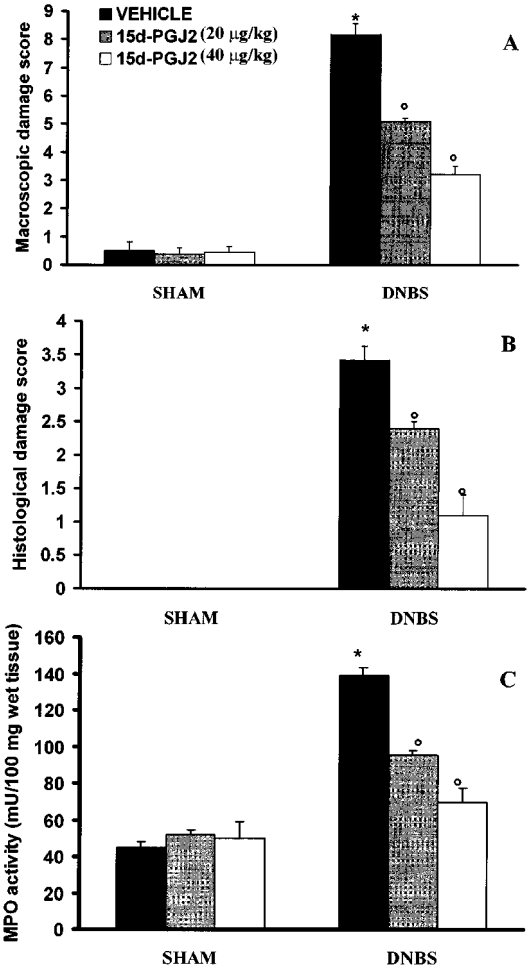

Figure 1 Effect of 15d-PGJ_2 treatment on the colon damage score and neuthophil infiltration. Macroscopic damage score (A), histological damage score (B) and myeloperoxidase (MPO) activity in the colon from DNBS-treated rats. Macroscopic damage score, histological damage score and MPO activity were significantly increased in DNBS-treated rats in comparison to sham. 15d-PGJ_2-treated rats show a significant reduction of macroscopic damage score, histological damage score and MPO activity. Macroscopic damage score ($n=10$ rats for each group), and histological damage score ($n=6$ section for each animals) were scored by two independent observers blinded to the experimental protocol MPO values are mean ± s.e.mean of 10 rats for each group. *$P<0.01$ *vs* SHAM; ○$P<0.01$ *vs* DNBS.

rats, which had received DNBS (Table 1). No significant increase in weight of either colon or spleen was observed in DNBS-rats, which had been treated with 15d- PGJ_2 (Table 1).

Effects of 15d-PGJ_2 on changes of body weight

In vehicle-treated rats, the severe colitis caused by DBNS was associated with a significant loss in body weight (Table 1). Treatment of DNBS-rats with 15d-PGJ_2 significantly reduced the loss in body weight (Table 1).

Effects of 15d-PGJ2 on cytokine production

The levels of TNFα and IL-1β were significantly elevated in the colon at 4 days after DNBS treatment (Figure 3). In contrast, the levels of these cytokines were significantly lower

in rats treated with 15d-PGJ_2 (Figure 3). No significant increase in the levels of cytokines was observed in the colon of sham-operated rats.

Effects of 15d-PGJ_2 on iNOS expression

At 4 days after DNBS treatment, colon sections were taken in order to determine the immunohistological staining for iNOS. While there was negligible staining in the intestinal sections of control animals (Figure 4A), immunohistochemical analysis, using a specific anti-iNOS antibody revealed a positive staining primarily localized in the infiltrated inflammatory cells and in disrupted epithelial cells (Figure 4B). 15d-PGJ_2 reduced the degree of positive staining for iNOS in the colon of DNBS-treated rats (Figure 4C).

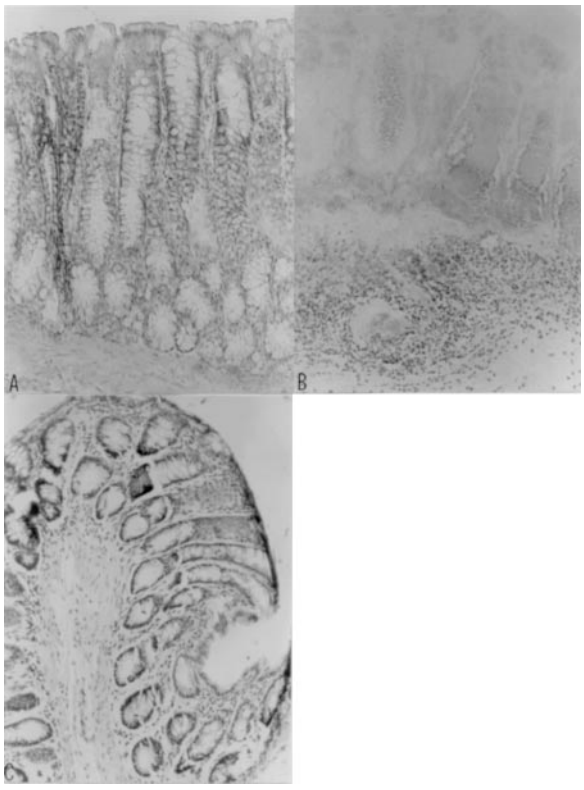

Figure 2 Effect of 15d-PGJ_2 on colon injury. No histological modification was observed in mucosal from sham-operated rats (A). Mucosal injury was produced after DNBS administration characterized by absence of epithelium and a massive mucosal and submucosal infiltration of inflammatory cells (B). Treatment with 15d-PGJ_2 (40 μg kg^{-1}) (C) corrected the disturbances in morphology associated with DNBS administration. Original magnification: $\times 125$. Figure is representative of at least three experiments performed on different experimental days.

Effects of 15d-PGJ_2 on nitrotyrosine formation and PARP activity

To determine the localization of 'peroxynitrite formation' and/or other nitrogen derivatives produced during colitis, nitrotyrosine, a specific marker of nitrosative stress, was measured by immunohistochemical analysis in the distal colon. At 4 days after DNBS treatment, sections of the colon were taken in order to determine the immunohistological staining for PAR. Sections of colon from sham-administered rats did not stain for either nitrotyrosine or PAR (Figure 5A,D). Colon sections obtained from vehicle-treated DNBS-rats exhibited positive staining for nitrotyrosine and PAR (Figure 5B,E) localized in inflammatory cells and in disrupted epithelial cells. 15d-PGJ_2 reduced the degree of positive staining for nitrotyrosine and PAR in the colon of DNBS-treated rats (Figure 5C,F).

Effects of 15d-PGJ_2 on ICAM-1 expression and PMN infiltration

The colitis caused by DNBS was also characterized by an increase in myeloperoxidase activity, an indicator of the polymorphonuclear neutrophils (PMNs) accumulation in the colon (Figure 1C). This finding is consistent with the

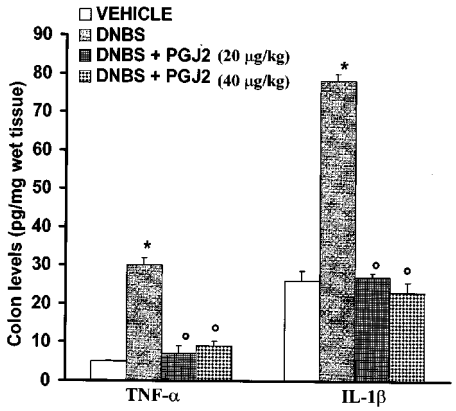

Figure 3 Effects of 15d-PGJ_2 on the levels of cytokines in the colon. Colon levels of TNF-α and IL-1β were significantly increased at 4 days after DNBS administration. 15d-PGJ_2 treatment significantly reduced in a dose dependent manner the cytokine levels. Values are mean$\pm$s.e.mean of 10 rats for each group. *$P<0.01$ *vs* SHAM; $\bigcirc P<0.01$ *vs* DNBS.

Table 1 Effect of 15-deoxy 12,14 PGJ_2 (15d-PGJ_2) on colon, spleen and body weight at 4 days after DNBS-induced colitis

	Colon weight (g)	*Spleen weight* (g)	*Body weight increase* (g)
Sham + Vehicle	1.2 $\pm$ 0.06	0.72 $\pm$ 0.04	48 $\pm$ 3.5
Sham + 15d-PGJ_2 (20 μg kg^{-1})	1.09 $\pm$ 0.04	0.85 $\pm$ 0.05	51 $\pm$ 2.1
Sham + 15d-PGJ_2 (40 μg kg^{-1})	1.01 $\pm$ 0.08	0.90 $\pm$ 0.07	45 $\pm$ 4.0
DNBS + Vehicle	5.44 $\pm$ 0.08*	1.37 $\pm$ 0.06*	$-$55 $\pm$ 4.4*
DNBS + 15d-PGJ_2 (20 μg kg^{-1})	3.2 $\pm$ 0.05°	0.93 $\pm$ 0.05°	$-$13 $\pm$ 3.0°
DNBS + 15d-PGJ_2 (40 μg kg^{-1})	2.01 $\pm$ 0.09°	0.77 $\pm$ 0.06°	9.0 $\pm$ 3.7°

Values are mean$\pm$s.e.mean of 10 rats for each group. *$P<0.01$ *vs* SHAM; $\bigcirc P<0.01$ *vs* DNBS.

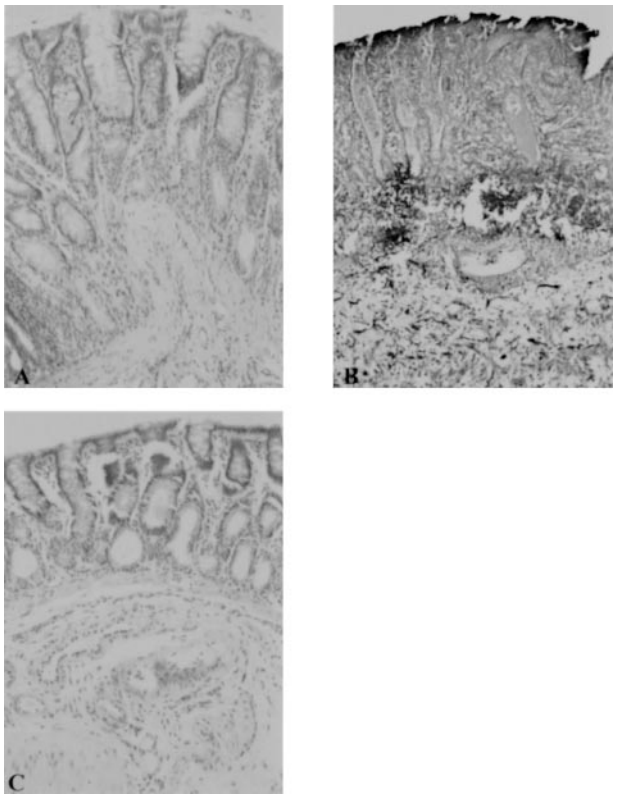

Figure 4 Immunohistochemical localization for iNOS in the colon. No positive staining for iNOS (A) was found in the colon section from sham-administered rats. Immunohistochemical analysis for iNOS (B) show positive staining localized in the injured area from DNBS-treated rats. The intensity of the positive staining for iNOS (C) was significantly reduced in the colon from 15d-PGJ_2 (40 μ kg^{-1})-treated rats. Original magnification: $\times 125$. Figure is representative of at least three experiments performed on different experimental days.

observation made with light microscopy that the colon of vehicle-treated DBNS-rats contained a large number of PMNs. 15d-PGJ_2 significantly reduced the degree of PMN infiltration (determined as increase in MPO activity) in inflamed colon (Figure 1C).

To further elucidate the effect of 15d-PGJ_2 on PMN accumulation in inflamed colon, we evaluated the intestinal expression of ICAM-1. Tissue sections obtained from sham-operated rats with anti-ICAM-1 antibody showed a specific staining along the vessels, demonstrating that ICAM-1 is expressed constitutively in endothelial cells (Figure 6A). After DNBS administration, the staining intensity substantially increased in the vessels of the lamina propria and submucosa. Immunohistochemical staining for ICAM-1 was also present in epithelial cells of injured colon and in infiltrated inflammatory cells in damaged tissues from DNBS-treated rats (Figure 6B). Section from 15d-PGJ_2-treated rats did not reveal any up-regulation of the constitutive ICAM-1, which was normally expressed in the endothelium along the vascular wall (Figure 6C).

Effects of 15d-PGJ_2 on lipid peroxidation in the colon

Infiltration of leukocytes into the mucosa has been suggested to contribute significantly to the tissue necrosis and mucosal dysfunction associated with colitis, as activated PMNs release large amounts of free radicals. The increase in myeloperoxidase activity in the colon correlated positively with the increase in tissue levels of malondialdehyde, indicating an increase in lipid peroxidation (Figure 7). Treatment of DBNS-rats with 15d- PGJ_2 significantly reduced the degree of lipid peroxidation (increase in tissue MDA levels) (Figure 7).

Effect of 15d-PGJ_2 on NF-κB activation in inflamed colon

To detect NF-κB/DNA binding activity, whole extracts from colon tissue of each rat were analysed by EMSA. A low basal level of NF-κB/DNA binding activity was detected in nuclear proteins from tissue of untreated rats (naïve). The DNA binding activity significantly increased in whole extracts obtained from inflamed colon of control animals 4 days after colitis induction. Treatment of rats with 20 μg kg^{-1} of 15d-PGJ_2 caused a significant inhibition of DNBS-induced NF-κB/DNA binding activity as revealed by specific EMSA (Figure 8).

The specificity of NF-κB/DNA binding complex was demonstrated by the complete displacement of the NF-κB/DNA binding in the presence of a 50 fold molar excess of unlabelled NF-κB probe (W.T. $50\times$) in the competition reaction. In contrast a 50 fold molar excess of unlabelled mutated NF-κB probe (Mut. $50\times$) or Sp-1 oligonucleotide (Sp-1 $50\times$) had no effect on this DNA-binding activity (Figure 8).

Effect of 15d-PGJ_2 on hsp72 *expression*

As shown in Figure 9, homogenates tissues from control animals showed a significant increase of *hsp72* protein expression as compared to naïve rats. Treatment of rats with 15d-PGJ_2 (20 μg kg^{-1}) significantly increased ($P<0.001$) the expression of *hsp72* protein when compared to the control group.

Discussion

IBD is a multi-factorial disorder of unknown aetiology. There is, however, very good evidence both from animal and clinical studies, which documents that an enhanced formation of reactive oxygen or nitrogen species importantly contribute to the pathophysiology of IBD. For instance, monocytes from patients with Crohn's disease (Kitahora *et al*., 1998) and PMNs from patients with ulcerative colitis (Shiratora *et al*., 1989) have an increased capacity to generate free oxygen radicals. Furthermore, advanced stages of bowel inflammation in humans (Middleton *et al*., 1993; Boughton-Smith *et al*., 1993; Nathan, 1996; Lundberg *et al*., 1994) and animals (Morris *et al*., 1989; Ikeda *et al*., 1997; Aiko & Grisham, 1995; Ribbons *et al*., 1995; Mourelle *et al*., 1996) are associated with an enhanced (local) formation of NO by iNOS. We demonstrate here that 15d-PGJ_2 attenuates: (i) the degree of haemorrhagic diarrhoea and weight loss, (ii) the activation of NF-κB, (iii) the degree of colonic injury, (iv) the infiltration of the colon PMNs, (v) the degree of lipid peroxidation in the colon, (vi) the increase in staining (immunohistochemistry) for iNOS, nitrotyrosine and PARP,

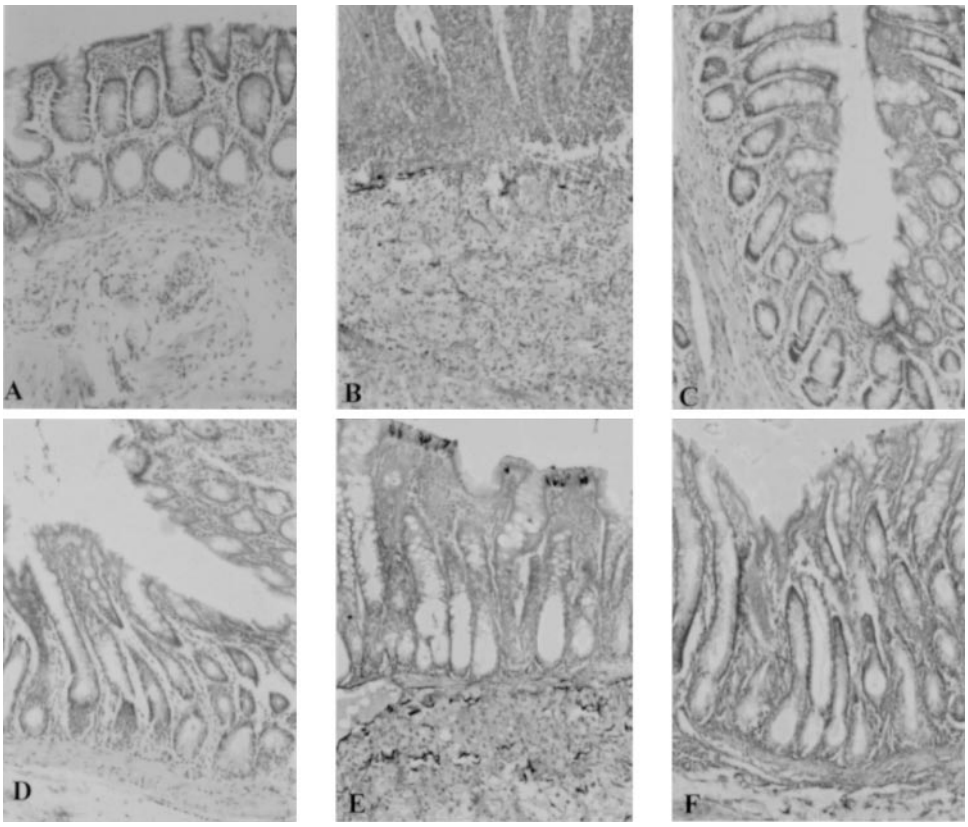

Figure 5 Immunohistochemical localization for nitrotyrosine and PAR in the colon. No positive staining for nitrotyrosine (A) and for PAR (D) was found in the colon section from sham-operated rats. Immunohistochemical analysis for nitrotyrosine (B) and for PAR (E) show positive staining localized in the injured area from a DNBS-treated rats. The intensity of the positive staining for nitrotyrosine (C) and for PAR (F) was significantly reduced in the colon from 15d-PGJ_2 (40 $\mu g\ kg^{-1}$)-treated rats. Original magnification: $\times 125$. Figure is representative of at least three experiments performed on different experimental days.

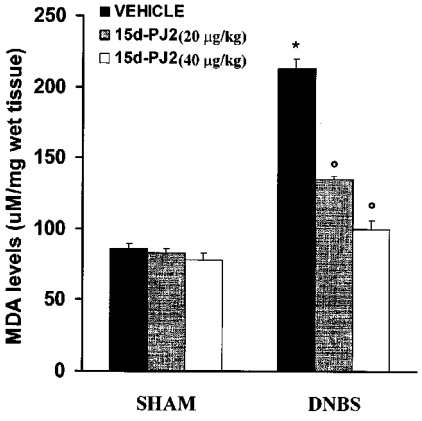

Figure 6 Effect of 15d-PGJ_2 on lipid peroxidation. Malondialdehyde (MDA) in the colon from DNBS-treated rats. MDA levels were significantly increased in DNBS-treated rats in comparison to sham. 15d-PGJ_2 -treated rats show a significant reduction of MDA levels. Values are mean$\pm$s.e.mean of 10 rats for each group. $^*P<0.01$ *vs* SHAM; $\bigcirc P<0.01$ *vs* DNBS.

as well as (vii) the increased expression of ICAM-1 caused by DNBS in the colon. Furthermore, 15d-PGJ_2 stimulates the expression of *hsp72* (immunoblot) in the inflamed colon. All of these findings support the view that 15d-PGJ_2 exerts potent anti-inflammatory effects and that this agent may be useful in the therapy of IBD. What, then, is the mechanism by which 15d-PGJ_2 inhibit the colon inflammation caused by injection of DNBS?

It has been recently reported that cyclopentenone prostaglandins can activate the inducible, phosphorylated form of HSF1, which in turn activates *hsp72* synthesis for extended periods (12–24 h) (Amici *et al*., 1992; Santoro *et al*., 1989). We confirm here that a significant increase of the expression of *hsp72* can be observed at 4 days after 15d-PGJ_2 treatment. In addition 15d-PGJ_2 reduces (among other effects) the biosynthesis and/or the effects of the pro-inflammatory cytokines TNF-α and IL-1. There is good evidence that TNF-α and IL-1 help to propagate the extension of a local or systemic inflammatory process (Guy *et al*., 1991; Saklatvala, 1986; Wooley *et al*., 1993). We confirm that the model of colitis used here leads to a substantial increase in the levels of TNF-α and IL-1 in the colon. Interestingly, the levels of these two proinflammatory cytokines are significantly lower in the animals, which were treated with 15d-PGJ_2. We propose that (at least some) of the anti-inflammatory effects of 15d-PGJ_2 reported here are due to prevention by this cyclopentenone prostaglandin of the formation of TNF-α and IL-1 in the colon. There is good evidence that TNFα (and IL-1) causes the activation and translocation of NF-κB into the nucleus (Bauerle & Henkel, 1994). Moreover, it has been recently shown *in vitro* that 15d-PGJ_2 inhibits the activation of NF-κB by preventing the phosphorylation of IK kinase (IKK)

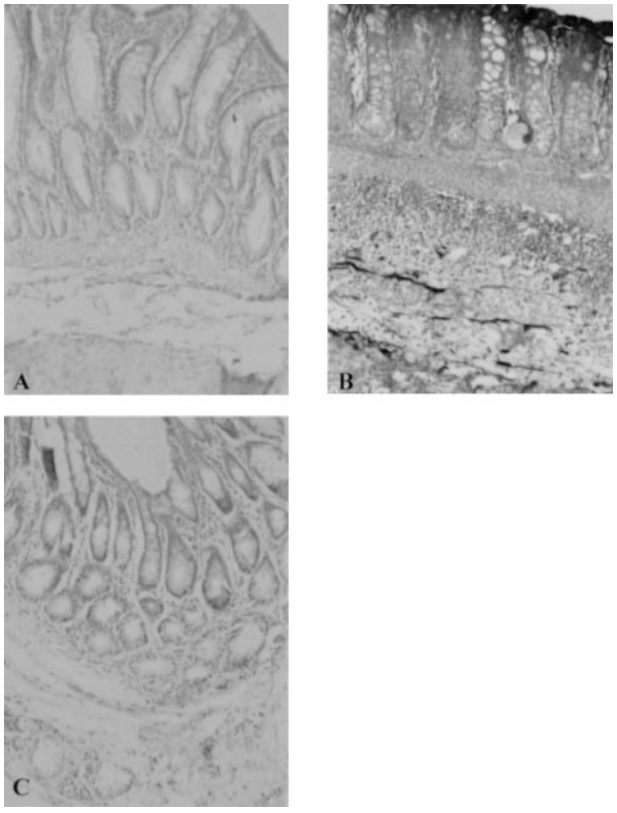

Figure 7 Immunohistochemical localization of ICAM-I in the colon. Staining of colon tissue sections obtained from sham-operated rats with anti-ICAM-1 antibody showed a specific staining along vessels, demonstrating that ICAM-1 is constitutively expressed (A). Section obtained from DNBS-treated rats showed intense positive staining for ICAM-1 (B) on endothelial cells. The degree of endothelial staining for ICAM-1 (C) was markedly reduced in tissue section obtained from 15d-PGJ_2 (40 μg kg^{-1})-treated rats. Original magnification: $\times$125. Figure is representative of at least three experiments performed on different experimental days.

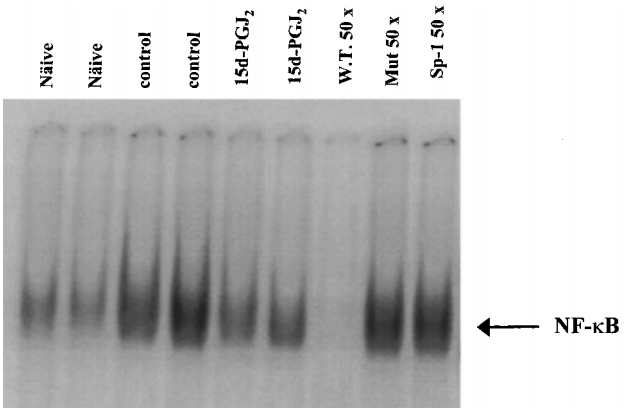

Figure 8 Effect of 15d-PGJ_2 on NF-κB/DNA binding activity in rat colon. Whole extracts from inflamed (control) or non-inflamed (naïve) rat colon were prepared as described in Methods and incubated with ^{32}P-labelled NF-κB probe. Representative EMSA of NF-κB show the effect of 15d-PGJ_2 (20 μg kg^{-1}) on NF-κB/DNA binding activity evaluated in tissue from colon rat 4 days after induction of colitis. In competition reaction whole cell extracts were incubated with radiolabelled NF-κB probe in absence or presence of identical but unlabelled oligonucleotides (W.T. 50$\times$), mutated non-functional κB probe (Mut. 50$\times$) or unlabelled oligonucleotide containing the consensus sequence for Sp-1 (Sp-1 50$\times$). Data illustrated are from a single experiment and are representative of three separate experiments.

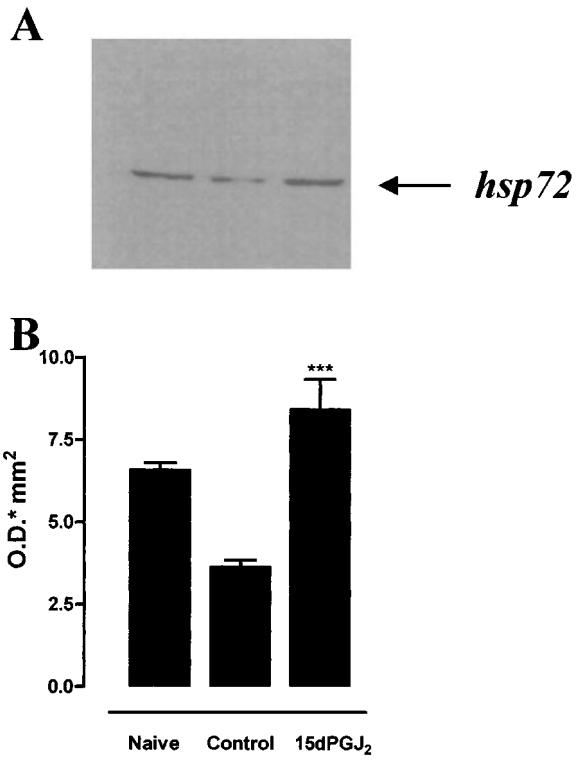

Figure 9 Effect of 15d-PGJ_2 on *hsp72* protein expression in rat colon. Representative Western blot of *hsp72* (A) as well as the densitometric analysis (B) shows the effect of 15d-PGJ_2 (20 μg kg^{-1}) on *hsp72* protein expression evaluated in rat colon tissue 4 days after colitis induction. Immunoblotting in panel A is representative of one colon out of five to six analysed. The results in panel B are expressed as mean$\pm$s.e.mean from five to six colons. ***$P<0.001$, *vs* control group.

and hence, preventing the degradation of IKB (Rossi *et al*., 1997).

In this study we demonstrate, *in vivo*, that systemic administration of 15d-PGJ_2 to rats inhibits the activation of NF-κB. Suppression of the activation of NF-κB by 15d-PGJ_2 may well result in a reduced expression of enzymes (e.g. iNOS), cytokines (TNFα, IL-1β, IL-6 etc), or adhesion molecules (ICAM-1, VCAM-1, E-selectin) known to play an important role in the pathophysiology of inflammation.

In the rat, DNBS causes an overproduction of NO due to induction of iNOS, which contributes to the inflammatory process (Zingarelli *et al*., 1998; 1999a). We demonstrate here that 15d-PGJ_2 attenuates the expression of iNOS in the colon from DNBS-treated rats. Our finding of a reduced NO-production by 15d-PGJ_2 *in vitro* is also in accordance with reports that 15d-PGJ_2 inhibits the expression of iNOS *in vitro* (see Introduction). Thus, the reduction of the expression of iNOS by 15d-PGJ_2 may contribute to the attenuation by this agent of the formation of nitrotyrosine in the colon from DNBS-treated mice. Nitrotyrosine formation, along with its detection by imunohistochemical staining, was initially proposed as a relatively specific marker for the detection of the endogenous formation 'footprint' of peroxynitrite (Beck-

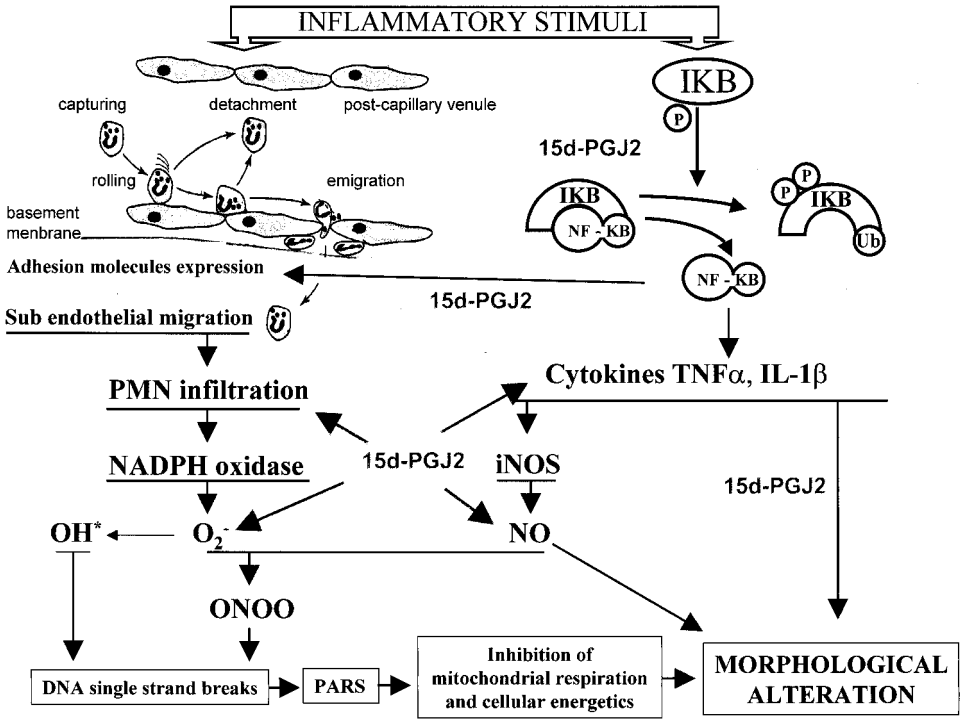

Figure 10 Proposed scheme of some of the delayed inflammatory pathways in DNBSinduced colitis, and potential sites of the anti-inflammatory actions of 15d-PGJ_2. DNBS, at least in part *via* activation of nuclear factor-κB. (NF-κB), triggers the expression of inducible NO synthase (iNOS), NO, in turn, combines with superoxide to form ONOO-. ONOO- or peroxynitrous acid (ONOOH) induce cellular injury. Part of the injury is related to the development of DNA single strand breakage, with consequent activation of PARS, leading to cellular dysfunction. Expression of the adhesion molecules (ICAM-1) is also dependent on the activation of NF-κB. In addition, endothelial dysfunction can directly induced the upregulation of ICAM-1, leading to the enhanced neutrophils infiltration. We propose that the anti-inflammatory effects of 15d-PGJ_2I may include (1) inhibition of the activation of NF-κB and prevention of the expression of iNOS and ICAM-1, (3) inhibition of ONOO formation, (4) prevention of the activation of PARP and (5) reduction of neutrophils infiltration. See Discussion for further explanations.

man, 1996). There is, however, recent evidence that certain other reactions can also induce tyrosine nitration; e.g., the reaction of nitrite with hypochlorous acid and the reaction of myeloperoxidase with hydrogen peroxide can lead to the formation of nitrotyrosine (Halliwell, 1997). Increased nitrotyrosine staining is considered, therefore, as an indication of 'increased nitrosative stress' rather than a specific marker of the generation of peroxynitrite. Thus, we propose that the reduction of the expression of iNOS protein, caused by 15d-PGJ_2, contributes to the reduction by this agent of the organ injury caused by acute and chronic inflammation in the rat.

ROS and peroxynitrite produce cellular injury and necrosis *via* several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage. ROS produce strand breaks in DNA that triggers energy-consuming DNA repair mechanisms and activates the nuclear enzyme PARP resulting in the depletion of its substrate NAD^+ *in vitro* and a reduction in the rate of glycolysis. As NAD^+ functions as a cofactor in glycolysis and the tricarboxylic acid cycle, NAD^+ depletion leads to a rapid fall in intracellular ATP. This process has been termed 'the PARP Suicide Hypothesis'. There is recent evidence that the activation of PARP may also play an important role in inflammation (Zingarelli *et al.*, 1999b; Szabó *et al.*, 1997; 1998; Cuzzocrea *et al.*, 1998a, b). We demonstrate here that 15d-PGJ_2 attenuates the increase in PARP activity in the colon DNBS-treated rat.

In conclusion, this study demonstrates that the degree of colitis caused by injection of DNBS is substantially reduced by treatment of rats with 15d-PGJ_2. The mechanisms of the anti-inflammatory effect of 15d-PGJ_2 are not entirely clear. 15d-PGJ_2 inhibits the activation of NF-κB (positive feedback; Figure 10), which mediates the expression of iNOS protein and ultimately the degree of peroxynitrite formation and tissue injury. In addition, 15d-PGJ_2 inhibits the formation of ICAM-1, which in turn may contribute to the recruitment of PMNs.

Moreover, this study shows that administration of 15d-PGJ_2 may provide protection against inflammation by inducing the expression of cytoprotective molecules such as *hsp72*. The question whether the observed anti-inflammatory effect of 15d-PGJ_2 are dependent on the activation of PPARγ warrants further investigation. However, our findings suggest that 15d-PGJ_2 treatment may be useful in conditions associated with local or systemic inflammation including inflammatory bowel disease.

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References

AIKO, S. & GRISHAM, M.B. (1995). Spontaneous intestinal inflammation and nitric oxide metabolism in HLA-B27 transgenic rats. *Gastroenterology,* **109,** 142–150.

AJUEBOR, M.N., SINGH, A. & WALLACE, J.L. (2000). Cyclooxygenase-2-derived prostaglandin D(2) is an early anti-inflammatory signal in experimental colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **279,** G238–G244.

AMICI, C., SISTONEN, L., SANTORO, M.G. & MORIMOTO, R.I. (1992). Antiproliferative prostaglandins activate heat shock transcription factor. *Proc. Natl. Acad. Sci. U.S.A.,* **89,** 6227–6231.

BAUERLE, P.A. & HENKEL, T. (1994). Function and activation of NF-κB in the immune system. *Ann. Rev. Immunol.,* **2,** 141–179.

BECKMAN, J.S. (1996). Oxidative damage and tyrosine nitration from peroxynitrite. *Chem. Res. Toxicol.,* **9,** 836–844.

BOUGHTON-SMITH, N.K., EVANS, S.M., HAWKEY, C.J., EVANS, S.M., HAWKEY, C.J., COLE, A.T., BALSITIS, M., WHITTLE, B.J. & MONCADA, S. (1993). Nitric oxide synthase activity in ulcerative colitis and Crohn's disease. *Lancet,* **341,** 338–340.

COLVILLE-NASH, P.R., QURESHI, S.S., WILLIS, D. & WILLOUGHBY, D.A. (1998). Inhibition of Inducible Nitric Oxide Synthase by Peroxisome Proliferator- Activated Receptor Agonists: Correlation With Induction of Heme Oxygenase 1. *J. Immunol.,* **161,** 978–984.

CUZZOCREA, S., CAPUTI, A.P. & ZINGARELLI, B. (1998a). Peroxynitrite-mediated DNA strand breakage activates poly (ADP-ribose) synthetase and causes cellular energy depletion in carrageenan-induced pleurisy. *Immunology,* **93,** 96–101.

CUZZOCREA, S., ZINGARELLI, B., GILAD, E., HAKE, P., SALZMAN, A.L. & SZABO, C. (1998b). Protective effects of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthase in carrageenan-induced models of local inflammation. *Eur. J. Pharmacol.,* **342,** 67–76.

DESREUMAUX, P., DUBUQUOY, L., NUTTEN, S., PEUCHMAUR, M., ENGLARO, W., SCHOONJANS, K., DERIJARD, B., DESVERGNE, B., WAHLI, W., CHAMBON, P., LEIBOWITZ, M.D., COLOMBEL, J.F. & AUWERX, J. (2001). Attenuation of colon inflammation through activators of retinoid X receptor (RXR)/peroxisome proliferator-activated receptor gamma (PPAR gamma) heterodimer. A basis for new therapeutic strategies. *J. Exp. Med.,* **193,** 827–838.

DREW, P.D. & CHAVIS, J.A. (2001). The Cyclopentenone Prostaglandin 15-Deoxy- Delta(12,14) Prostaglandin J2 Represses Nitric Oxide, TNF-Alpha, and IL-12 Production by Microglial Cells. *J. Neuroimmunol.,* **115,** 28–35.

GUY, G.R., CHUA, S.P., WONG, N.S., NG, S.B. & TAN, Y.H. (1991). Interleukin 1 and tumor necrosis factor activate common multiple protein kinases in human fibroblasts. *J. Biol. Chem.,* **266,** 14343–14352.

HALLIWELL, B. (1997). What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrite formation in vivo? *FEBS Lett.,* **411,** 157–160.

IANARO, A., IALENTI, A., MAFFIA, P., PISANO, B. & DI ROSA, M. (2001). HSF1/hsp72 pathway as an endogenous anti-inflammatory system. *FEBS Lett.,* **499,** 239–244.

IKEDA, I., KASAJIMA, T., ISHIYAMA, S., SHIMOJO, T., TAKEO, Y., NISHIKAWA, T., KAMEOKA, S., HIROE, M. & MITSUNAGA, A. (1997). Distribution of inducible nitric oxide synthase in ulcerative colitis. *Am. J. Gastroenterol.,* **92,** 1339–1341.

JIANG, C., TING, A.T. & SEED, B. (1998). PPAR-Gamma Agonists Inhibit Production of Monocyte Inflammatory Cytokines. *Nature,* **391,** 82–86.

KITAHORA, T., SUZUKI, K., ASAKURA, H., YOSHIDA, T., SUEMATSU, M., WATANABE, M., AISO, S. & TSUCHIYA, M. (1998). Active oxygen species generated by monocytes and polymorphonuclear cells in patients in Crohn's disease. *Dig. Dis. Sci.,* **33,** 951–955.

LEWIS, J.D., LICHTENSTEIN, G.R., STEIN, R.B., DEREN, J.J., JUDGE, T.A., FOGT, F., FURTH, E.E., DEMISSIE, E.J., HURD, L.B., SU, C.G., KEILBAUGH, S.A., LAZAR, M.A. & WU, G.D. (2001). An open-label trial of the PPAR-gamma ligand rosiglitazone for active ulcerative colitis. *Am. J. Gastroenterol.,* **96,** 3323–3328.

LUNDBERG, J.O., HELLSTROM, P.M., LUNDBERG, J.M. & ALVING, K. (1994). Greatly increased luminal nitric oxide in ulcerative colitis. *Lancet,* **344,** 1673–1674.

MIDDLETON, S.J., SHORTHOUSE, M. & HUNTER, J.D. (1993). Increased nitric oxide synthesis in ulcerative colitis. *Lancet,* **341,** 465–466.

MILLER, M.J., THOMPSON, J.H., ZHANG, X.J., SADOWSKA-KROWICKA, H., KAKKIS, J.L., MUNSHI, U.K., SANDOVAL, M., ROSSI, J.L., ELOBY-CHILDRESS, S. & BECKMAN, J.S. (1995). Role of inducible nitric oxide synthase expression and peroxynitrite formation in guinea pig ileitis. *Gastroenterology,* **109,** 1475–1483.

MORRIS, G.P., BECK, P.L., HERRIDGE, M.S., DEPEW, W.T., SZEWCZUK, M.R. & WALLACE, J.L. (1989). Hapten-induced model of chronic inflammation and ulceration in the rat. *Gastroenterology,* **96,** 795–803.

MOURELLE, M., VILASECA, J., GUARNER, F., SALAS, A. & MALAGELADA, J.R. (1996). Toxic dilatation of colon in a rat model of colitis is linked to an inducible form of nitric oxide synthase. *Am. J. Physiol.,* **33,** G425–G430.

MULLANE, K.M., KRAEMER, R. & SMITH, B. (1985). Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. *J. Pharmacol. Meth.,* **14,** 157–167.

NATHAN, C. (1996). Nitric oxide as a secretory product of mammalian cells. *FASEB J.,* **6,** 3051–3064.

OHKAWA, H., OHISHI, N. & YAGI, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.,* **95,** 351–358.

PASCERI, V., WU, H.D., WILLERSON, J.T. & YEH, E.T. (2000). Modulation of Vascular Inflammation in Vitro and in Vivo by Peroxisome Proliferator-Activated Receptor-Gamma Activators. *Circulation,* **101,** 235–238.

RIBBONS, K.A., ZHANG, X.J., THOMPSON, J.H., GREENBERG, S.S., MOORE, W.M., KORNMEIER, C.M., CURRIE, M.G., LERCHE, N., BLANCHARD, J. & CLARK, D.A. (1995). Potential role of nitric oxide in a model of chronic colitis in rhesus macaques. *Gastroenterology,* **108,** 705–711.

RICOTE, M., LI, A.C., WILLSON, T.M., KELLY, C.J. & GLASS, C.K. (1998). The Peroxisome Proliferator Activated Receptor-Gamma Is a Negative Regulator of Macrophage Activation. *Nature,* **391,** 79–82.

ROSSI, A., ELIA, G. & SANTORO, M.G. (1997). Inhibition of Nuclear Factor Kappa B by Prostaglandin A1: an Effect Associated With Heat Shock Transcription Factor Activation. *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 746–750.

SAKLATVALA, J. (1986). Tumour necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage. *Nature,* **322,** 547–549.

SANTORO, M.G. (2000). Heat shock factors and the control of stress response. *Biochem. Pharmacol.,* **59,** 55–63.

SANTORO, M.G., GARACI, E. & AMICI, C. (1989). Prostaglandins with antiproliferative activity induce the synthesis of a heat shock protein in human cells. *Proc. Natl. Acad. Sci. U.S.A.,* **86,** 8407–8411.

SHIRATORA, Y., AOKI, S., TAKADA, H., KIRIYAMA, H., OHTO, K., HAI, K., TERAOKA, H., MATANO, S., MATSUMOTO, K. & KAMII, K. (1989). Oxygenderived free radical generating capacity of polymorphonuclear cells in patients with ulcerative colitis. *Digestion,* **44,** 163–171.

STRAUS, D.S. & GLASS, C.K. (2001). Cyclopentenone Prostaglandins: New Insights on Biological Activities and Cellular Targets. *Med. Res. Rev.,* **21,** 185–210.

SU, C.G., WEN, X., BAILEY, S.T., JIANG, W., RANGWALA, S.M., KEILBAUGH, S.A., FLANIGAN, A., MURTHY, S., LAZAR, M.A. & WU, G.D. (1999). A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. *J. Clin. Invest.,* **104,** 383–389.

SZABÓ, C., LIM, L.H.K., CUZZOCREA, S., GETTING, S.J., ZINGARELLI, B., FLOWER, R.J., SALZMAN, A.L. & PERRETTI, M. (1997). Inhibition of poly (ADP-ribose) synthetase exerts anti-inflammatory effects and inhibits neutrophil recruitment. *J. Exp. Med.,* **186,** 1041 – 1049.

SZABÓ, C., VIRAG, L., CUZZOCREA, S., SCOTT, G.S., HAKE, P., O'CONNOR, M.P., ZINGARELLI, B., SALZMAN, A. & KUN, E. (1998). Protection against peroxynitrite-induced fibroblast injury and arthritis development by inhibition of poly (ADP-Ribose) synthetase. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 3867 – 3872.

TACCHINI, L., RADICE, L., POGLIAGHI, G. & BERNELLI-ZAZZERA, A. (1997). Differential activation of heat shock and nuclear factor kappaB transcription factors in postischemic reperfused rat liver. *Hepatology,* **26,** 186 – 191.

TSUBOUCHI, Y., KAWAHITO, Y., KOHNO, M., INOUE, K., HLA, T. & SANO, H. (2001). Feedback Control of the Arachidonate Cascade in Rheumatoid Synoviocytes by 15-Deoxy-Delta(12,14)-Prostaglandin J2. *Biochem. Biophys. Res. Commun.,* **283,** 750 – 755.

WALLACE, J.L., KEENAN, C.M., GALE, D. & SHOUPE, T.S. (1992). Exacerbation of experimental colitis by non-steroidal antinflammatory drugs is not related to elevate leukotriene B4 synthesis. *Gastroenterology,* **102,** 18 – 27.

WOOLEY, P.H., WHALEN, J.D., CHAPMAN, D.L., BERGER, A.E., RICHARD, K.A., ASPAR, D.G. & STAITE, N.D. (1993). The effect of an interleukin-1 receptor antagonist protein on type II collagen-induced arthritis and antigeninduced arthritis in mice. *Arthritis Rheum.,* **36,** 1305 – 1314.

ZHANG, X., WANG, J.M., GONG, W.H., MUKAIDA, N. & YOUNG, H.A. (2001). Differential Regulation of Chemokine Gene Expression by 15-Deoxy-Delta 12,14 Prostaglandin J2. *J. Immunol.,* **166,** 7104 – 7111.

ZINGARELLI, B., CUZZOCREA, S., SZABO', C. & SALZMAN, A.L. (1998). Mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, reduces trinitrobenzene sulfonic acid-induced colon damage in rats. *J. Pharmacol. Exp. Ther.,* **287,** 1048 – 1055.

ZINGARELLI, B., SQUADRITO, F., GRAZIANI, P., CAMERINI, R. & CAPUTI, A.P. (1993). Effects of zileuton, a new 5-lipoxygenase inhibitor, in experimentally induced colitis in rats. *Agents Actions,* **39,** 150 – 156.

ZINGARELLI, B., SZABÒ, C. & SALZMAN, A.L. (1999a). Reduced oxidative and nitrosative damage in murine experimental colitis in the absence of inducible nitric oxide synthase. *Gut,* **45,** 199 – 209.

ZINGARELLI, B., SZABO', C. & SALZMAN, A.L. (1999b). Blockade of Poly(ADP-ribose) synthetase inhibits neutrophil recruitment, oxidant generation, and mucosal injury in murine colitis. *Gastroenterology,* **16,** 335 – 345.

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