Beneficial effects of GW274150 treatment on the development of experimental colitis induced by dinitrobenzene sulfonic acid

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Abstract

Inflammatory bowel disease is associated with inducible nitric oxide synthase (iNOS) expression, oxidative and nitrosative stress, and leukocyte infiltration in the colon. Here, we investigate the effects of the selective iNOS-inhibitor (S)-2-amino-(1-iminoethylamino)-5-thiopentanoic acid (GW274150) on the development of experimental colitis induced by dinitrobenzene sulfonic acid. When compared to dinitrobenzene sulfonic acid-treated mice, GW274150 (5 mg/kg i.p.)-treated mice subjected to dinitrobenzene sulfonic ACID-induced colitis experienced a significantly lower rate of the extent and severity of the histological signs of colon injury. Dinitrobenzene sulfonic acid-treated mice experienced hemorrhagic diarrhoea and weight loss. At 4 days after the administration of dinitrobenzene sulfonic acid, the mucosa of the colon exhibited large areas of necrosis. Immunohistochemistry for nitrotyrosine and poly (ADP-ribose) (PAR) showed an intense staining in the inflamed colon. Treatment of dinitrobenzene sulfonic acid-treated mice with GW274150 significantly reduced the degree of hemorrhagic diarrhoea and weight loss caused by administration of dinitrobenzene sulfonic acid. GW274150 also caused a substantial reduction of (i) the degree of colon injury, (ii) the rise in myeloperoxidase (MPO) activity (mucosa), (iii) the increase in staining (immunohistochemistry) for nitrotyrosine, as well as (iv) PARP activation caused by dinitrobenzene sulfonic acid in the colon. Thus, GW274150 treatment reduced the degree of colitis caused by dinitrobenzene sulfonic acid. We propose that selective inhibition of iNOS activity with GW274150 may be useful in the treatment of inflammatory bowel disease.

Keywords: Inflammatory bowel disease; Nitric oxide synthase; Oxidative stress

1. Introduction

Reactive oxygen and nitrogen species (reactive oxygen and nitrogen species ) have been implicated in the pathogenesis of a variety of acute and chronic inflammatory states, including ischemia–reperfusion injury (Carden and Granger, 2000), atherosclerosis (Patel et al., 2000), rheumatoid arthritis (Bauerova and Bezek, 1999), and inflammatory bowel disease, e.g., Crohn’s disease and ulcerative colitis (Babbs, 1992; Grisham, 1994). The two most extensively studied reactive oxygen and nitrogen species, superoxide (O_2^-) and nitric oxide (NO^•), are known to exert profound, and often opposing, effects in inflamed tissues. These actions of O_2^- and NO^• can be manifested as impaired endothelium-dependent vasodilatation (Suzuki et al., 2000), activation of nuclear transcription factors, and the subsequent production of inflammatory cytokines (Flohe et al., 1997), enhanced recruitment and activation of leukocytes (Kubes et al., 1991), accelerated apoptosis (Zhai et al., 2000), and parenchymal cell necrosis (McKenzie et al., 2000).
Due to the opposing actions of $O_2^-$ and NO, which may result from their ability to chemically react with and decompose each other, it is widely held that conditions which alter the balance between $O_2^-$ and NO levels may promote inflammation. This concept has lead to extensive efforts to define the role of reactive oxygen and nitrogen species in different experimental models of inflammation and has stimulated the search for agents that may alter the production and/or bioavailability of reactive oxygen and nitrogen species (Krieglstein et al., 2001). The inflammatory bowel diseases are chronic, idiopathic disorders primarily of the ileum and/or colon that are characterized by abdominal pain, severe diarrhoea, rectal bleeding, and weight loss. Involved regions of the bowel often exhibit an intense infiltration of leukocytes (including granulocytes and lymphocytes), crypt cell hyperplasia, interstitial oedema, and mucosal ulcerations. Accompanying the extensive gut inflammation and tissue injury is an enhanced production of reactive oxygen species, such as superoxide, which appears to be largely generated by membrane-associated nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in activated neutrophils, and a corresponding increase in the expression of the inducible isoform of NO synthase (iNOS). Although several experimental strategies have been employed to address the importance of the enhanced production of $O_2^-$ and NO in the pathogenesis of inflammatory bowel disease, inconsistent findings have left this issue largely unresolved. For example, the use of inhibitors of iNOS activity has yielded mixed results in various, experimental models of inflammatory bowel disease (Rachmilewitz et al., 1995; Yoshida et al., 2000), and conflicting results have been recently reported using mice that are genetically deficient in iNOS (McCafferty et al., 1999; Zingarelli et al., 1999b; Mazzon and Cuzzocrea, 2003). It has been recently proposed that the cytotoxic effects of NO are mediated in part by peroxynitrite, a potent oxidant produced by the reaction of NO and $O_2^-$. As a highly toxic reactive species, peroxynitrite indiscriminately attacks biomolecules critical to function and viability of the cell. During inflammatory bowel disease, the simultaneous production of $O_2^-$ and NO is likely to produce peroxynitrite and to promote oxidative reactions and tissue alteration.

The realization of the cytotoxic potential of NO and peroxynitrite has stimulated efforts to discover novel therapeutic strategies to neutralize NO and peroxynitrite-induced damage by, i.e., inhibiting iNOS activity. The role of iNOS in pathologic conditions has stimulated the development of selective iNOS inhibitors like GW274150 ([S]-2-amino-(1-iminoethylamino)-5-thioheptanoic acid). This molecule is a novel inhibitor of iNOS activity (sulphur-substituted acetamide amino acid), which acts in competition with L-arginine and has a very high degree of selectivity for iNOS when compared to either eNOS (>300-fold) or nNOS (>100-fold). In addition, GW274150 is a long-acting (5-h half-life in rats) iNOS inhibitor and is also able to inhibit LPS-mediated increases in plasma nitrite/nitrate levels 14 h after a single intraperitoneal injection (ED$_{50}$ 3 mg kg$^{-1}$). The compound N-3-aminomethyl-benzylacetamidine (1400W) also identified by Garvey (Garvey et al., 1997) has proved to be a further step forward, since it is not only highly selective as an iNOS inhibitor versus both eNOS and nNOS, but also penetrates cells and tissues. Inhibition of human iNOS by 1400W was competitive with L-arginine, nicotinamide adenine dinucleotide 3'-phosphate (NADPH$^+$)-dependent and developed relatively slowly, and no significant reversal of this inhibition was observed after 2 h. Efficacy and selectivity were maintained in vivo in the stringent test of its differential effects on vascular leakage; unlike L-NIO [L-N$^2$-(1-iminoethyl)-ornithine] and amino-guanidine, it suppressed the late, iNOS-driven phase of endotoxin-provoked leakage with no exacerbation of the early phase, as it has been observed when eNOS and nNOS are inhibited (Garvey et al., 1997; Lazio and Whittle, 1997). Unfortunately, this compound exhibits an acute toxicity at high doses, which is likely to prevent its safe therapeutic use in humans, but there is a significant therapeutic window such that it can be used as a pharmacological tool in a variety of animal models (Garvey et al., 1997; Lazio and Whittle, 1997; Kankuri et al., 2001). Like with 1400W, the inhibition of iNOS activity caused by GW274150 is NADPH-dependent and develops very slowly, but is rapidly reversible, and recent studies report the role of this iNOS selective inhibitor in reducing organ injury in hemorrhagic shock, in collagen-induced arthritis, lung injury, and in renal ischemia/reperfusion (Medonald et al., 2002; Chatterjee et al., 2003; Cuzzocrea et al., 2002). Therefore, the aim of this study was to investigate the role of GW274150 in an experimental model of dinitrobenzene sulfonic acid-induced colitis.

2. Materials and methods

2.1. Animals

The study was carried out in 6- to 8-weeks-old CD1 male mice (Charles River, Calco, Italy). The animals 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 purposes (D.M. 116192), as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986).

2.2. Experimental groups

GW274150 was given daily as an i.p. bolus injection (5 mg/kg, dinitrobenzene sulfonic acid+GW274150 group) starting from day 2. In a vehicle-treated group of mice,
vehicle (saline) was given instead of GW274150 (dinitrobenzene sulfonic acid group). In a separate group of mice, surgery was performed in its every aspect identical to the one in the dinitrobenzene sulfonic acid group, except that 100 μl of 50% ethanol was injected instead of dinitrobenzene sulfonic acid (sham group). In an additional group of animals, sham surgery was combined with the administration of GW274150 (dose as above; sham+GW274150 group).

2.3. Induction of experimental colitis

Colitis was induced with a low dose of dinitrobenzene sulfonic acid (4 mg/mouse) by using a modification (Sturiale et al., 1999) of the method first described in rats (Lora et al., 1997). In preliminary experiments, this dose of dinitrobenzene sulfonic acid was found to induce reproducible colitis without mortality. Mice were anesthetized by Enflurane (Baker, Milan Italy). Dinitrobenzene sulfonic acid (4 mg in 100 μl of 50% ethanol) was injected into the rectum through a catheter inserted 4.5 cm proximally to the anus. The vehicle (100 μl of 50% ethanol) was administered in control experiments. 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 (40 μg/kg, Sigma, Milan, Italy), 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, localised 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.

2.4. 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, NJ). Thereafter, 7-μm sections were deparaffinised with xylene, stained with haematoxylin–eosin stain, and observed in a Dialux 22 Leitz (Wetzlar, 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.

2.5. Myeloperoxidase activity

Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte (polymorphonuclear leukocyte) accumulation, was determined as previously described (Mullane et al., 1985). At 4 days after intracolonic injection of dinitrobenzene sulfonic acid, the colon was removed and weighed. The colon was homogenised 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 x 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 H2O2. 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.

2.6. Localisation of nitrotyrosine and PAR by immunohistochemistry

At the end of the experiment, the tissues were fixed in 10% sodium phosphate buffer (PBS)-buffered formaldehyde, and 8 μm sections were prepared from paraffin embedded tissues. After deparaffinisation, endogenous peroxidase was quenched with 0.3% H2O2 in 60% methanol for 30 min. The sections were permeabilised with 0.1% Triton X-100 in PBS for 20 min. Nonspecific adsorption was minimised 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) antinitrotyrosine rabbit polyclonal antibody (1:500 in PBS) or with anti-PAR (1:500 in PBS, v/v; DBA). Specific labelling was detected with a biotin-conjugated goat antirabbit, donkey antigen or goat antimouse glycoprotein immunoglobulin G (IgG) and avidin–biotin peroxidase complex (DBA). To verify the binding specificity for PAR, 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.

2.7. Reagents

Recombinant human Erythropoietin (EPO) was obtained from St. Bartholomew’s Hospital Pharmacy. Biotin blocking kit, biotin-conjugated goat antirabbit IgG, and avidin–biotin peroxidase complex were obtained from Vector Laboratories (Burlingame, CA, USA). Primary antinitrotyrosine antibody was purchased from Upstate Biotech (Saranac Lake, NY, USA). Reagents and secondary and nonspecific IgG antibody for immunohistochemical analysis were from Vector Laboratories. All other reagents and compounds used were obtained from Sigma.

2.8. Statistical analysis

All values in the figures and text are expressed as mean±S.E.M. of N observations, where 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. Data sets were examined by one- and two-way analysis of variance, and individual group means were then compared with Student’s unpaired t-test. Nonparametric data were analysed with the Fisher’s Exact Test. A P-value less than 0.05 was considered to be significant.

3. Results

3.1. Effects of GW274150 treatment on the degree of colitis (histology and macroscopic assessment)

Four days after intracolonic administration of dinitrobenzene sulfonic acid, the colon appeared flaccid and filled with liquid stool. The macroscopic inspection of cecum, colon, and rectum showed presence of mucosal congestion, erosion, and haemorrhagic ulceraions (see damage score, Fig. 1A). The histopathological features included transmural necrosis, oedema, and a diffuse leukocyte cellular infiltrate in the submucosa of colon sections from dinitrobenzene sulfonic acid-treated mice (Figs. 1B and 2B). The observed inflammatory changes of the large intestine were associated with an increase in the weight of the colon (Fig. 3A). Four days after colitis induced by dinitrobenzene sulfonic acid treatment, all mice had diarrhoea and a significant reduction in body weight (compared with the control group of mice) (Fig. 3B). Daily treatment with GW274150 (5 mg/kg) resulted in a significant decrease in the extent and severity of damage (Figs. 1, 2C, and 3).

No histological alteration was observed in the colon tissue from sham-treated mice (Figs. 1, 2B, and 3). The daily treatment with GW274150 did not induce any alteration of the colon weight (Fig. 3A), as well as did not effect the body weight gain (data not shown) in sham-treated mice.

3.2. Effects of GW274150 treatment on polymorphonuclear leukocyte infiltration

The colitis caused by dinitrobenzene sulfonic acid was also characterised by an increase in MPO activity, an indicator of the polymorphonuclear leukocyte accumulation in the colon (Fig. 4). This finding is consistent with the observation made with light microscopy that the colon of vehicle-treated DBNSrats contained a large number of polymorphonuclear leukocytes. GW274150 treatment significantly reduced the degree of polymorphonuclear leukocyte infiltration (determined as an increase in MPO activity) in the inflamed colon (Fig. 4).

3.3. Effects of GW274150 treatment on nitrotyrosine and PAR formation

To determine the localisation 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. Sections of colon from sham-administered mice did not stain for nitrotyrosine (Fig. 5A; Table 1). Colon sections obtained from vehicle-treated dinitrobenzene sulfonic acid-treated mice exhibited positive staining for nitrotyrosine (Fig. 5B; Table 1) and for PAR (Fig. 6B; Table 1) localised in inflammatory cells and in disrupted epithelial cells. Sections from GW274150-treated mice did not reveal any positive staining for nitrotyrosine (Fig. 5C; Table 1) and for PAR (Fig. 6C; Table 1). No positive staining either for nitrotyrosine and for PAR was found in the colon section from sham-treated mice (Figs. 5A and 6A; Table 1).

4. Discussion

Inhibitors of NOS activity reduce the development of experimental colitis and support a role for NO$^{-}$ in the pathophysiology of inflammatory bowel disease (Kankuri et al., 2001; Krieglstein et al., 2001; Menchen et al., 2001;
Zingarelli et al., 1998), and, in addition to NO\textsuperscript{3}, peroxynitrite is also generated in dinitrobenzene sulfonic acid-induced colitis (Cuzzocrea et al., 2001).

Thus, NO\textsuperscript{3}, produced by iNOS, plays a pivotal role during inflammatory processes and iNOS selective inhibitors seem to be very useful in interfering with this NO\textsuperscript{3} production. The role of iNOS-derived NO\textsuperscript{3}, or peroxynitrite, as an amplifier of the inflammatory response is now also supported by previous observations: inhibition of iNOS suppresses tumor necrosis factor, alpha (TNF-\alpha) production in the delayed phase of allergic encephalomyelitis (Anggard, 1991); inhibition of iNOS suppresses interleukin-1 collagen and stromelysin production in arthritis (Parker, 1987); inhibition of iNOS suppresses interferon-gamma INF-\gamma production in a murine model of leishmaniasis (Stamler and Loscalzo, 1991), and the expression of certain chemokines is suppressed in the absence of iNOS in zymosan-induced peritonitis (Davidge et al., 1995).

GW274150 is a novel, potent, and selective inhibitor of iNOS activity, and previous studies have demonstrated its protective effect in organ injury in haemorragic shock, in renal ischemia and reperfusion, in a model of collagen-induced arthritis, and in a model of acute lung injury (Mcdonald et al., 2002; Chatterjee et al., 2003; Cuzzocrea et al., 2002; Dugo et al., 2004).

Table 1

Typical densitometry evaluation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Nitrotyrosine</th>
<th>PAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham+vehicle</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Sham+GW 274150</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>DNBS+vehicle</td>
<td>7.3±0.12*</td>
<td>6.8±0.14*</td>
</tr>
<tr>
<td>DNBS+GW 274150</td>
<td>2.2±0.10'</td>
<td>1.8±0.15'</td>
</tr>
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Densitometry analysis of for nitrotyrosine and PAR from colon sections was assessed. The assay was carried out by using Optilab Graftek software on a Macintosh personal computer (CPU G3-266). Data are expressed as means of percent of total tissue area ±S.E.M. of five immunocytochemistry photographs for each group.

* \( P<0.01 \) vs. SHAM; †\( P<0.01 \) vs. DNBS.

Fig. 4. Effect of GW274150 treatment on neutrophil infiltration. Myeloperoxidase (MPO) activity was significantly increased in dinitrobenzene sulfonic acid-treated mice in comparison to sham. Treatment with GW274150 (5 mg/kg day i.p.) significantly reduced the colon MPO activity. Data are means±S.E.M. of 10 mice for each group. *\( P<0.01 \) vs. SHAM; †\( P<0.01 \) vs. dinitrobenzene sulfonic acid.

Fig. 5. Immunohistochemical localization of nitrotyrosine in the colon. Immunohistochemical analysis for nitrotyrosine (B) shows positive staining localised in the injured area from dinitrobenzene sulfonic acid-treated mice. The intensity of the positive staining for nitrotyrosine (C) was markedly reduced in tissue section obtained from GW274150 (5 mg/kg day i.p.)-treated mice. No positive staining for nitrotyrosine (A) was observed in tissue section obtained from sham-treated mice. Figure is representative of at least three experiments performed on different experimental days.
Here, we demonstrate for the first time that GW274150 also reduces the development of experimental colitis. Specifically, we demonstrate here that treatment with GW274150 significantly reduced (i) the degree of haemorrhagic diarrhoea and weight loss, (ii) the degree of colonic injury, (iii) the infiltration of the colon with polymorphonuclear leukocytes, and (iv) the positive staining (immunohistochemistry) for nitrotyrosine and PAR caused by dinitrobenzene sulfonic acid in the colon. All of these findings support the view that GW274150 exerts potent antiinflammatory effects.

Neutrophils play a crucial role in the development and full manifestation of gastrointestinal inflammation, (Grisham, 1994; Shiratora et al., 1989), and neutrophil infiltration into inflamed tissue plays a crucial role in the destruction of foreign antigens and in the breakdown and remodelling of injured tissue (Lefer and Lefer, 1993).

In this study, it is reported that dinitrobenzene sulfonic acid-induced colitis results in a significant infiltration of inflammatory cells in the injured colon. We have demonstrated that treatment with GW274150 reduces this inflammatory cell infiltration as assessed by the specific granulocyte enzyme, MPO, and with the moderation of the tissue damage as evaluated by histological examination. Neutrophils are recruited into the tissue by local production of cytokines and can then contribute to tissue destruction by the production of reactive oxygen metabolites, granule enzymes, and cytokines that further amplify the inflammatory response by their effects on macrophages and lymphocytes (Lawrence and Spinger, 1991). There is, however, very good evidence both from animal and clinical studies, which documents that an enhanced formation of reactive oxygen and nitrogen species importantly contribute to the pathophysiology of inflammatory bowel disease (Shiratora et al., 1989; Kitahora et al., 1988; Middleton et al., 1993; Boughton-Smith et al., 1993; Nathan, 1996; Lundberg et al., 1994; Morris et al., 1989; Ikeda et al., 1997; Aiko and Grisham, 1995; Ribbons et al., 1995; Mourelle et al., 1996) and are associated with an enhanced (local) formation of NO by iNOS. In this study, we confirm that the mucosal damage induced by intracolonic administration of dinitrobenzene sulfonic acid was associated with immunohistochemical expression of nitrotyrosine mostly localised on epithelial cells and in the area of infiltrated inflammatory cells, suggesting that peroxynitrite or other nitrogen derivatives and oxidants are formed in vivo and may contribute to tissue injury. These data are consistent with previous findings that immunohistochemical staining for nitrotyrosine was localized on epithelial cells in a dinitrobenzene sulfonic acid model of guinea pig ileitis (Miller et al., 1995) or rat colitis (Zingarelli et al., 1999a, b) and in active Crohn’s lesions in humans (Singer et al., 1996). The pathogenic role of nitrogen derived species, such as peroxynitrite (Beckman et al., 1990; Ischiropoulos et al., 1992), in inflammatory bowel disease is further supported by the fact that intracolonic administration of exogenous peroxynitrite induces a severe colonic inflammation which mimics the features of both ulcerative colitis and Crohn’s disease (Rachmilewitz et al., 1993). In this study, we observed that epithelial disruption was significantly less in

Fig. 6. Immunohistochemical localization of PAR in the colon. Immunohistochemical analysis for PAR (B) shows positive staining localised in the injured area from dinitrobenzene sulfonic acid-treated mice. The intensity of the positive staining for PAR (C) was markedly reduced in the tissue section obtained from GW274150-treated mice. No positive staining for PAR (A) was observed in the tissue section obtained from sham-treated mice. Figure is representative of at least three experiments performed on different experimental days.
mice treated with GW274150. Indeed, GW274150 treatment prevented the formation of tissue nitrotyrosine staining in dinitrobenzene sulfonic acid-treated animals. This result of the effects of GW274150 on free radical production is in agreement with previous studies (McDonald et al., 2002; Chatterjee et al., 2003; Cuzzocrea et al., 2002; Dugo et al., 2004). Superoxide and peroxynitrite cause DNA single-strand damage, leading to PAR synthetase activation and cell death (Szabo and Dawson, 1998). Some evidence exists to support the possible role of PAR synthetase activation in inflammatory bowel disease (Zingarelli et al., 1999a; Szabo et al., 1997). As shown in Fig. 6C, GW274150 reduced PAR synthetase immunostaining, an effect that might account for the overall protective action of GW274150.

Our results demonstrate for the first time that GW274150 is protective in experimental colitis. Furthermore, our data provide evidence that GW274150 reduces the degree of oxidative stress and of PARS activation during dinitrobenzene sulfonic acid-induced colitis. Thus, we demonstrate here that the mechanisms underlying the protective effects of GW274150 are dependent by a reduction of (i) iNOS activity and the nitration of proteins by peroxynitrite, (ii) the PARP activation, and (iii) inflammatory cell infiltration.

References


