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Celecoxib, a selective cyclo-oxygenase-2 inhibitor reduces the severity of experimental colitis induced by dinitrobenzene sulfonic acid in rats

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Abstract

Inflammatory bowel disease is characterised by oxidative and nitrosative stress, leukocyte infiltration, upregulation of the expression of intercellular adhesion molecule 1 (ICAM-1) and upregulation of P-selectin in the colon. Here, we investigate the effects of the selective cyclo-oxygenase-2 inhibitor, celecoxib, in rats subjected to experimental colitis. Colitis was induced in rats by intracolonic instillation of dinitrobenzene sulfonic acid (DNBS). Rats experienced hemorrhagic diarrhoea and weight loss. At 4 days after administration of DNBS, the mucosa of the colon exhibited large areas of necrosis. Neutrophil infiltration (determined by histology, as well as an increase in myeloperoxidase activity in the mucosa) was associated with upregulation of ICAM-1 and P-selectin, as well as high tissue levels of malondialdehyde. Immunohistochemistry for nitrotyrosine and poly(ADP-ribose) polymerase showed intense staining in the inflamed colon. Celecoxib (5 mg/kg twice a day orally) significantly reduced the degree of hemorrhagic diarrhoea and the weight loss caused by administration of DNBS. Celecoxib also caused a substantial reduction of (i) the degree of colonic injury, (ii) the rise in myeloperoxidase activity (mucosa), (iii) the increase in the tissue levels of malondialdehyde, (iv) the increase in staining (immunohistochemistry) for nitrotyrosine, as well as (v) the upregulation of ICAM-1 and P-selectin caused by DNBS in the colon. Thus, we provide the first evidence that a selective cyclo-oxygenase-2 inhibitor celecoxib reduces the degree of colitis caused by DNBS.

Keywords: DNBS (Dinitrobenzene sulfonic acid); Inflammation; Nitric oxide (NO); Peroxynitrite; Poly(ADP-ribose) polymerase; Colon damage; Free radicals; Cyclo-oxygenase-2

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit prostanoid generation and their effects vary in different tissues and organs. In the upper gastrointestinal tract, prostanoids are essential for the maintenance of mucosal integrity and, therefore, inhibition of their generation induced by NSAIDs results in gastrointestinal bleeding and ulceration (Whittle et al., 1986). The function of the two cyclooxygenase isoforms, cyclooxygenase-1 and cyclooxygenase-2 in maintaining mucosal homeostasis and modulating inflammation in the digestive tract remains uncertain. Colonic prostanoid generation is increased in both experimental colitis and in inflammatory bowel disease (Sharon et al., 1978; Rachmilewitz et al., 1989) and correlates well with disease activity (Sharon et al., 1978; Rachmilewitz et al., 1989; Allgayer et al., 1989). Therefore, prostaglandins also play a protective role against gastrointestinal injury (Hoult and Moore, 1978) and downregulation of the expression of proinflammatory cytokines (Knudsen et al., 1986; Marcinkiewicz, 1991). Indeed, experimental colitis can be attenuated by pretreatment with exogenous prostaglandins (Allgayer et al., 1989; Fedorak et al., 1990), and prostaglandin E_2 plays a major role in the regeneration of the epithelial crypts after dextran sodium sulfate (DSS)- and radiation-induced intestinal damage in mice (Cohn et al., 1997). Furthermore, inhibition of prostaglandin synthesis by indomethacin induces acute and chronic enterocolitis in genetically susceptible

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rats (Yamada et al., 1993), which is consistent with the gastrointestinal ulceration seen in patients treated with nonsteroidal anti-inflammatory drugs (NSAIDs) (Kaufmann and Taubin, 1987; Bjarnson et al., 1993). This effect is attributed to the inhibition of constitutive mucosal prostaglandins, which have cytoprotective properties (Wallace et al., 1992). The relative contribution of cyclooxygenase-1 and cyclooxygenase-2 isoforms to the biological actions of prostaglandins in the gastrointestinal mucosa is less clear. Several observations led to the hypothesis that inducible cyclooxygenase-2 drives the proinflammatory actions of prostaglandins during mucosal injury, whereas cyclooxygenase-1 regulates gastrointestinal homeostasis through the synthesis of cytoprotective prostaglandins (Masferrer et al., 1996). In mice, cyclooxygenase-1 has been reported to play a protective role against small intestine (Cohn et al., 1997) and colon (Tessner et al., 1998) mucosal injury through the synthesis of prostaglandins that promote epithelial regeneration. In contrast, cyclooxygenase-2 expression is induced during inflammation (Seibert et al., 1994), and its expression is inhibited by endogenous glucocorticoids (O'Banion et al., 1991). Based on these observations, some researchers have attributed the anti-inflammatory action of NSAIDs to the inhibition of cyclooxygenase-2, and the harmful effects of NSAIDs on the gastrointestinal mucosa are attributed to the blockade of cyclooxygenase-1 activity (Xie et al., 1992; Chan et al., 1995). This association of cyclooxygenase-2 with inflammatory events led to the development of selective cyclooxygenase-2 inhibitors expected to display systemic anti-inflammatory properties, while avoiding gastrointestinal toxicity. Recently, a clinical trial in patients with osteoarthritis emphasized the safety of the selective cyclooxygenase-2 inhibitor celecoxib, which caused significantly less gastroduodenal ulceration than did the NSAIDs (Goldstein et al., 2000). However, cyclooxygenase-2 inhibitors have also been shown to be harmful when there is preexisting gastrointestinal inflammation because they delay gastric ulcer healing in mice (Mizuno et al., 1997) and exacerbate colon mucosal inflammation in rats (Reuter et al., 1996). In addition, it has been recently demonstrated that some cyclooxygenase-2 inhibitor did not exerts any beneficial effect in an experimental model of colitis induced by tinitrobenzene sulfonic acid (TNBS) (Lesch et al., 1999) and that cyclooxygenase-2 derived prostaglandin D2 is an early anti-inflammatory signal in experimental colitis.

The role of NSAIDs in the possible modulation of colon inflammation is controversial and remain uncertain.

In this study, we have investigated the effect of celecoxib, a new cyclooxygenase-2 selective inhibitor, on the inflammatory response (colitis) caused by intracolonic administration of DNBS. In particular, we investigated the effects of celecoxib on the colon injury associated with DNBS-induced colitis. In order to gain a better insight into the mechanism of action of celecoxib, we have determined the following endpoints of the inflammatory response: (1) polymorphonucleates infiltration, (2) peroxynitrite formation (immunohistochemistry), (3) activation of the nuclear enzyme poly(ADP-ribose) polymerase, (4) expression of cyclooxygenase-2 protein (immunohistochemistry) and activity, (5) lipid peroxidation and (6) colon injury.

2. Materials and methods

2.1. 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 the protection of animals used for experimental and other scientific purposes (D.M. 116192), as well as with EEC regulations (O.J. of E.C. L 358/1 12/18/1986).

2.2. Experimental groups

Celecoxib was given twice a day as an oral bolus (5 mg/kg, DNBS + celecoxib group; $n = 10$), the first dose was given immediately after damage induction. In a vehicle-treated group of rats, vehicle was given instead of celecoxib (DNBS group; $n = 10$). A separate groups of rats (sham-colitis group; $n = 10$) received an enema with vehicle alone (50% ethanol, 0.8 ml). In an additional group of animals, sham-colitis was combined with the administration of celecoxib (dose as above) (Sham-colitis + celecoxib; $n = 10$).

2.3. Induction of experimental colitis

Colitis was induced by using a technique of acid-induced inflammation of the colon as described previously (Fries et al., 1999). Fasted rats were lightly anesthetised with isoflurane, a 3.5-F catheter was inserted into the colon via the anus up to the splenic flexure (8 cm from the anus). 2,4,6-dinitrobenzene sulfonic acid (DNBS; 25 mg/rat) was dissolved in 50% ethanol (total volume, 0.8 ml). Thereafter, the animals were kept for 15 min in a Trendelenburg position to avoid reflux. After induction of colitis or sham-colitis, the animals were observed for 3 days. On Day 4, the animals were weighed and anesthetised with chloral hydrate (400 mg/kg, i.p.), and the abdomen was opened by a midline incision. The descending colon was removed, freed from surrounding tissues, opened along the anti-mesenteric border, rinsed, weighed and processed for histology and immunohistochemistry. The macroscopic damage score, according to Wallace et al. (1992), was assessed. In an additional experiment, colitis and sham-colitis were induced in 28 rats (7 ani-

mals/group). The animals were monitored for evaluation of mortality for 7 days.

2.4. Light microscopy

After fixation for 1 week at room temperature in Dietrich solution (14.25% ethanol, 1.85% formaldehyde, 1% acetic acid), the samples were dehydrated in graded ethanol and embedded in Paraplast (Sigma, Milan, Italy). Thereafter, 7-μm sections were deparaffinized with xylene, stained with hematoxylin–eosin and trichromic van Gieson's stain, and observed in a Dialux 22 Leitz (Wetzlar, Germany) microscope.

2.5. Localisation of nitrotyrosine, poly(ADP-ribose) synthase, P-selectin, intercellular adhesion molecules 1 (ICAM-1) by immunofluorescence

Tissues were fixed in 10% buffered formalin and sections were permeabilized with 0.1% Triton X-100 in phosphate-buffered saline for 20 min. Sections were coincubated overnight with (1) anti-nitrotyrosine rabbit polyclonal antibody (1:500 in phosphate-buffered saline, v/v) or with anti-poly(ADP-ribose) goat polyclonal antibody rat (1:500 in phosphate-buffered saline (PBS), v/v) or (2) with rabbit anti-human polyclonal antibody directed at P-Selectin (CD62P) which react with rat and with mouse anti-rat antibody directed at ICAM-1 (CD54) (1:500 in PBS, v/v) (DBA, Milan, Italy) . Sections were washed with PBS and coincubated with secondary antibody (TRITC-conjugated anti-goat and with FITC-conjugated anti-rabbit (Jackson, West Grove, PA) antibody (1:80 in PBS, v/v) for 2 h at RT. Sections were washed as before, mounted with 90% glycerol in PBS and observed with a Nikon RCM8000 confocal microscope equipped with a 40× oil objective.

2.6. Myeloperoxidase activity

Myeloperoxidase activity, an indicator of polymorphonuclear leukocyte accumulation, was determined as previously described (Mullane et al., 1985). At 4 days after intracolonic injection of DNBS, 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 × *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. Myloperoxydase activity was defined as the quantity of enzyme degrading 1 μmol of peroxide min^{-1} at 37 °C and was expressed in milliunits per gram weight of wet tissue.

2.7. Malondialdehyde measurement

The levels of malondialdehyde in the colon were determined as an indicator of lipid peroxidation (Ohkawa et al., 1979). At 4 days after intracolonic injection, the colon was removed, weighed and homogenised in 1.15% KCl solution. An aliquot (100 μl) of the homogenate was added to a reaction mixture containing 200 μl of 8.1% sodium dodecyl sulphate, 1500 μl of 20% acetic acid (pH 3.5), 1500 μl of 0.8% thiobarbituric acid and 700 μl distilled water. The 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 650 nm.

2.8. Measurement of cytokines

The levels of tumour necrosis factor-α (TNF-α) and interleukin-1β were evaluated in the colon 4 days after intracolonic injection of DNBS. The assay was carried out by using a commercial colorimetric kit (Calbiochem-Novabiochem, Milan, Italy).

2.9. Assessment of cyclooxygenase activity

Colon tissues were removed at 4 days after the induction of colitis by DNBS injection. The material was homogenised at 4 °C in a buffer containing the following protease inhibitors (HEPES 20 mM, pH 7.2 + saccarosio 320 mM, DTT 1 mM, STY 10 ug/ml, aprotinin 2 ug/ml, leupeptin 10 ug/ml: in ration of 5:1 (v/w). The protein concentration in the homogenates was measured by the Bradford assay (Bradford, 1976), with bovine serum albumin used as standard. Homogenates were incubated at 37 °C for 30 min in the presence of excess arachidonic acid (30 μM). The samples were boiled and centrifuged at

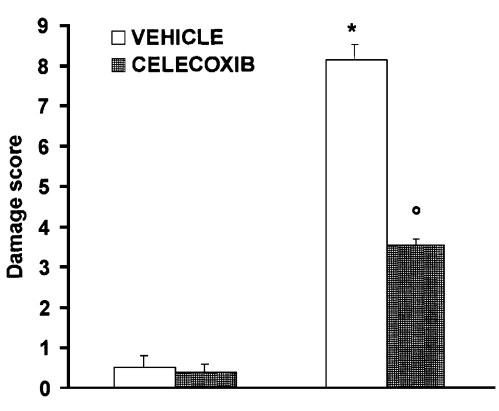

Fig. 1. Effect of celecoxib treatment on the damage score. Colonic damage was scored on a 0 (normal) to 10 (severe) scale by two independent observers. Values are means ± S.E.M. for 10 rats from each group. $^{*}P < 0.01$ vs. sham; $^{\circ}P < 0.01$ vs. DNBS.

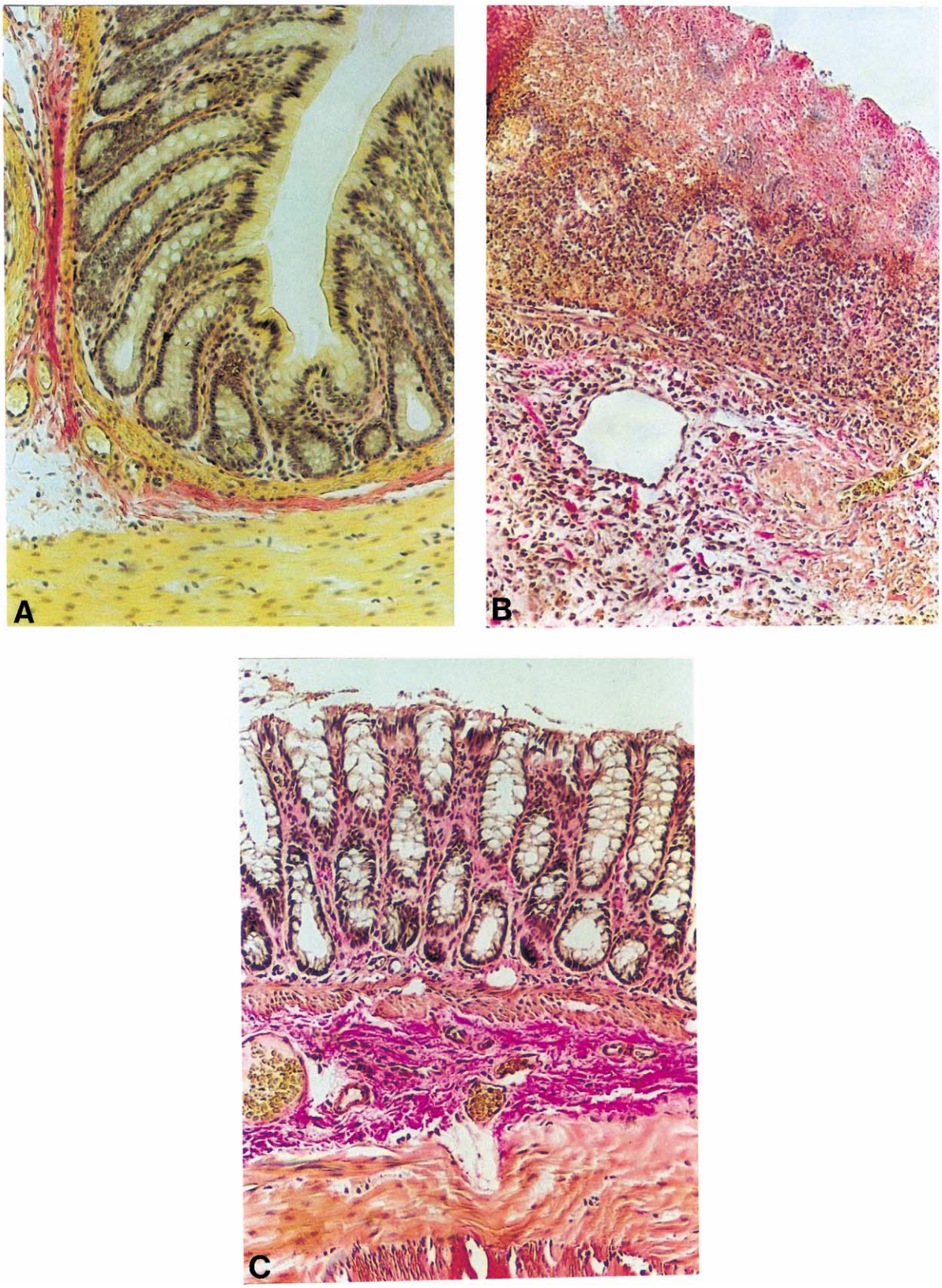

Fig. 2. Effect of celecoxib on colon injury. No histological modification was present in the control animals (A). Mucosal injury was produced after DNBS administration, characterized by necrosis of epithelium and a massive mucosal and submucosal infiltration of inflammatory cells (B). Treatment with celecoxib (C) reduced the morphological alteration associated with DNBS administration. Original magnification: 150 ×. Figure is representative of at least three experiments performed on different experimental days.

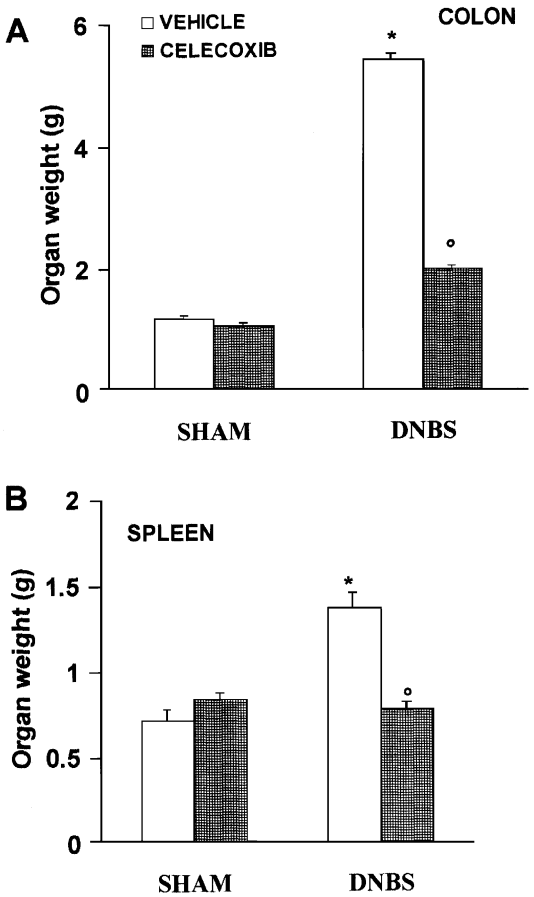

Fig. 3. Organ weight. A significant increase was consistently seen at 4 days after DNBS injection in spleen (A) and colon (B). The weight of the organs was significantly reduced in the rats treated with celecoxib. Values are means ± S.E.M. for 10 rats from each group. * $P < 0.01$ vs. sham; ° $P < 0.01$ vs. DNBS.

10,000 × *g* for 5 min. The concentration of 6-keto-prostaglandin $F_{1\alpha}$ present in the supernatant was measured by radioimmunoassay as previously described (Cuzzocrea et al., 1999).

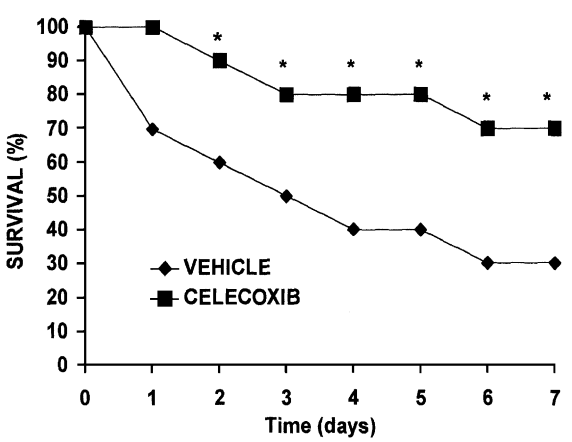

Fig. 4. Effect of celecoxib treatment on DNBS-induced mortality. Survival was significantly improved in celecoxib-treated rats in comparison to the high mortality rate of the DNBS-treated rat. $n = 10$ rats for each group. * $P < 0.01$ vs. DNBS.

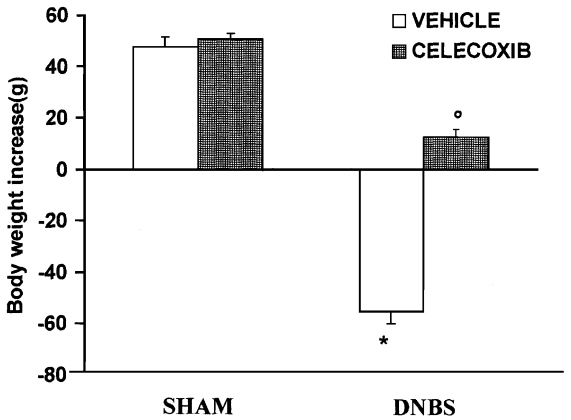

Fig. 5. Effect of celecoxib treatment on body weight changes 4 days after DNBS intracolonic administration. Body weight was recorded immediately before DNBS administration and at the end of the experimental period. celecoxib treatment significantly prevented the decrease in body weight. Values are means ± S.E.M. for 10 rats from each group. * $P < 0.01$ vs. sham; ° $P < 0.01$ vs. DNBS.

2.10. Materials

Biotin blocking kit, biotin-conjugated goat anti-rabbit immunoglobulin G and avidin–biotin peroxidase complex were obtained from Vector Laboratories (Burlingame, CA). Primary anti-nitrotyrosine antibody was purchased from Upstate Biotech (Saranac Lake, NY). Primary antibodies for P-selectin (CD62P) and ICAM-1 (CD54) were purchased from Pharmingen (DBA). All other reagents and compounds used were purchased from Sigma (St. Louis, MO).

2.11. Data analysis

All values in the figures and text are expressed as means ± S.E.M. of *n* observations. For the in vivo studies,

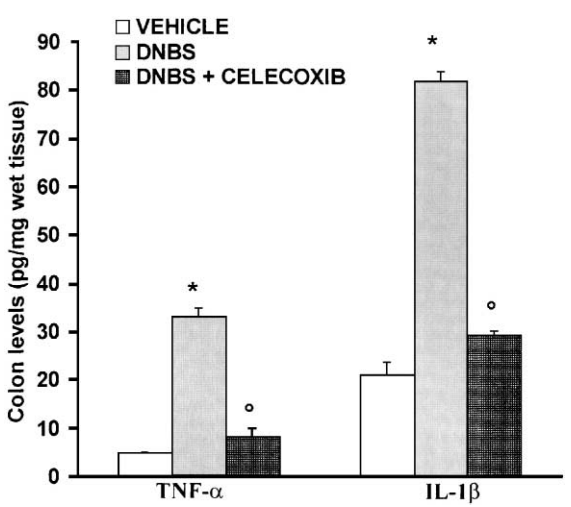

Fig. 6. Colon levels of TNFα (A) and IL1β (B). Cytokine levels were significantly reduced in the colon from celecoxib-treated rats. Values are means ± S.E.M. for 10 rats from each group. * $P < 0.01$ vs. sham; ° $P < 0.01$ vs. DNBS.

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 days. The results were analysed by one-way analysis of variance followed by a Bonferroni post hoc test for multiple comparisons. A P value less than 0.05 was considered significant.

3. Results

3.1. Effects of celecoxib on the degree of colitis (histology)

Four days after intracolonic administration of DNBS, the colon appeared flaccid and filled with liquid stool. The cecum, colon and rectum showed evidence of mucosal

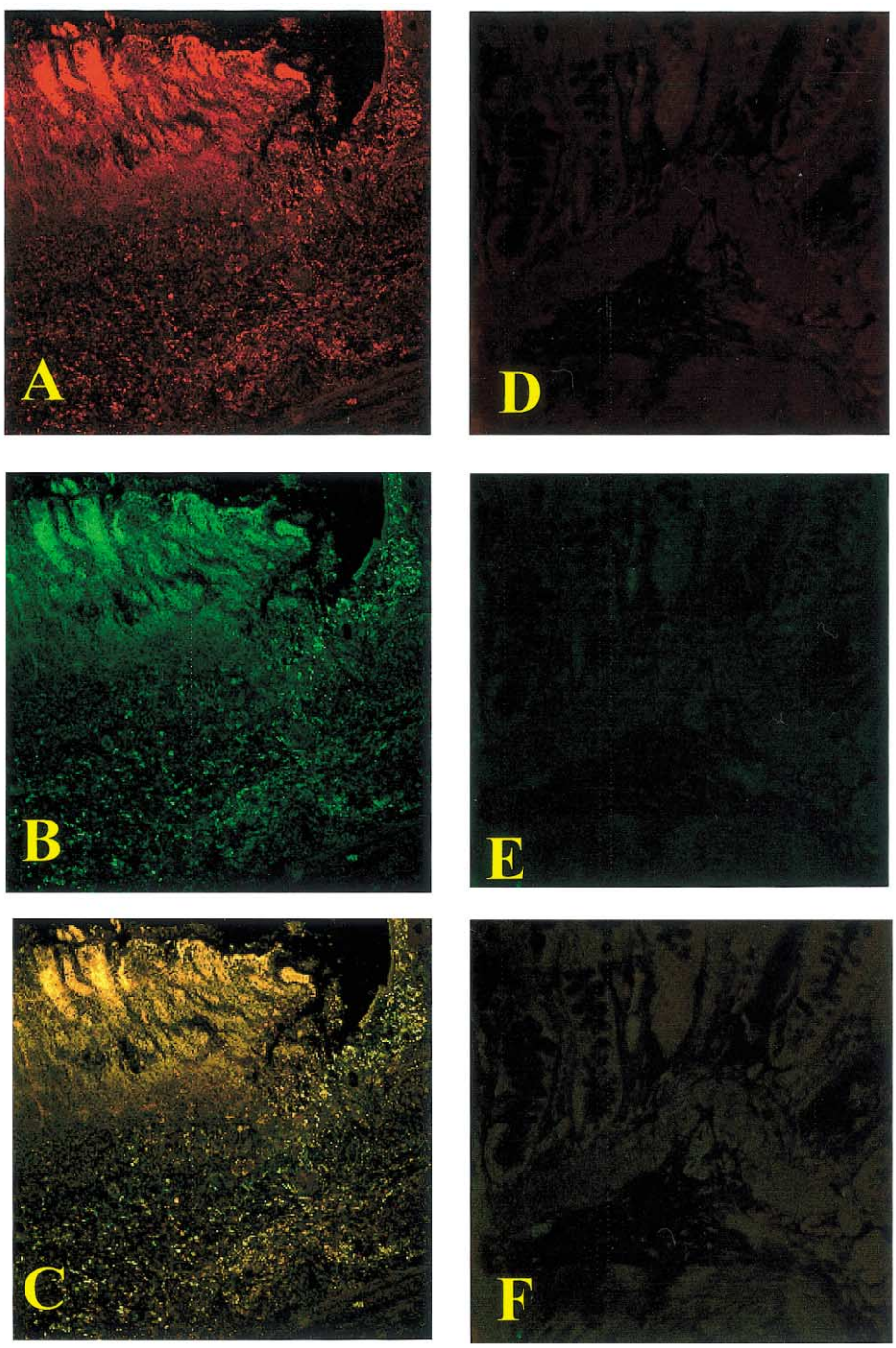

Fig. 7. Immunohistochemical localisation for nitrotyrosine and for PARP in the colon. Immunohistochemical determination for nitrotyrosine (A) and for PARP (B) show positive staining localised in the injured area from a DNBS-treated rat. The intensity of the positive staining for nitrotyrosine (D) and for PARP (E) was significantly reduced in the ileum from celecoxib-treated rats. (C) and (F) represent the staining combination of panels (A)–(B) and (D)–(E), respectively. Original magnification: ×125. Figure is representative of at least three experiments performed on different days.

congestion, erosion and hemorrhagic ulcerations (see Fig. 1 for damage score). The histopathological features included necrosis and edema and diffuse inflammatory cells infiltration in the mucosa (Fig. 2B). The inflammatory changes of the intestinal tract were associated with an increase in the weight of the colon (Fig. 3B). Treatment of rats with celecoxib significantly attenuated the extent and severity of the histological signs of colonic injury (Figs. 2B and 3B). A significant increase in the weight of the spleen, an indicator of inflammation, was also noted in vehicle-treated rats, which had received DNBS (Fig. 3A). No significant increase in weight of either colon or spleen was observed in DNBS-treated rats, which had been treated with celecoxib (Fig. 3). Survival of animals was monitored for 7 days. DNBS-treated rats, which had received vehicle, developed severe hemorrhagic diarrhoea, and 50% and 80% of these animals died within 2 and 6 days, respectively, after DNBS administration. In contrast, only 20% of the rats treated with celecoxib had hemorrhagic diarrhoea and died (Fig. 4). The surviving rats appeared healthy and showed very mild diarrhoea.

3.2. Effects of celecoxib on changes of body weight

In vehicle-treated rats, the severe colitis caused by DBNS was associated with a significant loss in body weight (Fig. 5). Treatment of DNBS-treated rats with celecoxib significantly prevented the loss in body weight.

3.3. Effect of celecoxib on cytokines production

The levels of TNF-α and interleukin-1β were significantly elevated in the colon at 4 days after DNBS treatment. In contrast, the levels of these cytokines were significantly lower in rats treated with celecoxib (Fig. 6). No significant increase in the levels of cytokines was observed in the colon of sham-operated rats.

3.4. Effect of celecoxib on nitrotyrosine formation and poly(ADP-ribose) polymerase activity

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. At 4 days after DNBS treatment, sections of the colon were taken in order to determine the immunohistological staining for poly(ADP-ribose) polymerase. Colon sections obtained from vehicle-treated DNBS-treated rats exhibited positive staining for nitrotyrosine and poly(ADP-ribose) polymerase (Fig. 7A and B) which was colocalised in inflammatory cells and in disrupted epithelial cells. Celecoxib reduced the degree of immunostaining for nitrotyrosine and poly(ADP-ribose) polymerase in the colon of DNBS-treated rats (Fig. 7D and E).

3.5. Effect of celecoxib on myeloperoxidase activity and lipid peroxidation in the colon

The colitis caused by DNBS was also characterised by an increase in myeloperoxidase activity, an indicator of the infiltration (accumulation) of the colon with polymorphonuclear neutrophils (Fig. 8A). This finding is consistent with the light microscopy observation that the colon of vehicle-treated DNBS-treated rats contained a large number of polymorphonuclear neutrophils. Infiltration of leukocytes into the mucosa has been suggested to contribute significantly to the tissue necrosis and mucosal dysfunction associated with colitis, as activated polymorphonuclear neutrophils 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 (Fig. 8B). Treatment of DNBS-treated rats with cele-

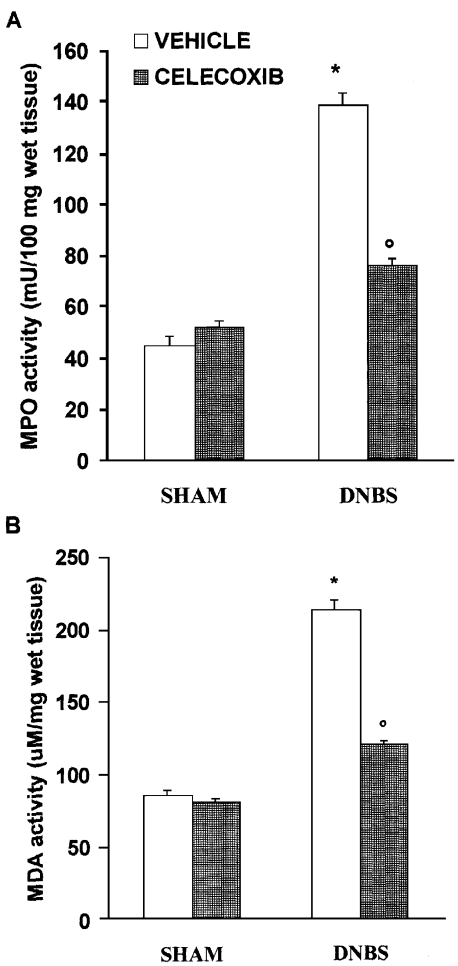

Fig. 8. Effect of celecoxib on neutrophil infiltration and lipid peroxidation. Myeloperoxidase activity (A) and malondialdehyde (B) in the colon from DNBS-treated rats. Myeloperoxidase activity and malondialdehyde levels were significantly increased in DNBS-treated rats in comparison to sham. Celecoxib-treated rats show a significant reduction of Myeloperoxidase activity and malondialdehyde levels. Values are means ± S.E.M. for 10 rats from each group. * $P < 0.01$ vs. sham; ° $P < 0.01$ vs. DNBS.

coxib, however, significantly reduced both the degree of polymorphonuclear neutrophils infiltration (determined as increase in myloperoxidase activity) and the associated lipid peroxidation (increase in tissue malondialdehyde levels) (Fig. 8).

3.6. Effect of celecoxib on P-selectin and ICAM-1 expression

To further elucidate the effect of celecoxib on neutrophil accumulation in inflamed colon, we evaluated the

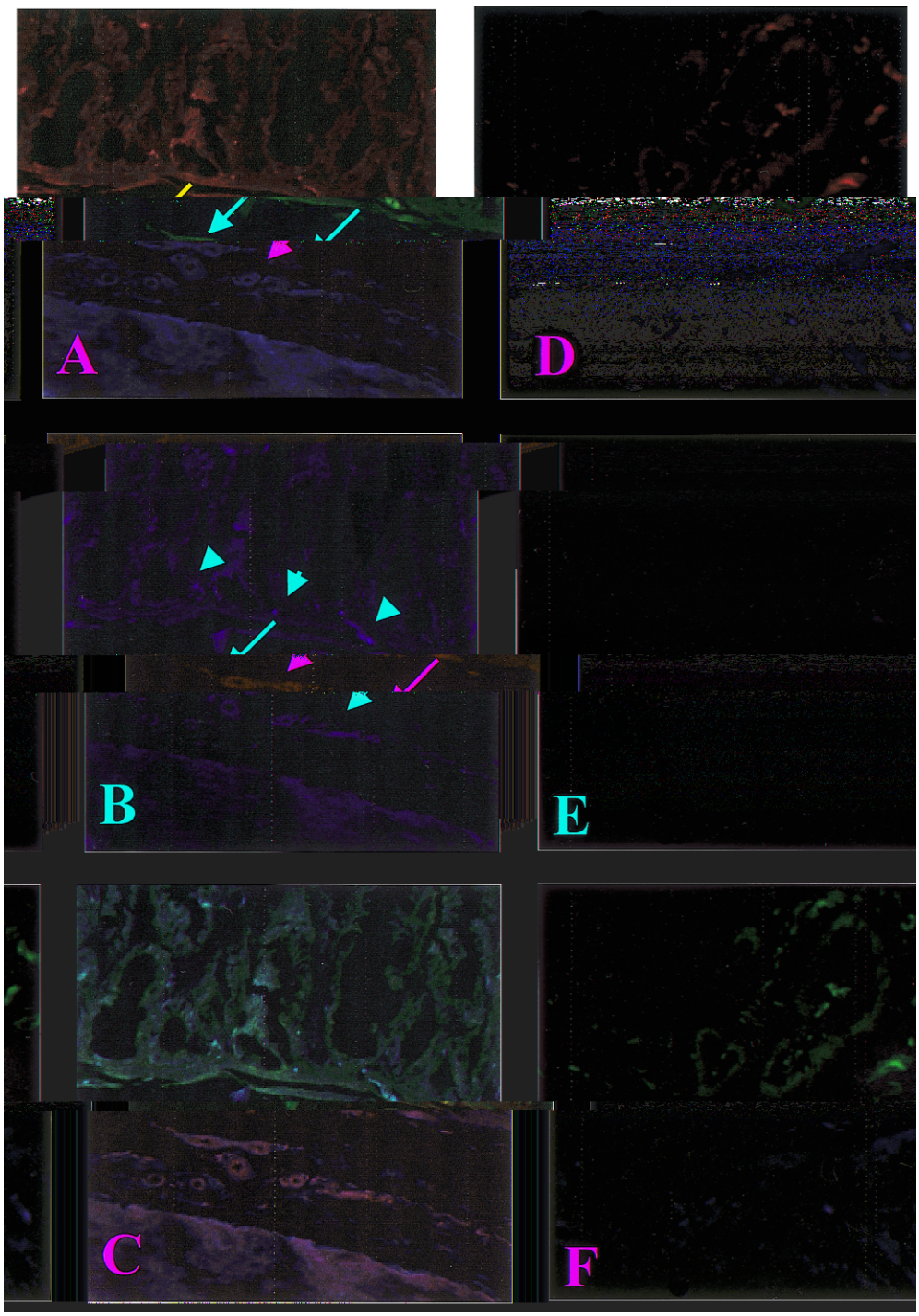

Fig. 9. Immunohistochemical localisation of P-selectin in the colon. Section obtained from DNBS-treated rats showed intense positive staining for ICAM-1 (A) and for P-selectin (B) on the vessels. The degree of vessel staining for ICAM-1 (D) and for P-selectin (E) was markedly reduced in tissue sections obtained from celecoxib-treated rats. (C) and (F) represent the staining combination of panels (A)–(B) and (D)–(E), respectively. Original magnification: ×150. Figure is representative of at least three experiments performed on different experimental days.

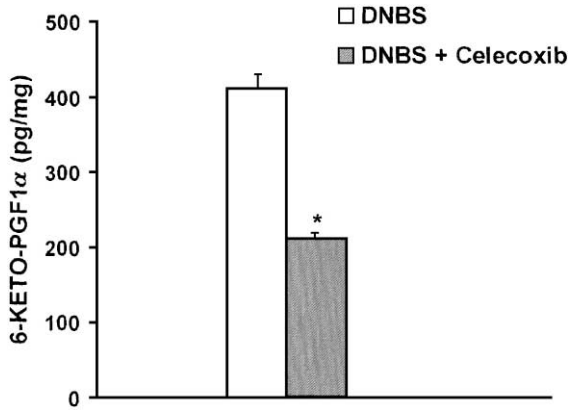

Fig. 10. Effect of celecoxib on cyclooxygenase activity. 6-keto-$PGF_{1\alpha}$ was significantly increased in the colons from DNBS-treated rats. The amounts of 6-keto-$PGF_{1\alpha}$ were significantly reduced in rats treated with celecoxib. Values are means ± S.E.M. for 10 rats from each group. $^{*}P < 0.01$ vs. sham; $^{\circ}P < 0.01$ vs. DNBS.

intestinal expression of ICAM-1 and P-selectin. Immunohistochemical staining for ICAM-1 was present in the vessels of the submucosa (see arrows) in damaged tissues from DNBS-treated rats (Fig. 9A). Sections from celecoxib-treated rats showed no upregulation of the constitutive ICAM-1, which was normally expressed in the endothelium along the vascular wall (Fig. 9D). Tissue sections of the colon from DNBS-treated rats showed positive staining for P-selectin localised in the vascular endothelium (see arrows) and in inflammatory cells (arrowhead) (Fig. 9B). In tissue from celecoxib-treated rats, no expression of P-selectin (Fig. 9E) was found. As can be seen in Fig. 9C, the positive staining for P-selectin and for ICAM-1 were colocalised in the in the endothelium.

3.7. Effect of celecoxib on cyclooxygenase activity

Cyclooxygenase activity in DNBS-induced colitis was assessed by measuring the increase in the formation of 6-keto-prostaglandin $F_{1\alpha}$ in the colon from DNBS-treated rats. In colons from DNBS-treated rats, the amount of 6-keto-prostaglandin $F_{1\alpha}$ was 412 ± 13 pg/mg/tissue (Fig. 10). The amount of 6-keto-prostaglandin $F_{1\alpha}$ was significantly reduced in the colons from DNBS-treated rats pretreated with celecoxib (Fig. 10).

4. Discussion

Our data show that rats treated with celecoxib, a new cyclooxygenase-2 selective inhibitor, are significantly more resistant to lethality and pathological changes in the colon and rectum associated with DNBS-induced colitis.

Prostanoid generation was found to be enhanced in the colonic mucosa of patients with ulcerative colitis and Crohn's disease (Sharon et al., 1978). In view of the role of prostanoids as mediators of symptoms associated with inflammation, trials to modulate inflammatory bowel disease with NSAIDs were conducted, all without success (Gibson et al., 1992; Kaufmann and Taubin, 1987; Jozeau et al., 1997). Obviously, the NSAIDs used in these trials inhibited cyclooxygenase activity but were not selective towards cyclooxygenase-1 or cyclooxygenase-2. The results was reported indicate that celecoxib, a selective cyclooxygenase-2 inhibitor, is effective to modulate the extent of experimental colitis and, therefore, may also be beneficial in the treatment of inflammatory bowel disease. Moreover, celecoxib, as well as other selective inhibitors of cyclooxygenase-2 activity, induces to a lesser extent the NSAID-related gastric and renal side-effects which are due to inhibition of the constitutive isoform, cyclooxygenase-1 (Marini and Spotti, 1993; Dreiser and Benvelli, 1993). It is important to note that, in the present study, celecoxib did not induce any gastric mucosal insult. Determination of colon 6-keto-$PGF_{1\alpha}$ activity, as we now performed, represents cyclooxygenase activity. Therefore, the significant reduction in activity of colon 6-keto-$PGF_{1\alpha}$ by celecoxib treatment confirms the important role of cyclooxygenase-2 in colon inflammation. This observation is different from the claim that cyclooxygenase-1 makes an important contribution to inflammatory responses (Wallace et al., 1998).

The pathogenesis of colitis was also associated with increased TNF-α and interleukin-1β, since these cytokines are present in colon tissues and can be detected immunohistochemically in the inflamed tissues (Carty et al., 2000; Negoro et al., 1999). Recently, it has been demonstrated in both animal models of experimental colitis and in patients with inflammatory bowel disease or with Crohn's disease that blocking of the action of these cytokines delays the onset, suppresses inflammation, and reduced colon destruction that corresponds to the anti-inflammatory response (Andborn and Hanauer, 1999; Urthy et al., 1999; Present et al., 1999; Targan et al., 1997; Ricart et al., 1999; D'Haens et al., 1999; Moreland et al., 1996). In the present study, the increase in TNF-α and interleukin-1β in the colon was reduced by celecoxib. This beneficial effect observed with this new selective cyclooxygenase-2 inhibitor is consistent with results of recent studies showing that Nimesulide significantly reduces the levels of these two pro-inflammatory cytokines (Karmeli et al., 2000). In addition, treatment with celecoxib markedly reduced (1) the cellular infiltrate in the colon and (2) the inflammation of colon tissue. The ability of cyclooxygenase inhibitors to partially reduce the inflammatory cell infiltrate in the colon could in part explain the observed reduction in the levels of cyclooxygenase-2 and cytokine production. In addition we have observed that celecoxib significantly reduced P-selectin and ICAM-I in the colon from DNBS-treated rats.

Neutrophils have been considered to play a crucial role in the development and full manifestation of gastrointestinal inflammation, as they represent a major source of free radicals in the inflamed colonic mucosa (Grisham, 1994; Shiratora et al., 1989).

Colon inflammation is usually characterised by extensive infiltration of colon tissue by polymorphonuclear leukocytes, which is more marked in bronchoalveolar lavage fluid during acute, infectious exacerbations. Neutrophil activation represents an important source of reactive oxygen and nitrogen species. It has been proposed that reactive oxygen and nitrogen species play a key role in inflammatory bowel disease (Grisham, 1994). These species are cytotoxic agents, inducing lipid peroxidation and other cellular oxidative stress by cross-linking proteins, lipids and nucleic acids, which then cause cellular dysfunction, damage and eventually death. Evidence consistent with damage by reactive radical species is provided by the increase in lipid peroxides in rectal biopsy specimens from patients with ulcerative colitis (Grisham, 1994; Simmonds et al., 1992; McKenzie et al., 1996). In the present study, we found that the mucosal damage induced by intracolonic administration of DNBS was associated with high concentrations of malondialdehyde, which is considered a good indicator of lipid peroxidation (Ohkawa et al., 1979).

Recent evidence indicates that nitration of tyrosine can result from a number of chemical actions, and can be considered as a global marker of nitrosative stress (Halliwell, 1997). Nitrotyrosine can be formed from the reaction of nitrite with hypochlorous acid or the reaction of nitrite with myeloperoxidase and hydrogen peroxide (Eiserich et al., 1998). In our experiments, we found an increased 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. In the present, study we observed that epithelial disruption was significantly less in rats treated with celecoxib. Indeed, celecoxib treatment prevented the formation of tissue malondialdehyde and nitrotyrosine staining in DNBS treated animals. Furthermore, celecoxib-treated rats are more resistant to DNBS induced lethal disease with a significant resolution of the macroscopic and histological signs of the inflammatory process. Superoxide and peroxynitrite can also cause DNA single-strand damage that is the obligatory trigger for poly(ADP-ribose) polymerase activation (Szabo and Dawson, 1998) resulting in the depletion of its substrate NAD^+ in vitro and a reduction in the rate of glycolysis. Since NAD^+ functions as a cofactor in glycolysis and the tricarboxylic acid cycle, NAD^+ depletion leads to a rapid fall in intracellular ATP and, ultimately, cell injury (Szabo and Dawson, 1998). Furthermore, substantial evidence supports the fact that poly(ADP-ribose) polymerase activation is important in inflammatory bowel disease as shown by the use of poly(ADP-ribose) polymerase inhibitors such as nicotinamide and 3-aminobenzamide (Zingarelli et al., 1999; Szabo et al., 1997). As shown in Fig. 5 celecoxib reduced poly(ADP-ribose) polymerase immunofluorescence. In view of the role of poly(ADP-ribose) polymerase in inflammatory bowel disease, it is possible that poly(ADP-ribose) polymerase inhibition by celecoxib accounts for its beneficial effect.

Together, our data demonstrate that celecoxib exert a beneficial effect in an experimental model of colitis induced by DNBS in the rat. These results are at variance with results of other recent studies demonstrating a lack of effect of different cyclooxygenase-2 inhibitors or a protective effect of a cyclooxygenase-2-derived prostaglandin D_2 (Lesch et al., 1999; Ajuebor et al., 2000). However, in the present study, we observed that the treatment with celecoxib reduced the oxidative stress associated with experimental colitis. Although it is not clear at this time whether the protection by celecoxib is primarily due to inhibition of cyclooxygenase-2 or to some associated mechanism(s), such as inhibition of oxidative stress. Confirmation of this hypothesis needs further investigation. The protective role played by natural antioxidants in diets, such as green tea (Challa et al., 1997; Narisawa and Fukaura, 1993; Tsujii et al., 1997), flavonoids (Duthie and Dobson, 1999) or by natural cyclooxygenase-2 inhibitors such as curcumin (Zhang et al., 1999), berberine (Fukuda et al., 1999) or resveratrol (Chanvitayapongs et al., 1997), may possibly be understood in this respect. In conclusion, our results demonstrate that celecoxib, a selective cyclooxygenase-2 inhibitor, is protective in experimental colitis. The anti-inflammatory effects of celecoxib are associated with a reduction of (i) the upregulation of P-selectin and ICAM-1 leading to attenuation of the recruitment of neutrophils, (ii) lipid peroxidation, (iii) peroxynitrite formation, (iv) poly(ADP-ribose) polymerase activation and (v) ultimately tissue injury.

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