ELSEVIER

Biochemical Pharmacology 64 (2002) 327–337

Biochemical Pharmacology

GPI 6150, a PARP inhibitor, reduces the colon injury caused by dinitrobenzene sulfonic acid in the rat

Emanuela Mazzon[a], Laura Dugo[b], Jia-He Li[c], Rosanna Di Paola[b], Tiziana Genovese[b], Achille P. Caputi[b], Jie Zhang[c], Salvatore Cuzzocrea[b,*]

[a]*Department of Biomorphology, School of Medicine, University of Messina, Messina, Italy*
[b]*Institute of Pharmacology, School of Medicine, University of Messina, Torre Biologica-Policlinico Universitario Via C. Valeria-Gazzi, 98100 Messina, Italy*
[c]*Guilford Pharmaceuticals Inc., Baltimore, MD 21224, USA*

Received 28 January 2002; accepted 18 April 2002

Abstract

Poly (ADP-ribose) polymerase, a nuclear enzyme activated by DNA strand breaks, has been shown to play an important role in the pathogenesis of inflammatory bowel disease. Here we investigate the effects of 1,11b-dihydro-[2H]benzopyrano [4,3,2-*de*]isoquinolin-3-one (GPI 6150), a new poly (ADP-ribose) polymerase inhibitor, in animal models of experimental colitis. Colitis was induced in rats by intra-colonic instillation of dinitrobenzene sulfonic acid. Rats experienced hemorrhagic diarrhea and weight loss. At 4 days after administration of dinitrobenzensulfonic acid, the mucosa of the colon exhibited large areas of necrosis. Neutrophil infiltration (determined by histology and an increase in myeloperoxidase activity in the mucosa) was associated with up-regulation of ICAM-1. Immunohistochemistry for poly (ADP-ribose) showed an intense staining in the inflamed colon. GPI 6150 (20 or 40 mg/kg daily, i.p.) significantly reduced the degree of hemorrhagic diarrhea and weight loss caused by administration of dinitrobenzensulfonic acid. GPI 6150 also caused a substantial reduction of (i) the degree of colon injury, (ii) the rise in myeloperoxidase activity (mucosa), (iii) the increase in the tissue levels of malondialdehyde, (iv) the increase in staining (immunohistochemistry) for poly (ADP-ribose), as well as (v) the upregulation of ICAM-1 and P-selectin caused by dinitrobenzensulfonic acid in the colon. Thus, GPI 6150 reduces the degree of colitis caused by dinitrobenzensulfonic acid. We propose that GPI 6150 may be useful in the treatment of inflammatory bowel disease.

Keywords: Poly (ADP-ribose) synthethase; GPI 6150; Free radicals; Nitric oxide; Inflammation; Bowel disease

1. Introduction

Reactive oxygen (ROS) and nitrogen species have been implicated as mediators of the disruption of the intestinal barrier in inflammatory bowel diseases [1–3]. In addition to reactive oxygen species, an overproduction of nitric oxide (NO) due to the expression of the inducible isoform of NO synthase (iNOS) also plays an important role in various models of inflammation including colitis [4,5]. Evidence obtained using cultured cells demonstrated that reactive oxygen species produce strand breaks in DNA which triggers energy-consuming DNA repair mechanisms and activates the nuclear enzyme poly (ADP-ribose) polymerase [EC 2.4.2.30, also referred to as poly (ADP-ribose) synthetase (PARP) or poly (ADP-ribose) transferase (pADPRT)] [6]. PARP is an abundant, chromatin-bound enzyme constitutively expressed in numerous cell types [7] which, when activated, catalyses the transfer of ADP-ribose moieties from NAD to nuclear proteins including histones and onto PARP itself (automodification) with the concomitant formation of nicotinamide [8]. However, there is now good evidence that exposure of cells to oxidant stress in the form of superoxide anions, hydrogen peroxide (H_2O_2), and hydroxyl radicals causes strand breaks in DNA leading to an excessive activation of PARP resulting in the depletion of its substrate NAD *in vitro* and a reduction in the rate of glycolysis [9,10]. As NAD functions as a cofactor in glycolysis and the tricarboxylic acid cycle, NAD depletion leads to a rapid fall in intracellular ATP levels [9–13]. Furthermore, nicotinamide formed by PARP

* Corresponding author. Tel.: +39-090-2213644; fax: +39-090-2213300.
E-mail address: salvator@unime.it (S. Cuzzocrea).

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01075-4

activation can be recycled back to NAD *via* a mechanism, which also consumes ATP [12]. Thus, activation of PARP leads to a fall in ATP *via* two different mechanisms leading to cellular dysfunction and ultimately cell death [9–13]. Overall, this process has been termed '*the PARP Suicide Hypothesis*' [14].

Benzamide analogues such as 3-aminobenzamide (3-AB) and nicotinamide (Nic) were first recognized as PARP inhibitors over 20 years ago and have been commonly used for this purpose in both *in vivo* and *in vitro* studies investigating their effects in many pathophysiological conditions including colitis, ischemia–reperfusion injury and inflammation [15–17]. They display higher selectivity for PARP compared to mono-(ADP-ribose) transferase and low toxicity in *in vivo* studies [18,19].

GPI 6150 is a novel PARP inhibitor, which has been shown to be neuroprotective in rat model of cerebral focal ischemia, rat lateral fluid percussion model of traumatic brain injury, cardioprotective in rat model of regional heart ischemia, increase survival in mouse models of septic shock, and reduce tissue damage in streptozotocin-induced diabetes and MPTP-neurotoxicity [20–23]. Therefore, recently we have demonstrated that this new PARP inhibitor exerts an anti-inflammatory effect in different models of acute and chronic inflammation [24].

Here we investigate the effects of GPI 6150 on the inflammatory response (colitis) caused by intra-colonic administration of dinitrobenzensulfonic acid. In particular, we investigate the effects of GPI 6150 on the colon injury associated with dinitrobenzensulfonic acid-induced colitis. In order to gain a better insight into the mechanism of action of GPI 6150, we also investigate the effects of GPI 6150 on tissue polymorphonuclear neutrophils immigration (assessed by the measurement of tissue myeloperoxidase (MPO) activity), ICAM-I expression, cytokines production, and morphological changes in the bowel.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (300–350 g; Charles River) 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).

2.2. Experimental groups

In the treated two groups of animals, GPI 6150 was given daily as an intraperitoneally (i.p.) bolus at 20 or 40 mg/kg (dinitrobenzensulfonic acid + GPI 6150 group). In a vehicle-treated group of rats, vehicle (dimethylsulfoxide) was given instead of GPI 6150 (dinitrobenzensulfonic acid group). In separate groups of rats, surgery was performed in its every aspect identical to the one in the dinitrobenzensulfonic acid group, except that the saline vehicle alone (50% ethanol) was injected instead of dinitrobenzensulfonic acid (Sham group, Sham). In an additional group of animals, Sham-surgery was combined with the administration of GPI 6150 (dose as above; Sham + GPI 6150). Colitis and Sham-colitis were induced in 60 rats (10 animals/group).

2.3. Induction of experimental colitis

Colitis was induced by using a technique of acid-induced colon inflammation as described previously [25]. After fasted rats were lightly anaesthetized with isoflurane, a 3.5 F catheter was inserted into the colon *via* the anus until approximately the splenic flexure (8 cm from the anus) to deliver 2,4,6-dinitrobenzene sulfonic acid (dinitrobenzensulfonic acid, 25 mg/rat) dissolved in 50% ethanol (total volume, 0.8 mL). Thereafter, the animals were kept for 15 min in a Trendelenburg position to avoid reflux. Seven animals (Sham-colitis) received an enema with vehicle alone (50% ethanol, 0.8 mL). After colitis and Sham-colitis induction, the animals were observed for 3 days. On day 4, the animals were weighed and anaesthetized with chloralium hydrate (400 mg/kg, i.p.), and the abdomen was opened by a midline incision. The 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.* [26], was assessed.

2.4. Light microscopy

After fixation for 1 week at room temperature in buffered formaldehyde solution (10% in phosphate buffered saline), samples were dehydrated in graded ethanol, and embedded in Paraplast (Sherwood Medical). Thereafter, 7-mm sections were deparaffinized with xylene, stained with trichromic van Giesson's stain, and observed in a Dialux 22 Leitz (Wetziar) microscope. Colon damage was scored by two independent observers as described previously [25,27,28] according to the following morphological 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 site of inflammation and ulceration extending >1 cm along the length of the colon; and 6–10, one point is added for each centimeter of ulceration beyond an initial 2 cm.

2.5. Immunohistochemical localization of ICAM-1 and PAR

Immunohistochemical staining was performed on 7 mm thick sections of frozen colon tissues. Sections were cut in

with a Slee and London cryostat at −30°, transferred onto clean glass slides, and dried overnight at room temperature. Sections were permeabilized with acetone at −20° for 10 min and rehydrated in PBS (phosphate buffered saline, 150 mM NaCl, 20 mM sodium phosphate pH 7.2) at room temperature for 45 min. Sections were incubated overnight with (1) ICAM-1 (hamster anti-mouse CD54) at a dilution 1:500 in PBS, v/v, or (2) with anti-poly (ADP-ribose) monoclonal antibody (1:500 in PBS, v/v). Sections were washed with PBS, and incubated with secondary antibody for 2 hr at room temperature. Specific labeling was detected with an avidin–biotin peroxidase complex. To verify the binding specificity for ICAM-1 or poly (ADP-ribose) (PAR), some sections were also incubated with only the primary antibody (no secondary) or with only the secondary antibody (no primary). In these situations no positive staining was found in the sections indicating that the immunoreaction was positive in all the experiments carried out.

2.6. MPO activity

MPO activity, an indicator of polymorphonuclear leukocyte (polymorphonuclear neutrophils) accumulation, was determined as previously described [29]. At 4 days after intra-colonic injection of dinitrobenzensulfonic acid, the colon was removed and weighed. The colon was homogenized in a solution containing 0.5% hexa-decyltrimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at 20,000 *g* at 4°. An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl-benzidine (1.6 mM) and 0.1 mM H_2O_2. The rate of change in absorbance was measured spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 μmol of peroxide per minute at 37° and was expressed in milliunits per gram weight of wet tissue.

2.7. Measurement of cytokines

Tumor necrosis factor (TNF-α) and IL-1β levels were evaluated in the colon tissues at 4 days after intra-colonic injection of dinitrobenzensulfonic acid [30,31]. The assay was carried out by using a colorimetric, commercial kit (Calbiochem-Novabiochem Corporation). The enzyme linked immunosorbent assay has a lower detection limit of 5 pg/mL.

2.8. Materials

Primary monoclonal ICAM-1 (CD54) for immunohistochemistry was purchased from Pharmingen. Reagents and secondary and nonspecific IgG antibody for immunohistochemical analysis were from Vector Laboratories Inc. Primary monoclonal anti-PAR antibody was purchased by Alexis. All other reagents and compounds used were obtained from Sigma Chemical Co.

2.9. Data analysis

All values in the figures and text are expressed as mean ± standard error of the mean (SEM) of N observations. For the *in vivo* studies N represents the number of animals studied. In the experiments involving histology or immunohistochemistry, the figures shown are representative of at least three experiments performed on different experimental days. The results were analyzed by one-way ANOVA 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 GPI 6150 on the degree of colitis (histology)

Four days after intra-colonic administration of dinitrobenzensulfonic 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 hemorrhagic ulcerations (see Fig. 1).

No histological alteration was observed in colon tissues from Sham-treated rats (Fig. 2(A)). The histopathological features included a transmural necrosis and edema and a diffuse leukocyte cellular infiltrate in the submucosa (Fig. 2(B)). The inflammatory changes of the intestinal tract were associated with an increase in the weight of the colon (Fig. 3). Treatment of rats with GPI 6150 significantly attenuated in a dose dependent manner the extent and severity of the histological signs of colon injury (Figs. 1 and 2(C)). A significant increase in the weight of the spleen, an indicator of inflammation, was also noted in vehicle-treated rats, which had received dinitrobenzensulfonic acid (Fig. 3). GPI 6150 significantly reduced DNBS-induced increase in colon or spleen weight (Fig. 3).

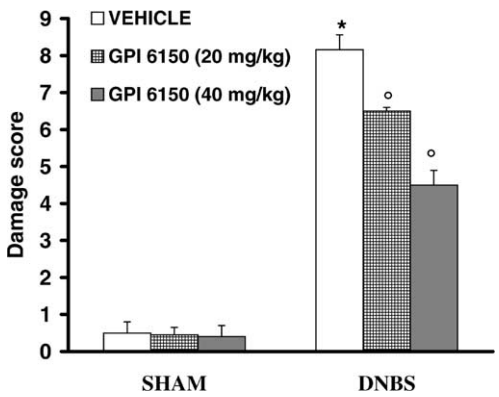

Fig. 1. Effect of GPI 6150 treatment on the damage score. Colonic damage was scored on a scale of 0 (normal) to 10 (severe) by two independent observers. Values are mean ± SE of 10 rats for each group. (★) $P < 0.01$ vs. Sham and (○) $P < 0.01$ vs. DNBS + vehicle.

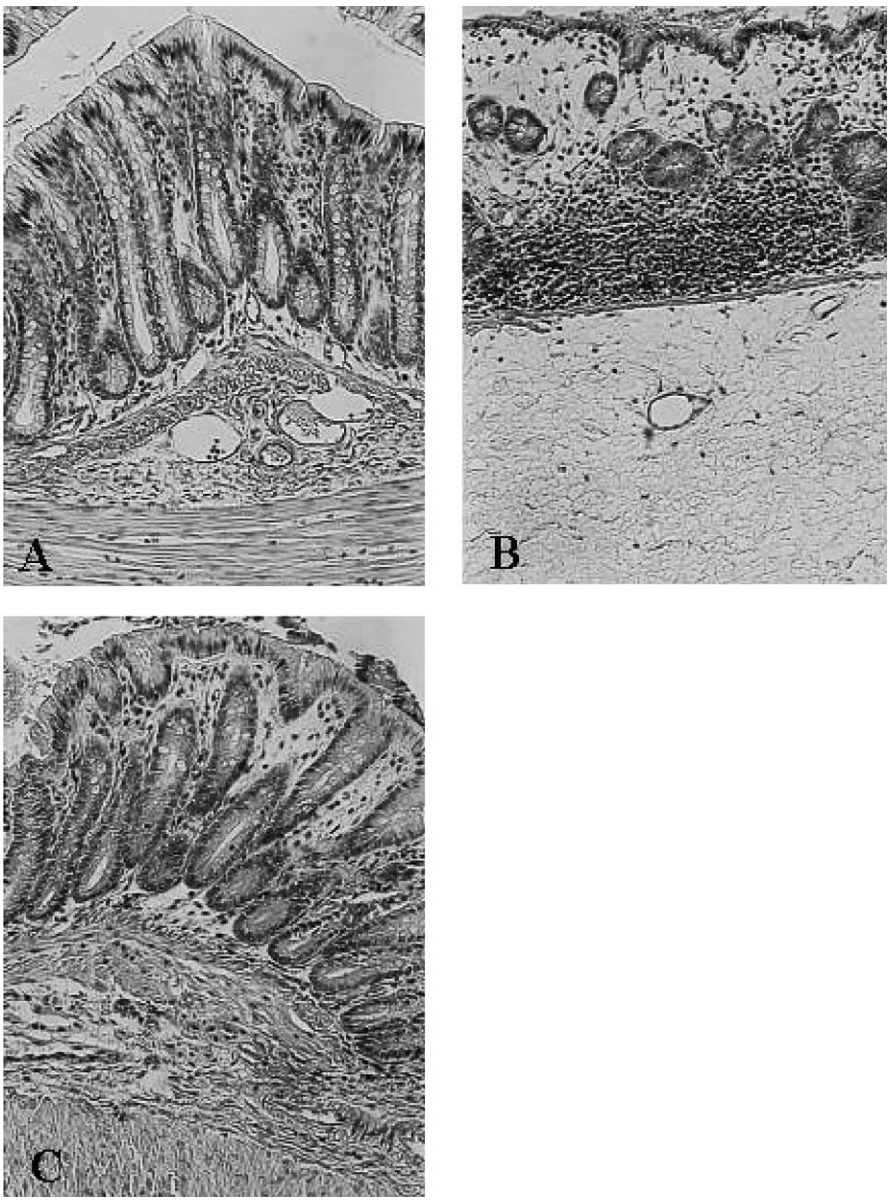

Fig. 2. Effect of GPI 6150 on colon injury. No histological modification was observed in mucosal from Sham-operated rats (A). Mucosal injury was produced after DNBS administration characterized by absence of epithelium and a massive mucosal and submucosal infiltration of inflammatory cells (B). Treatment with GPI 6150 (40 mg/kg) corrected the disturbances in morphology associated with DNBS administration (C). Original magnification: 125×. Figure is representative of at least three experiments performed on different experimental days.

3.2. Effects of GPI 6150 on changes of body weight

In vehicle-treated rats, the severe colitis caused by dinitrobenzensulfonic acid was associated with a significant loss in body weight (Fig. 4). Treatment of dinitrobenzensulfonic acid-treated rats with GPI 6150 significantly reduced in a dose dependent fashion the loss in body weight (Fig. 4).

3.3. Effect of GPI 6150 on PAR accumulation

At 4 days after dinitrobenzensulfonic acid 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 PAR (Fig. 5(A)). Colon sections obtained from vehicle-treated dinitrobenzensulfonic acid-treated rats exhibited positive staining for PAR (Fig. 5(B)), which was localized in inflammatory cells and in disrupted epithelial cells. GPI 6150 (40 mg/kg, i.p.) reduced the degree of immunostaining for PAR in the colon of dinitrobenzensulfonic acid-treated rats (Fig. 5(C)).

3.4. Effect of GPI 6150 on neutrophils infiltration and MPO activity in the colon

The colitis caused by dinitrobenzensulfonic acid was also characterized by an increase in MPO activity, an

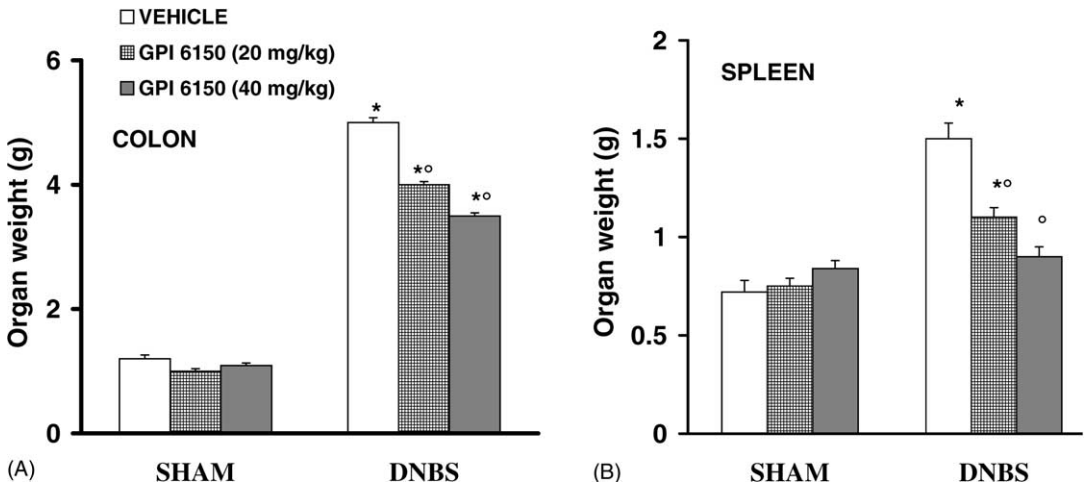

Fig. 3. Effect of GPI 6150 treatment on spleen (A) and colon (B). A significant increase was consistently seen at 4 days after DNBS injection in colon (A) and spleen (B). GPI 6150 treatment significantly reduced the organ weight (A and B). Values are mean ± SE of 10 rats for each group. (★) $P < 0.01$ vs. Sham and (○) $P < 0.01$ vs. DNBS + vehicle.

indicator of the infiltration (accumulation) of the colon with polymorphonuclear neutrophils (Fig. 6). This finding is consistent with the observation made with light microscopy that the colon of vehicle-treated dinitrobenzensulfonic acid-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. Treatment of dinitrobenzensulfonic acid-treated rats with GPI 6150, however, significantly reduced in a dose dependent way the degree of polymorphonuclear neutrophils infiltration (determined as increase in MPO activity, Fig. 6).

To further elucidate the effect of GPI 6150 treatment on neutrophil accumulation in inflamed colon, we next 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 (Fig. 7(A)). After dinitrobenzensulfonic acid administration, the staining intensity substantially increased in the endothelium along the vascular wall as well as in epithelial cells (Fig. 7(B)). Section from GPI 6150-treated (40 mg/kg, i.p.) rats did not reveal any upregulation of the constitutive ICAM-1, which was normally expressed in the endothelium along the vascular wall (Fig. 7(C)).

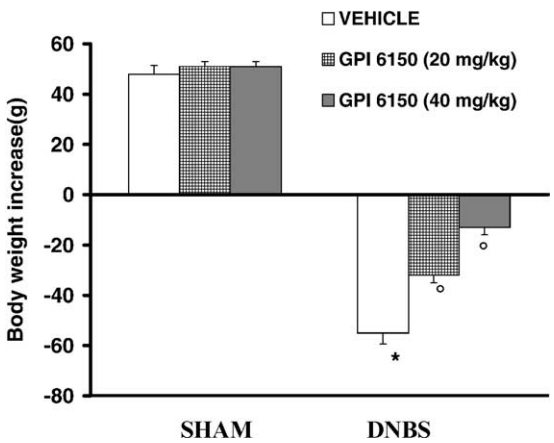

Fig. 4. Effect of GPI 6150 treatment on body weight. A significant loss of body weight was observed at 4 days after DNBS administration. GPI 6150 treatment significantly prevents in a dose dependent manner the loss of body weight. Values are mean ± SE of 10 rats for each group. (★) $P < 0.01$ vs. Sham and (○) $P < 0.01$ vs. DNBS + vehicle.

3.5. Effects of GPI 6150 on cytokine release

Colonic injury by dinitrobenzensulfonic acid administration was also characterized by an increase of pro-inflammatory cytokines (TNF-α and IL-1β) in the colon (Fig. 8). As shown in Fig. 8, GPI 6150 reduced in a dose dependent fashion the increase in TNF-α and IL-1β as observed in colonic tissues.

4. Discussion

Inflammatory bowel disease is a multi-factorial disorder of unknown etiology. There is, however, very good evidence both from animal and clinical studies, which documents that an enhanced formation of reactive oxygen or nitrogen species contribute to the pathophysiology of inflammatory bowel disease. For instance, monocytes from patients with Crohn's disease [32] and polymorphonuclear leukocytes from patients with ulcerative colitis [33] have an increased capacity to generate free oxygen radicals. *In vivo* experimental data have demonstrated that reactive oxygen species and peroxynitrite, by causing strand breaks in DNA, trigger an energy-consuming DNA repair mechanisms and activate the nuclear enzyme PARP. Activation of PARP results in the depletion of its substrate

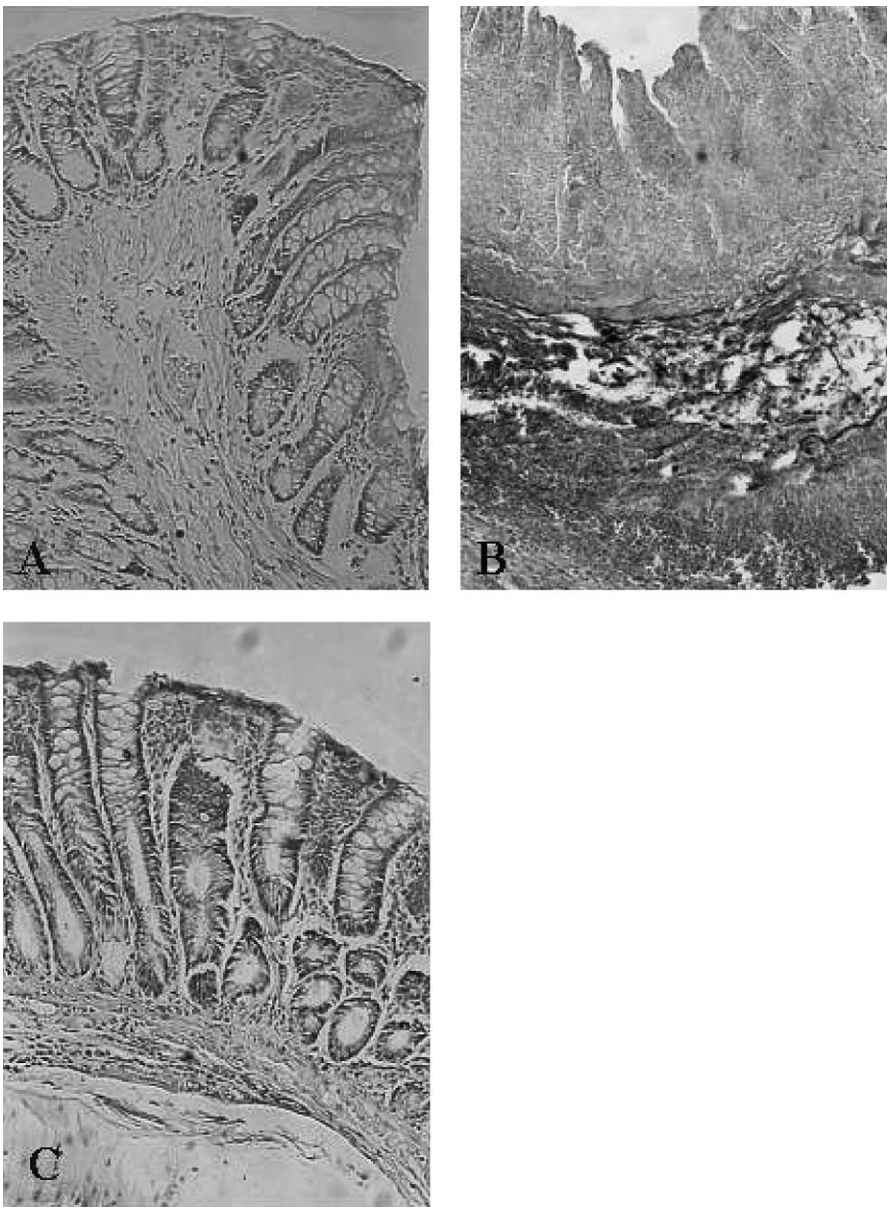

Fig. 5. Immunohistochemical localization for PAR in the colon. No positive staining for PAR (A) was found in the colon section from Sham-administered rats. Immunohistochemistry for PAR (B) show positive staining localized in the injured area from a DNBS-treated rats. The intensity of the positive staining for PAR (C) was significantly reduced in the colon from GPI 6150-treated rats. Original magnification: 150×. Figure is representative of at least three experiments performed on different experimental days.

NAD leading to a rapid fall in intracellular ATP. This process has been termed '*the PARP or poly (ADP-ribose) polymerase Suicide Hypothesis*' [14]. There is recent evidence that the activation of PARP may also play an important role in inflammation [5,16,34–37]. Recently Zingarelli *et al.* have demonstrated using mice lacking of functional gene for PARP that this *PARP-dependent suicide cycle* plays an important role also in experimental colitis [38]. As shown in Fig. 5 GPI 6150 reduced PAR immunostaining, confirming the effect of this new compound on PARP activity *in vivo*.

PARP inhibition has proved to be a useful approach to delineate the physiologic functions of this abundant nuclear protein. Many functions attributed to PARP were initially deduced from experimental consequences of its inactivation. Experiments using benzamide or other PARP inhibitors also implicated that PARP participates, either directly or indirectly, in gene expression, DNA replication, DNA rearrangement, differentiation, and mutagenesis [11].

There are recent reports from our laboratory and from other groups showing the protective effect of PAR synthethase inhibitors in experimental models of stroke [39], endotoxic shock [40,41], ischemia–reperfusion injury [42], and inflammation [36,43]. Because extensive activation of PARP due to massive oxidant-mediated DNA injury can lead to pronounced NAD^+ and ATP depletion in various tissues, it was generally assumed that the mode

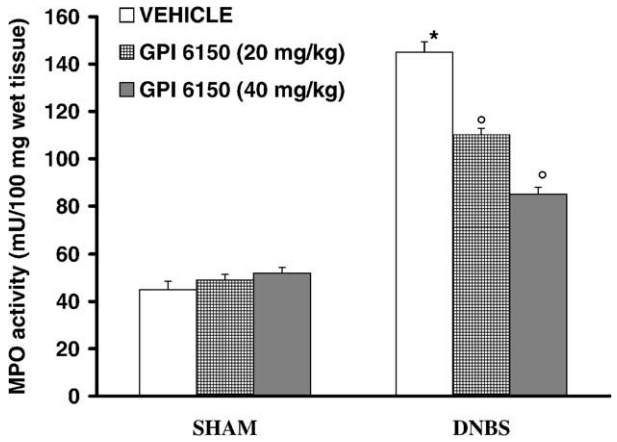

Fig. 6. Effect of GPI 6150 on neutrophil infiltration. MPO activity in the colon from DNBS-treated rats. MPO activity was significantly increased in DNBS-treated rats in comparison to Sham. GPI 6150-treated rats show a significant reduction of MPO activity. Values are mean ± SE of 10 rats for each group. (★) $P < 0.01$ vs. Sham and (○) $P < 0.01$ vs. DNBS + vehicle.

of protection by inhibitors of PARP is directly related to improved metabolic status of the target tissues in these models [12,36,40,41,44–48]. In fact, in *in vitro* studies, our group and other investigators have observed that hydrogen peroxide, oxyradical- or peroxynitrite-induced cellular injury is ameliorated by pharmacological inhibition of PARP [12,36,40,41,44–48] or in cells derived from the PARP −/− mice, when compared to corresponding wild-type controls [36,47,49].

Further development of PARP inhibitors for therapeutic purposes demands substantial improvement of the pharmacological profiles of compounds in terms of potency, specificity, solubility, bioavailability, and toxicity. Despite the wide use of benzamide family of PARP inhibitors, at high concentration, these compounds have been associated with side effects that were not mediated by PARP [28]. In search for specific, potent, small molecule PARP inhibitors, we identified GPI 6150, 1,11 b-dihydro-[2TA benzopyrano] 4,3,2-del-isoquinolin-3-one, which exhibited remarkable efficacy in reducing tissue damage in rodent models of focal cerebral ischemia, traumatic brain injury, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine damage to dopaminergic neurons, regional myocardial ischemia, streptozotocin-induced diabetes, septic shock, gut ischemia, and reperfusion and inflammation [20–24,50].

We confirm in this study that dinitrobenzensulfonic acid causes a substantial degree of inflammation and tissue injury (histology) in the rat colon, which is associated with an infiltration of the colon with polymorphonuclear neutrophils (histology and MPO activity) as well as cytokine production. The degree of inflammation, tissue injury and cytokines production caused by dinitrobenzensulfonic acid was substantially reduced in rats treated with the new PARP inhibitor GPI 6150. What then, is the mechanism by which GPI 6150 protects the colon against the injury and inflammation caused by dinitrobenzensulfonic acid?

The main finding of the current study is that inhibition of PARP by a pharmacological approach using GPI 6150 reduces polymorphonuclear neutrophils recruitment and accumulation into inflammatory colon tissue. Extravasated polymorphonuclear neutrophils become activated once in the inflammatory sites to secrete a variety of substances such as growth factors, chemokines and cytokines, complement components, proteases, NO, reactive oxygen metabolites and peroxynitrite, which are important mediators of tissue injury [51–53]. Prevention of neutrophil-dependent inflammatory pathways is likely to contribute to the reduced fluid extravasation and improved histological status after inhibition of PARP. Therefore, based on the data presented in the current study as well as by observation made by other groups using PARP-deficient mice, we propose that a reduced neutrophil recruitment represents an important additional mechanism for the anti-inflammatory effects provided by inhibition of PARP in colon inflammation.

GPI 6150 treatment produced a significant reduction of polymorphonuclear neutrophils influx into rats colon challenged with dinitrobenzensulfonic acid. Neutrophils 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 [3,33]. Neutrophil infiltration into inflamed tissue play a crucial role in the destruction of foreign antigens and in the breakdown and remodeling of injured tissue [54]. Polymorphonucleates accumulation is an important inflammatory mechanism in the model of dinitrobenzensulfonic acid-induced colitis used here [55,56]. Similarly, there is good evidence for an important contribution of extravasated neutrophils to the edema caused by carrageenan [57–61] and, to the massive leukocyte infiltration seen in the various organs after injection of zymosan, thus leading to a pivotal role for this cell type in mediating inflammation [51–53,62–64]. Although inhibition of PAR synthethase was effective in reducing polymorphonuclear neutrophils recruitment in all inflammatory models tested, paradoxically, the protective effects appeared to be more pronounced in more severe forms and more delayed stages of inflammation [36,47] This may be related to the fact that PARP activation and related cellular alterations mainly occur under conditions of more severe oxidant stress.

What, then, is the mechanism of the protection against polymorphonuclear neutrophils recruitment provided by inhibition of PARP using GPI 6150? The data presented in the current study provide evidence that the effects of GPI 6150 treatment are mainly due to interference with polymorphonuclear neutrophils post-adhesion/emigration phenomena. The strongest indication for this conclusion derives from our experiments assessing ICAM-1 expression. Furthermore, ICAM-1 was expressed in endothelial and epithelial cells, and neutrophils in the distal colon in dinitrobenzensulfonic acid treated rats. Treatment of rats subjected to dinitrobenzensulfonic acid-induced colitis

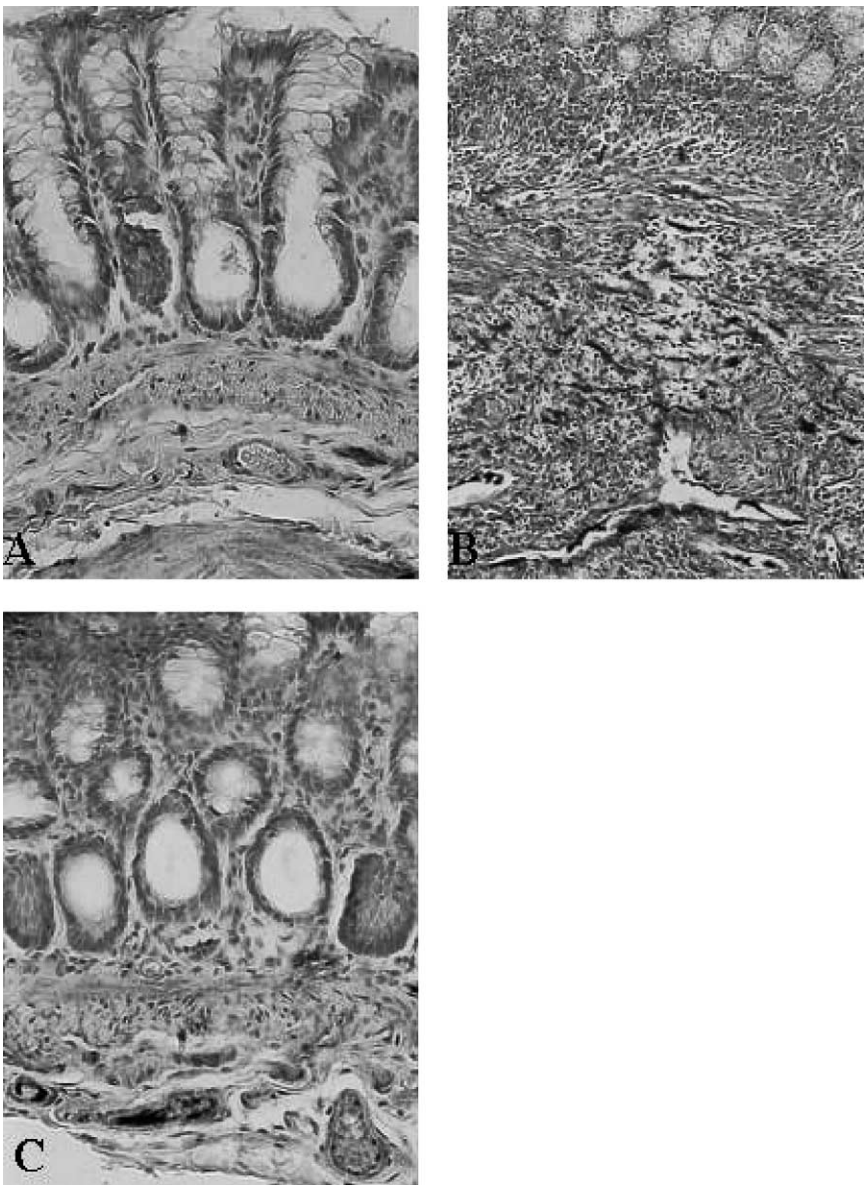

Fig. 7. Immunohistochemical localization of ICAM-I in the colon. Staining of colon tissue sections obtained from Sham-operated rats with anti-ICAM-1 antibody showed a specific staining along vessels, demonstrating that ICAM-1 is constitutively expressed (A). Section obtained from DNBS-treated rats showed 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 GPI 6150-treated rats. Original magnification: 150×. Figure is representative of at least three experiments performed on different experimental days.

with GPI 6150 was associated with a significant reduction of ICAM-1 expression in endothelial and epithelial cells. Our data confirm that PARP activation contribute to the regulation of neutrophil infiltration and is consistent with previously published data in models of acute inflammation and reperfusion injury [5,36,65].

The mechanisms regulating leukocyte emigration through the gap formed between adjacent endothelial cells in inflammatory conditions are incompletely understood. Since PAR synthethase regulates the expression of various genes [66–68], the possibility that PARP may alter the expression of adhesion receptors involved in post-adhesion/emigration processes may be proposed. Endothelial-derived NO inhibits the infiltration of polymorphonuclear neutrophils into the inflammatory tissue sites.

Thus, we propose that GPI 6150 may exert anti-inflammatory effects by two distinct mechanisms. First, extensive activation of PARP due to massive oxidant-mediated DNA injury can lead to pronounced NAD^+ and ATP depletion in various tissues, and GPI 6150 may reduce this injury. Second, a reduced neutrophil recruitment may represent an important additional mechanism for the anti-inflammatory effects provided by inhibition of PARP. An additional mechanism, which may be considered as a contributor to the anti-inflammatory effects of GPI 6150, may be related to inhibition of TNF-α production. TNF-α is clearly

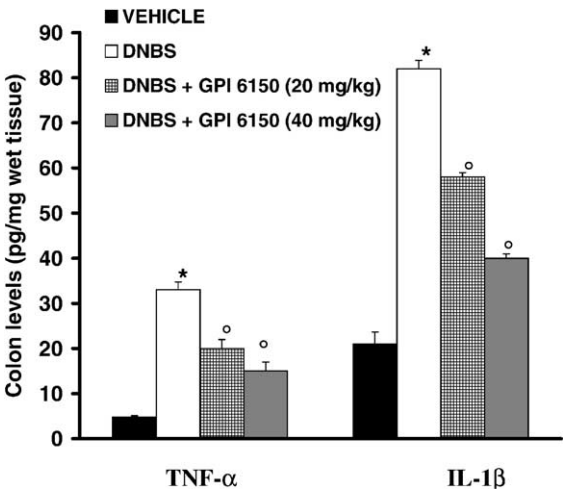

Fig. 8. Effects of GPI 6150 on the levels of cytokines in the colon. Colon levels of TNF-α (A) and IL-1β (B). Cytokine levels were significantly reduced in the colon from GPI 6150-treated rats. Values are mean ± SEM of 10 rats for each group. (✱) $P < 0.01$ vs. Sham and (○) $P < 0.01$ vs. DNBS + vehicle.

involved in the pathogenesis of colitis since this cytokine is present in colon tissues and can be detected immunohistochemically in the inflamed tissues [69,70]. Direct evidence that TNF-α plays a role in the pathogenesis of experimental colitis has been obtained in animal models in which blocking of the action of these cytokines has been shown to delay the onset of experimental colitis, suppress inflammation, and ameliorate colon destruction that corresponds to the anti-inflammatory response [71,72]. The mechanism of this reduced TNF-α production is not clear at present. It may be related to the reduced neutrophils infiltration. The mechanism of this reduced neutrophil recruitment is not clear at present. It may be related to prevention by GPI 6150 of endothelial oxidant injury. In other words, we propose the following positive feedback cycle in colitis: early oxidative stress ⇒ PARP activation ⇒ endothelial injury ⇒ PMN infiltration ⇒ more free radicals and cytokines production. PARP inhibition by GPI 6150 would interrupt this cycle preventing endothelial injury. This model would explain the reduction of ICAM-1 as well as the neutrophil infiltration leading to reduced cytokines production.

Based on the present data, we propose that GPI 6150 may represent a novel strategy for anti-inflammatory therapy under conditions of oxidant stress such us inflammatory bowel disease. A number of approaches are available to reduce oxidant- and neutrophil-related cytotoxicity (blockers of adhesion receptors, nitric oxide synthase inhibitors, peroxynitrite scavengers, oxyradical scavengers, etc.). Inhibition of PARP appears to be a viable anti-inflammatory strategy for the following reasons: (1) inhibition of PARP is not likely to interfere with the physiological roles of NO (an effect frequently seen with NOS inhibitors); (2) inhibition of PARP would affect multiple aspects of the inflammatory response, such as endothelial and epithelial dysfunction, vascular hypocontractility, and cellular energetic failure [40,41,44–47,49,73,74] as well as polymorphonucleates recruitment (current study); and finally (3) inhibition of PARP is effective in severe models of inflammation, even when applied in the post-treatment, rather than pre-treatment. However, as with most pharmacological inhibitors, we cannot exclude that additional, PARP-independent effects that may contribute to the anti-inflammatory effects observed with GPI 6150 in the current study. Further studies are needed to evaluate the others possible anti-inflammatory mechanisms of GPI 6150.

References

[1] Rachmilewitz D, Stamier JS, Karmeli F, Mullins ME, Singel DJ, Loscaizo J, Xavier RJ, Pocidolsky DK. Peroxynitrite-induced rat colitis—a new model of colonic inflammation. Gastroenterology 1993;105:1681–8.
[2] Middleton SJ, Shorthouse M, Hunter JD. Increased nitric oxide synthesis in ulcerative colitis. Lancet 1993;341:465–6.
[3] Grisham MB. Oxidants and free radicals in inflammatory bowel disease. Lancet 1994;344:859–61.
[4] Nathan C. Nitric oxide as a secretory product of mammalian cells. FASEB J 1996;6:3051–64.
[5] Cuzzocrea S, Caputi AP, Zingarelli B. Peroxynitrite-mediated DNA strand breakage activates poly (ADP-ribose) synthetase and causes cellular energy depletion in carrageenan-induced pleurisy. Immunology 1998;93:96–101.
[6] Kloner RA, Przyklenk K, Whittaker P. Deleterious effects of oxygen radicals in schema/reperfusion. Resolved and unresolved issues. Circulation 1989;80:1115–27.
[7] Paller MS. The cell biology of reperfusion injury in the kidney. J Investig Med 1994;42:632–9.
[8] Oei SL, Griesenbeck J, Schweiger M. The role of poly (ADP-ribosyl)ation. Rev Physiol Biochem Pharmacol 1997;131:127–73.
[9] Ikai K, Ueda K. Immunohistochemical demonstration of poly (adenosine diphosphate-ribose) synthetase in bovine tissues. J Histochem Cytochem 1983;31:1261–4.
[10] De Murcia JM, Niedergang C, Trucco C, Ricoul M, Dutrillaux B, Mark M, Oliver FJ, Masson M, Dierich A, LeMeur M, Waltzinger C, Chambon P, DeMurcia G. Requirement of poly (ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. Proc Natl Acad Sci USA 1997;94:7303–7.
[11] Ueda K, Hayaishi O. ADP-ribosylation. Annu Rev Biochem 1985;54:73–100.
[12] Schraufstatter IU, Hinshaw DB, Hyslop PA, Spragg RG, Cochrane CG. Oxidant injury of cells: DNA strand-breaks activate polyadenosine diphosphate-ribose polymerase and lead to depletion of nicotinamide adenine dinucleotide. J Clin Invest 1986;77:1312–20.
[13] Satoh M, Lindahl T. Role of poly (ADP-ribose) formation in DNA repair. Nature 1992;356:356–8.
[14] Carson DA, Seto S, Wasson B, Carrera CJ. DNA strand breaks NAD metabolism and programmed cell death. Exp Cell Res 1986;164:273–81.
[15] Cuzzocrea S, Zingarelli B, Costantino G, Szabo A, Salzman AL, Caputi AP, Szabo C. Beneficial effects of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase in a rat model of splanchnic artery occlusion and reperfusion. Br J Pharmacol 1997;121:1065–74.
[16] Szabó C, Viràg L, Cuzzocrea S, Scott GS, Hake P, O'Connor M, Zingarelli B, Ma Y, Hirsch R, Boiovin GP, Salzman AL, Kun E. Protection against peroxynitrite-induced fibroblast injury and arthritis development by inhibition of poly (ADP-Ribose) synthetase. Proc Natl Acad Sci USA 1998;95:3867–72.

[17] Zingarelli B, Cuzzocrea S, Zsengeller Z, Salzman AL, Szabo C. Protection against myocardial schema and reperfusion injury by 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase. Cardiovasc Res 1997;36:205–15.
[18] Hyslop PA, Hinshaw DB, Halsey WA, Schraufstatter IU, Sauerheber RD, Spragg RG, Jackson JH, Cochrane CG. Mechanisms of oxidant-mediated cell injury: the glycolytic and mitochondrial pathways of ADP phosphorylation are major intracellular targets inactivated by hydrogen peroxide. J Biol Chem 1988;263:1665–75.
[19] Thies RL, Autor AP. Reactive oxygen injury to cultured pulmonary artery endothelial cells: mediation by poly (ADP-ribose) polymerase activation causing NAD depletion and altered energy balance. Arch Biochem Biophys 1991;286:353–63.
[20] Zhang J, Li JH, Lautar S. Soc Neurosci Abstr 1998;24:1226.
[21] La Placa MC, Zhang J, Raghupathi R, Smith F, Bareyre FM, Li JH, Graham DI, Snyder SH, McIntosh TK. Temporal patterns of poly(-ADP-ribose) polymerase activation in the cortex following experimental brain injury in the rat. J Neurochem 1999;73:205–13.
[22] Walles T, Wang P, Pieper A, Li JH, Zhang J, Snyder SH, Zweier J. Demonstration that poly(ADP-ribose) accumulation occurs in the post-ischemic heart and is associated with myocardial necrosis. Circ Suppl 1998;98:I260.
[23] Zhang J. Beneficial effect of GPI 6150 treatment in multiple animal models of disease. In: Zhang J, editor. PARP as a therapeutic target. Boca Raton, FL, USA: CRC Press LLC, in press.
[24] Mazzon E, Dugo L, De Sarro A, Li JH, Caputi AP, Zhang J, Cuzzocrea S. Beneficial effects of GPI 6150 an inhibitor of poly(ADP-ribose) polymerase in a rat model of splanchnic artery occlusion and reperfusion. Shock 2002;17:222–7.
[25] Morrs GP, Bek PL, Herridge MS, Drew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat. Gastroenterology 1989;96:795–803.
[26] Wallace JL, Keenan CM, Gale D, Shoupe TS. Excerbation of experimental colitis by non-steroidal antinflammatory drugs is not related to elevate leukotriene B4 synthesis. Gastroenterology 1992;102: 18–27.
[27] Cuzzocrea S, McDonald MC, Mazzon E, Mota-Filipe H, Centorrino T, Terranova ML, Ciccolo A, Britti D, Caputi AP, Thiemermann C. Calpain inhibitor I reduces colon injury caused by dinitrobenzene sulphonic acid in the rat. Gut 2001;48:478–88.
[28] Milam KM, Cleaver JE. Inhibitors of poly(adenosine diphosphate-ribose) synthesis: effect on other metabolic processes. Science 1984;223:589–91.
[29] Mullane KM, Kraemer R, Smith B. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. J Pharmacol Methods 1985;14:157–67.
[30] Nielsen OH, Gionchetti P, Ainsworth M, Vainer B, Campieri M, Borregaard N, Kjeldsen L. Rectal dialysate and fecal concentrations of neutrophil gelatinase-associated lipocalin, interleukin-8, and tumor necrosis factor-alpha in ulcerative colitis. Am J Gastroenterol 1999; 94:2923–8.
[31] Tountas NA, Casini-Raggi V, Yang H, Di Giovine FS, Vecchi M, Kam L, Melani L, Pizarro TT, Rotter JI, Cominelli F. Functional and ethnic association of allele 2 of the interleukin-1 receptor antagonist gene in ulcerative colitis. Gastroenterology 1999;117:806–13.
[32] Kitahora T, Suzuki IR, Asakura H, Yoshida T, Suematsu M, Watanabe M, Aiso S, Tsuchiya M. Active oxygen species generated by monocytes and polymorphonuclear cells in patients in Crohn's disease. Dig Dis Sci 1998;33:951–5.
[33] Shiratora Y, Aoki S, Takada H, Kiriyama H, Ohto K, Hai K, Teraoka H, Matano S, Matsumoto K, Kamii K. Oxygenderived free radical generating capacity of polymorphonuclear cells in patients with ulcerative colitis. Digestion 1989;44:163–71.
[34] Salgo MG, Bermudez E, Squadrito G, Pryor W. DNA damage and oxidation of thiols peroxynitrite causes in rat thymocytes. Arch Biochem Biophys 1995;322:500–5.
[35] Lih-Brody L, Powell SR, Collier KP, Reddy GM, Cerchia R, Kahn E, Weissman GS, Katz S, Floyd RA, McKinley MJ, Fisher SE, Mullin G. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. Dig Dis Sci 1995;41:2078–86.
[36] Szabó C, Cuzzocrea S, Lim LHK, Getting SJ, Zingarelli B, Flower RJ, Salzman AL, Perretti M. Inhibition of poly (ADP-ribose) synthetase inhibits neutrophil recruitment and exerts anti-inflammatory effects. J Exp Med 1997;186:1041–9.
[37] Cuzzocrea S, Zingarelli B, Gilard E, Hake P, Salzman AL, Szabó C. Protective effects of 3-aminobenzamide an inhibitor of poly (ADP-ribose) synthase in carrageenan-induced models of local inflammation. Eur J Pharmacol 1998;342:67–76.
[38] Zingarelli B, Szabo C, Salzman A. Blockade of poly(ADP-ribose) synthetase inhibits neutrophil recruitment oxidant generation and mucosal injury in murine colitis. Gastroenterology 1999;116:335–45.
[39] Kazushi T, Greenberg JH, Jackson P, Maclin K, Zhang J. Neuroprotective effects of inhibiting poly (ADP-ribose) synthetase on focal cerebral schema in rats. J Cereb Blood Flow Metab 1997;17:1137–42.
[40] Szabó C, Zingarelli B, Salzman AL. Role of poly-ADP ribosyltransferase activation in the nitric oxide- and peroxynitrite-induced vascular failure. Circ Res 1996;78:1051–63.
[41] Szabó C, Zingarelli B, O'Connor M, Salzman AL. DNA strand breakage, activation of poly-ADP ribosyl synthetase, and cellular energy depletion are involved in the cytotoxicity in macrophages and smooth muscle cells exposed to peroxynitrite. Proc Natl Acad Sci USA 1996;93:1753–8.
[42] Thiemermann C, Bowes J, Mynny FP, Vane JR. Inhibition of the activity of poly(ADP ribose) synthase reduces ischaemia–reperfusion injury in the heart and skeletal muscle. Proc Natl Acad Sci USA 1997;94:679–83.
[43] Cuzzocrea S, Zingarelli B, Gilard E, Hake P, Salzman AL, Szabó C. Anti-inflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. Free Radic Biol Med 1998;24:450–9.
[44] Cochrane C. Mechanism of oxidant injury of cells. Mol Aspects Med 1991;12:137–47.
[45] Radons J, Heller B, Burkle A, Hartmann B, Rodriguez M, Kroncke K, Burkart V, Kolb H. Nitric oxide toxicity in islet cells involves poly (ADP-ribose) polymerase activation and concomitant NAD depletion. Biochem Biosphys Res Comm 1994;199:1270–7.
[46] Zhang J, Dawson V, Dawson T, Snyder S. Nitric oxide activation of poly (ADP-ribose) synthetase in neurotoxicity. Science 1994;263:687–9.
[47] Szabó C, Cuzzocrea S, Zingarelli B, O'Connor M, Salzman AL. Endothelial dysfunction in endotoxic shock: importance of the activation of poly (ADP-Ribose) synthetase (PARS) by peroxynitrite. J Clin Invest 1997;100:723–35.
[48] Cosi C, Suzuki H, Milani D, Facci L, Menegazzi M, Vantini G, Kanai C, Skaper SD. Poly (ADP-ribose) polymerase: early involvement in glutamate-induced neurotoxicity in cultured cerebellar granule cells. J Neurosci Res 1994;39:38–46.
[49] Heller B, Wang ZQ, Wagner EF, Radons J, Burkle A, Fehsel K, Burkart V, Kolb H. Inactivation of the poly(ADP-ribose) polymerase gene affects oxygen radical and nitric oxide toxicity in islet cells. J Biol Chem 1995;270:11176–80.
[50] Mazzon E, Serraino I, Li JH, Dugo L, Caputi AP, Zhang J, Cuzzocrea S. GPI 6150 a poly (ADP-ribose) polymerase inhibitor exhibits an anti-inflammatory effect in rat models of inflammation. Eur J Pharmacol 2001;415:85–94.
[51] McMillen MA, Huribal M, Sumpio B. Common pathway of endothelial–leukocyte interaction in shock schema and reperfusion. Am J Surg 1993;166:557–62.
[52] Fujishima S, Aikawa N. Neutrophil-mediated tissue injury and its modulation. Int Care Med 1995;21:277–85.
[53] Schlag G, Redl H. Mediators of injury and inflammation. World J Surg 1996;20:406–10.

[54] Lefer AM, Lefer DJ. Pharmacology of the endothelium in schema–reperfusion and circulatory shock. Annu Rev Pharmacol Toxicol 1993;33:71–90.
[55] Cuzzocrea S, McDonald MC, Mazzon E, Dugo L, Lepore V, Fonti MT, Ciccolo A, Terranova ML, Caputi AP, Thiemermann C. Tempol a membrane-permeable radical scavenger reduces dinitrobenzene sulfonic acid-induced colitis. Eur J Pharmacol 2000;406:127–37.
[56] Cuzzocrea S, McDonald MC, Mazzon E, Mota-Filipe H, Lepore V, Ciccolo A, Terranova ML, Britti D, Caputi AP, Thiemermann C. The tyrosine kinase inhibitor tyrphostin AG 126 reduced the development of colitis in the rat. Lab Invest 2000;80:1439–53.
[57] Wojtecka-Lukasik E, Maslinski S. Polymorphonuclear leukocyte collagenase in carrageenin - induced inflammation: effect of nonsteroidal antiinflammatory drugs. Int J Tissue React 1986;8:321–5.
[58] Sopata I, Wojtecka-Lukasik E, Wize J, Maslinski S. Neutrophil enzyme activities in carrageenan-induced inflammation in rats. Agents Actions 1989;28:89–92.
[59] Vinegar R, Truax JF, Selph JL, Johnston PR, Venable AL, McKenzie KK. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Fed Proc 1987;46:118–26.
[60] Salvemini D, Wang ZQ, Bourdon DM, Stern MK, Currie MG, Manning PT. Evidence of peroxynitrite involvement in the carrageenan-induced rat paw edema. Eur J Pharmacol 1996;303:217–20.
[61] Salvemini D, Wang ZQ, Wyatt DM, Bourdon MH, Marino PT, Currie MG. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br J Pharmacol 1996;118: 829–38.
[62] Goris RJ, Boekholtz WK, Van Bebber IP, Nuytinck JK, Schillings PH. Multiple-organ failure and sepsis without bacteria. An experimental model. Arch Surg 1986;121:897–901.
[63] Van Bebber IP, Boekholz WK, Goris RJ, Schillings PH, Dinges HP, Bahrami S, Redl H, Schlag G. Neutrophil function and lipid peroxidation in a rat model of multiple organ failure. J Surg Res 1989;47: 471–5.
[64] Shayevitz JR, Miller C, Johnson KJ, Rodriguez JL. Multiple organ dysfunction syndrome: end organ and systemic inflammatory response in a mouse model of nonseptic origin. Shock 1995;4:389–96.
[65] Cuzzocrea S, Mazzon E, Dugo L, Caputi AP, Aston K, Riley DP, Salvemini D. Protective effects of a new stable, highly active SOD mimetic, M40401 in splanchnic artery occlusion and reperfusion. Br J Pharmacol 2001;132:19–29.
[66] Pellat-Seceunyk C, Wietzerbin J, Drapier JC. Nicotinamide inhibits nitric oxide synthase mRNA induction in activated macrophages. Biochem J 1994;297:53–8.
[67] Bauer PI, Kirsten E, Young LJT, Varadi G, Csonka E, Buki KG, Mikala G, Hu R, Comstock JA, Mendeleyev J, Hakam A, Kun E. Modification of growth related enzymatic pathways and apparent loss of tumorigenicity of a ras-transformed bovine endothelial cell line by treatment with 5-iodo-6-amino-12-benzopyrone. Int J Oncol 1995;8:239–52.
[68] Meisterernst M, Stelzer G, Roeder RG. Poly (ADP-ribose) polymerase enhances activator-dependent transcription in vitro. Proc Natl Acad Sci USA 1997;94:2261–5.
[69] Carty E, De Brabander M, Feakins RM, Rampton DS. Measurement of in vivo rectal mucosal cytokine and eicosanoid production in ulcerative colitis using filter paper. Gut 2000;46:487–92.
[70] Negoro K, Kinouchi Y, Hiwatashi N, Takahashi S, Takagi S, Satoh J, Shimosegawa T, Toyota T. Crohn's disease is associated with novel polymorphisms in the 5′-flanking region of the tumor necrosis factor gene. Gastroenterology 1999;117:1062–8.
[71] Andborn WJ, Hanauer SB. Antitumor necrosis factor therapy for inflammatory bowel disease: a review of agents, pharmacology, clinical results, and safety. Inflamm Bowel Dis 1999;2:119–33.
[72] Urthy S, Cooper HS, Yoshitake H, Meyer C, Meyer CJ, Murthy NS. Combination therapy of pentoxifylline and TNF-α monoclonal antibody in dextran sulphate-induced mouse colitis. Aliment Pharmacol Ther 1999;13:251–60.
[73] Schraufstatter IU, Hinshaw DB, Hyslop PA, Spragg G, Cochrane CG. Oxidant injury of cells: DNA strand-breaks activate polyadenosine diphosphate-ribose polymerase and lead to depletion of nicotinamide adenine dinucleotide. J Clin Invest 1986;77:1312–20.
[74] Kirkland JB. Lipid peroxidation, protein thiol oxidation and DNA damage in hydrogen peroxide-induced injury to endothelial cells: role of activation of poly(ADP-ribose)polymerase. Biochim Biophys Acta 1991;1092:319–25.