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Treatment with PARP-1 inhibitors, GPI 15427 or GPI 16539, ameliorates intestinal damage in rat models of colitis and shock

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Abstract

Poly (ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme activated by DNA strand breaks, plays a detrimental role during inflammation. As inflammation is important in the development of colitis and ischemia/reperfusion (I/R) injury of the intestine, we investigated the effects of 10-(4-methyl-piperazin-1-ylmethyl)-2H-7-oxa-1,2-diaza-benzo[de]anthracen-3-one (GPI 15427) and 2-(4-methyl-piperazin-1-yl)-5H-benzo[c][1,5] naphthyridin-6-one (GPI 16539), two novel and potent inhibitors of PARP-1, in a rat model of gut injury and inflammation, splanchnic artery occlusion (SAO)shock and dinitrobenzene sulfonic acid (DNBS)-induced colitis. We report here for the first time that post-injury administration of GPI 15427 and GPI 16539 exerts potent anti-inflammatory effects by reducing inflammatory cell infiltration and histological injury, and delaying the development of clinical signs in both in vivo models. Furthermore, GPI 15427 and GPI 16539 treatment diminished the accumulation of poly (ADP-ribose) in the ileum of splanchnic artery occlusion-shocked rats and in the colons of dinitrobenzene sulfonic acid-treated rats. Thus, GPI 15427 and GPI 16539 exhibited anti-inflammation activity against damage caused by intestinal ischemia/reperfusion and colitis. GPI 15427 and GPI 16539 may be useful for treating gut ischemia and inflammation.

Keywords: PARP-1; GPI 15427; GPI 16539; Myeloperoxidase; Colitis; Ischemia/Reperfusion

1. Introduction

Poly(ADP-ribose) polymerase-1 (PARP-1) is an abundant nuclear enzyme with an important role in the cellular life cycle (Herceg and Wang, 1999; Decker et al., 2000; Yu et al., 2002). It contains three domains: the DNA-binding domain consisting of two zinc fingers, the automodification site, and the catalytic center (Rolli et al., 1997; de Murcia et al., 1994). Upon activation by DNA damage, PARP-1 converts nicotinamide adenine dinucleotide (NAD^+) into nicotinamide and ADP-ribose , the latter is polymerized and bound to the substrate proteins (Jeggo, 1998). Over-activation of PARP-1 can cause a decline in NAD^+ level and subsequently a drop in intracellular ATP level. This depletion of ATP results in necrotic cell death such as that seen during ischemic tissue damage (Takahashi et al., 1999; Nagayama et al., 2000). Many studies with PARP-1 "knockout" mice have established resistance against ischemia and reperfusion injury (Eliasson et al., 1997; Pieper et al., 2000; Cuzzocrea et al., 2002). More recently, studies have demonstrated protection by PARP-1 inhibitors in rat models of gut ischemia (Cuzzocrea et al., 2002; Mazzon et al., 2002a,b), suggesting that PARP-1 is a clinically relevant target for this indication.

Several PARP inhibitors have previously been examined as potential novel therapeutics against colon injury associated with experimental colitis. Specifically, these studies have demonstrated that the chemically distinct PARP inhibitors GPI 6150, PJ-34 and 3-aminobenzamide (3AB) can attenuate PARP activation and provide benefits against colon injury and dysfunction in vivo (Jijon et al., 2000; Mabley et al., 2001; Mazzon et al., 2002a,b). Although the potency of recently developed PARP inhibitors has improved greatly, the majority still lack water solubility. Without an aqueous formulation, it is difficult

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to administer the compounds i.v., a clinically relevant route in most situations, or test the compounds' effect in post-injury treatment. A lack of pharmacokinetic data for most PARP inhibitors also makes it impossible to design dosing regimens and correlate in vivo effect with exposure to the compounds.

We have synthesized 10-(4-methyl-piperazin-1-ylmethyl)-2H-7-oxa-1,2-diaza-benzo[de]anthracen-3-one (GPI 15427) and 2-(4-methyl-piperazin-1-yl)-5H-benzo[c][1,5]naphthyridin-6-one (GPI 16539), PARP inhibitors, which are potent, selective, orally bioavailable and capable of penetrating the blood–brain barrier, as revealed by pharmacokinetic analysis (Tentori et al., 2003; Ferraris et al., 2003). We have demonstrated that these two families of PARP inhibitors provide cardioprotection in a rat model of regional myocardial ischemia and neuroprotection in a rat model of transient as well as permanent focal cerebral ischemia. Representative compounds, such as GPI 15427 and GPI 18180, have been shown to enhance the chemotherapeutic effects of temozolomide against melanoma, lymphoma and glioma in mouse brain (Tentori et al., 2005).

Previously, we have shown that a water-soluble PARP inhibitor, 5-aminoisoquinolinone (5-AIQ), reduces gut ischemia injury when given 5 min before ischemia (Di Paola et al., 2005). However, a lack of pharmacokinetic data on 5-AIQ hinders the dosing design and testing of the compound effect as post-ischemia treatment, which is more relevant in a clinical scenario. The present study was designed to investigate whether two new PARP-1 inhibitors, GPI 15427 and GPI 16539, when given post-injury, reduce the intestinal damage and inflammation caused by splanchnic artery occlusion shock and the chronic inflammatory response (colitis) caused by injection of dinitrobenzene sulfonic acid in the rat.

2. Matherial 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. Induction of experimental colitis

Colitis was induced by using a technique of acid-induced colon inflammation as described previously (Sturiale et al., 1999). In fasted rats lightly anesthetized 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-dinitrobenzenea 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. After colitis and sham-colitis induction, the animals were observed for 3 days. On Day 4, the animals were weighed and killed with chloral 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 antimesenteric border, rinsed, weighed, and processed for histology and biochemical studies. The macroscopic damage score, according to Wallace et al. (1992), was assessed.

2.3. Splanchnic artery occlusion shock (splanchnic artery occlusion-shock)

Male Sprague–Dawley rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.). Following anesthesia, catheters were placed in the carotid artery and jugular vein as described previously (Caputi et al., 1980). Blood pressure was monitored continuously with a Maclab A/D converter (AD Instruments), and stored and displayed on a Macintosh personal computer. After midline laparotomy, the celiac and superior mesenteric arteries were isolated near their aortic origins. During this procedure, the intestinal tract was maintained at 37 °C by placing it between gauze pads soaked with warmed 0.9% NaCl solution.

The rats were observed duringr a 30-min stabilization period before either splanchnic ischemia or sham ischemia. Splanchnic artery occlusion shock was induced by clamping both the superior mesenteric artery and the celiac trunk, resulting in a total occlusion of these arteries for 45 min. After this period of occlusion, the clamps were removed. In one study, the various groups of rats were killed 60 min after the start of reperfusion for histological examination of the bowel and for biochemical studies, as described below.

2.4. Experimental groups (Colitis Study)

Upon completion of experimental procedures, rats were randomly allocated to the following groups: (i) *dinitrobenzene sulfonic acid+saline group,* rats were given dinitrobenzene sulfonic acid ($N=10$); (ii) *dinitrobenzene sulfonic acid+GPI 15427 group*, rats were subjected to treatment as above and *GPI 15427* was given daily as an intravenous (i.v.) bolus at 40 mg/kg starting 24 h after dinitrobenzene sulfonic acid administration for 3 days ($N=10$); (iii) *Sham+ saline group*, rats were subjected to procedures as above except that the vehicle alone (50% ethanol, 0.8 ml) was injected instead of dinitrobenzene sulfonic acid and the rats were kept under anesthesia for the duration of the experiment ($N=10$); (iv) *Sham+ GPI 15427 group*, identical to sham-operated rats except for the administration of *GPI 15427* (40 mg/kg/day, i.v.) ($N=10$).

2.5. Experimental groups (splanchnic artery occlusionStudy)

Upon completion of surgical procedures, rats were randomly allocated to the following groups: (i) *ischemia/reperfusion+saline group*, rats were subjected to splanchnic artery occlusion shock (45 min) followed by reperfusion (1 h) ($N=10$); (ii) *ischemia/reperfusion+GPI 15427 group*, rats were subjected to surgical procedures described as above and administered *GPI 15427* (10 mg/kg, i.v.) 30 min after to ischemia ($N=10$); (iii) *ischemia/reperfusion+GPI 16539 group*, rats were subjected to surgical procedures as above and administered *GPI 16539* (10 mg/kg, i.v.)

30 min after ischemia ($N=10$); (iv) *Sham+ saline group*, rats were subjected to identical surgical procedures except for splanchnic artery occlusion shock and were kept under anesthesia for the duration of the experiment ($N=10$); (v) *Sham+16539 group*, identical to sham-operated rats except for the administration of *16539* (40 mg/kg, i.v.) ($N=10$); (vi) *Sham+15427 group*, identical to sham-operated rats except for the administration of *15427* (40 mg/kg, i.v.) ($N=10$).

2.6. Myeloperoxidase activity

Myeloperoxidase activity, an indicator of polymorphonuclear leucocyte accumulation, was determined as previously described (Mullane et al., 1985). At the specified time point the ileum and the colon were removed and weighed. The tissues were homogenized in a solution containing 0.5% hexa-decyltrimethyl-ammonium bromide and 10 mM 3-(*N*-morpholino)-propane-sulfonic acid dissolved in 80 mM sodium 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 (16 mM) and 1 mM hydrogen peroxide. The rate of change in absorbance was measured with a spectrophotometer at 650 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmol of peroxide/min at 37 °C and is expressed in units per gram weight of wet tissue.

2.7. Localization of PAR (Poly ADP ribose) by immunohistochemistry

At the end of the experiment, the tissues were fixed in 10% formaldehyde in phosphate buffered saline (PBS) and 8 μm sections were prepared from paraffin-embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% H_2O_2 in 60% methanol for 30 min. The sections were permeabilized with 0.1% Triton X-100 in PBS for 20 min. Non-specific adsorption was minimized by incubating the section in 2% normal goat serum in PBS for 20 min. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin (DBA, Milan, Italy). Sections were incubated overnight with goat anti-poly(ADP-ribose), an indicator of PARP activation, (PAR; 1:500 in PBS, v/v) (Alexis; DBA, Milan, Italy). Specific labeling was detected with a biotin-conjugated goat anti-rabbit, and avidin–biotin peroxidase complex (DBA, Milan, Italy). 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.

2.8. Light microscopy

Ileum was collected after 1 h of reperfusion from the rats subjected to splanchnic artery occlusion shock. The colon was collected 4 days after dinitrobenzene sulfonic acid administration. After fixation for 1 week at room temperature in Dietrich solution (14.25% ethanol, 1.85% formaldehyde, 1% acetic acid), samples were dehydrated in graded ethanol and embedded in Paraplast (Sherwood Medical, Mahwah, New Jersey). Thereafter, 7-μm sections were deparaffinized with xylene, stained with hematoxylin–eosin (ileum section) or trichrome stain (colon section) and examined under in a Dialux 22 Leitz (Wetziar, Germany) microscope. Colon damage was scored by two independent observers as described previously (Liaudiet et al., 2000a,b), according to the following morphological criteria: 0, no damage; 1, localized hyperemia without ulcers; 2, linear ulcers with no significant inflammation; 3, linear ulcers with

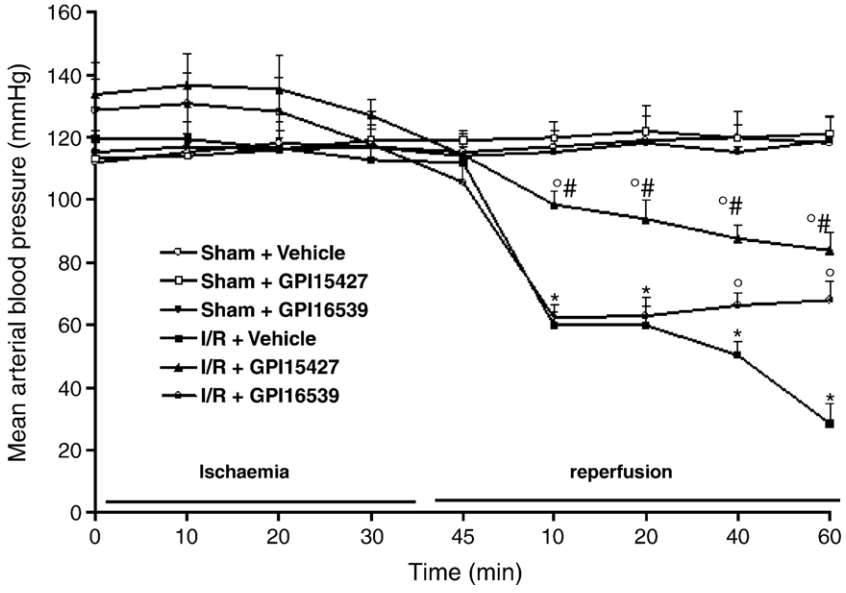

Fig. 1. Effect of GPI 15427 and GPI 16539 treatment on mean blood pressure. No significant alteration of MAP was observed in sham-operated rats. The fall in MAP and the mortality in splanchnic artery occlusion rats were significantly reduced by GPI 15427 and GPI 16539. Values are means± S.E.M. of 10 rats for each group. *$P<0.01$ *versus* sham, °$P<0.01$ versus ischemia/reperfusion. °#$P<0.05$ versus GPI16539.

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 centime of ulceration beyond an initial 2 cm.

2.9. Materials

All other reagents and compounds used were purchased from Sigma Chemical Company (Sigma, St. Louis, MO).

2.10. Data analysis

All values in the figures and text are expressed as means ± standard error (S.E.M.) of the mean of *N* observations. For the in vivo studies *N* represents the number of animals studied. In the experiments involving histology, the figures shown are representative of at least three experiments performed on different experimental days. The results were analyzed by one-way ANOVA (analysis of variance) followed by a Bonferroni post-hoc test for multiple comparisons. A *p*-value of less of than 0.05 was considered significant.

3. Results

3.1. Protective effects of GPI 15427 and GPI 16539 in splanchnic artery occlusion shock

Occlusion of the splanchnic arteries produced a continuous decline in mean arterial blood pressure (Fig. 1). At histological examination of the small intestine after 60 min of reperfusion (see representative section in Fig. 2), we found the following pathologic changes. The ileum showed infiltration with neutrophils, lymphocytes and plasma cells, which extending through the entire wall, with a number of cells being concentrated below the epithelial layer. We also found evidence of villi tip alteration (Fig. 2B). No histological alterations were found in tissue sections obtained from sham-operated rats (2A).

The PARP inhibitors GPI 15427 and GPI 16539, given 30 min post-ischemia, significantly reduced the fall in blood pressure (Fig. 1) seen after reperfusion and also reduced the degree of tissue injury (Fig. 2C and D, respectively). Assessment of neutrophil infiltration into the ileum was performed by measuring the activity of myeloperoxidase, an enzyme that is contained in, and specific for, polymorphonuclear leucocytes. Myeloperoxidase activity was significantly elevated after splanchnic ischemia/reperfusion in splanchnic artery occlusion-shocked rats (Fig. 3). In splanchnic artery occlusion-shocked rats treated with GPI 15427 and GPI 16539, tissue myeloperoxidase activity was markedly reduced in comparison to that in rats treated with vehicle (Fig. 3). Intestinal sections were also taken in order to determine the immunohistological staining for poly(ADP-ribosyl)ated (PAR) proteins (an indicator of PARP activation). Immunohistochemical analysis of intestinal sections obtained from rats subjected to splanchnic ischemia/reperfusion revealed positive staining for PAR, which was mainly localized in inflammatory and epithelial cells (Fig. 4B). In contrast, significantly less positive PAR staining was found in the intestine of rats treated with GPI 15427 (Fig. 4C) and GPI 16539 (Fig. 4D) also subjected to splanchnic ischemia/reperfusion. It is important to underline that there was no staining for

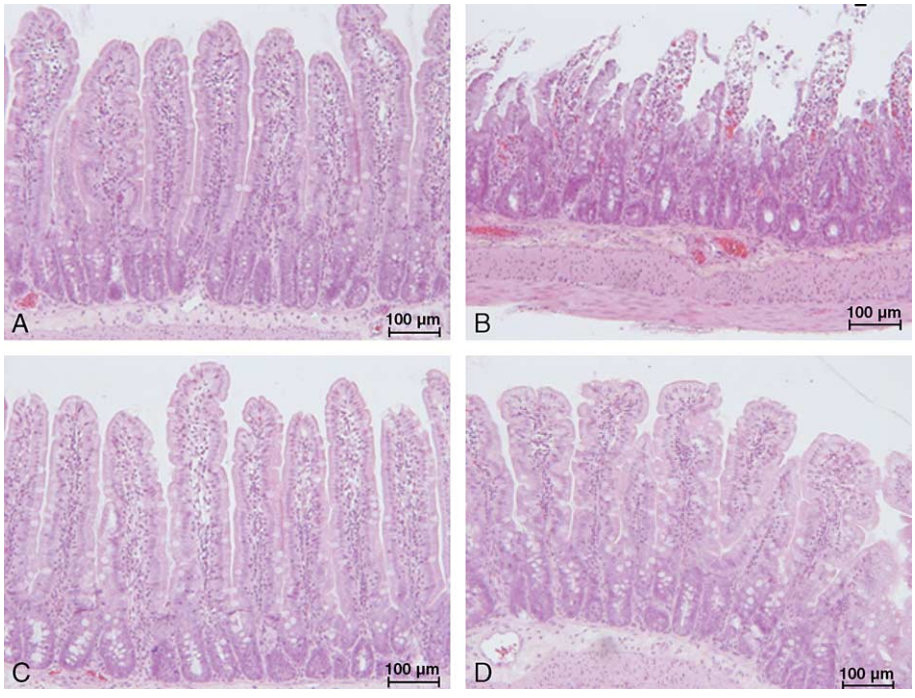

Fig. 2. Effect of GPI 15427 and GPI 16539 treatment on the tissue damage. No histological alterations were found in tissue sections obtained from sham-operated rats (A). Distal ileum section from splanchnic artery occlusion shocked-rats showed inflammatory cell infiltration extending through the wall and concentrated below the epithelial layer and demonstrating edema of the distal portion of the villi (B). Distal ileum from GPI 15427 (C) and GPI 16539-treated rats (D) shows reduced splanchnic artery occlusion-induced organ injury. The figure is representative of at least 3 experiments performed on different experimental days.

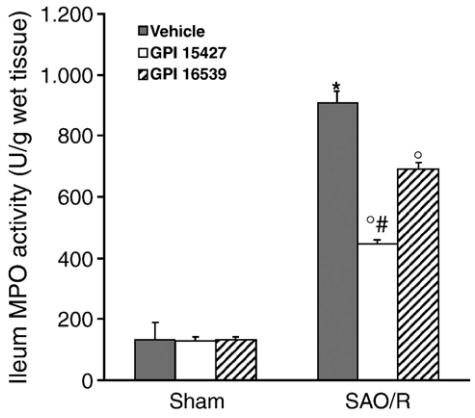

Fig. 3. Myeloperoxidase tissue levels. Reperfusion of the ischemic splanchnic circulation leads to a profound increase in myeloperoxidase level in ileum tissues which is inhibited by *GPI 15427 and GPI 16539* treatment. Values are means± S.E.M. for 10 rats in each group. *$P<0.01$ versus sham, °$P<0.01$ versus ischemia/reperfusion; °#$P<0.05$ versus GPI16539.

PAR in intestine obtained from sham-operated rats (Fig 4A). It also appears that the anti-inflammatory effect observed with the GPI 15427 treatment was significantly more potent than that with the GPI 16539 treatment.

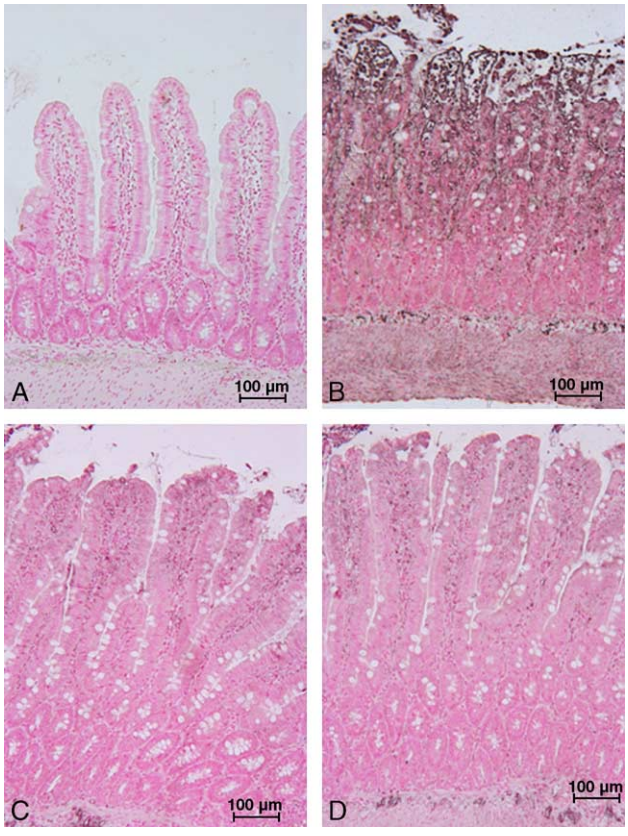

Fig. 4. Immunohistochemical staining of PAR. No staining for PAR was observed in the intestine section obtained from sham-operated rats (A). After reperfusion, PAR (B) staining was localized in the injured area from a splanchnic artery occlusion-shocked rat. There was no detectable immunostaining for PAR in the ileum from GPI 15427 (C) and GPI 16539-treated rats (D). The figure is representative of at least 3 experiments performed on different experimental days.

3.2. Effect of GPI 15427 treatment for colitis

Four days after intra-colonic administration of dinitrobenzene sulfonic acid, the colon appeared flaccid and filled with liquid stool. Macroscopic inspection of the cecum, colon and rectum showed the presence of mucosal congestion, erosion and hemorrhagic ulcerations (see damage score Fig. 5AC). The histopathologic features included transmural necrosis and edema (Fig. 6B) and a diffuse leukocyte cellular infiltrate in the submucosa (see particle Fig. 6B1) of colon sections from dinitrobenzene sulfonic acid-treated rats. The observed inflammatory changes of the large intestine were associated with an increase in the weight of the colon (Fig. 7A). Four days after colitis induced by dinitrobenzene sulfonic acid treatment, all rats had diarrhea and a significant reduction in body weight (compared with the control group of rats) (Fig. 7B). Treatment with GPI 15427 resulted in a significant decrease in the extent and severity of damage (Figs. 5BC, 6C, 7). No histological alteration was observed in the colon tissue from vehicle-treated

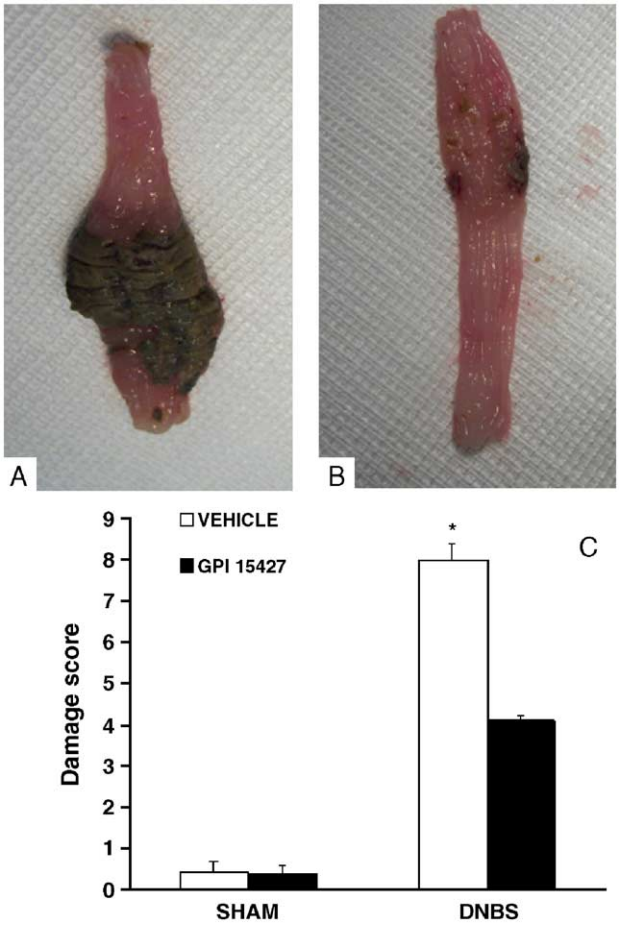

Fig. 5. Effect of GPI 15427 treatment on the damage score and on colon injury. Colon tissues from dinitrobenzene sulfonic acid-treated rats at 4 days post dinitrobenzene sulfonic acid administration (A) and the colon tissues collected from dinitrobenzene sulfonic acid-treated mice, which have received GPI 15427 treatment (B). The macroscopic damage (C) was scored by two independent observers. Treatment with GPI 15427 significantly reduced the damage score. Values are means±S.E.M. for 10 rats for each group. *$P<0.01$ vs. sham; °$P<0.01$ vs. dinitrobenzene sulfonic acid.

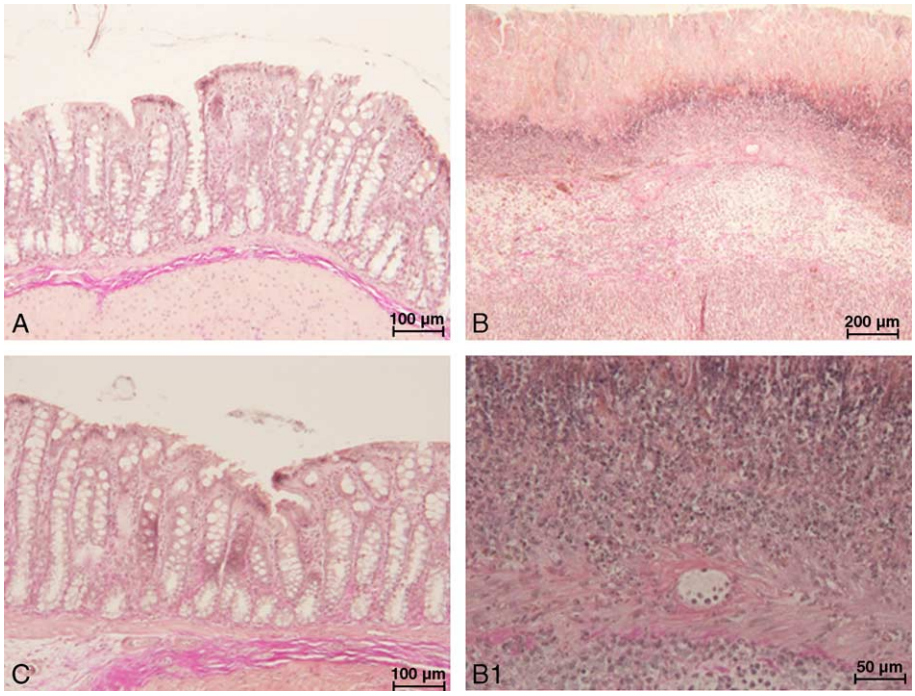

Fig. 6. Effect of GPI 15427 treatment on colon injury. No histological alterations were observed in the colon tissues collected from sham-treated rats (A). In contrast, histological examination of descending colon from dinitrobenzene sulfonic acid-treated rats revealed a complete alteration of the epithelial layer, muscularis mucosa and submucosal (B) as well as a diffuse inflammatory cell infiltration in the perilesional area (B1). Treatment with GPI 15427 (C) significantly reduced and corrected the disturbances in morphology and reduced the inflammatory cell infiltration associated with dinitrobenzene sulfonic acid administration. The figure is representative of at least 3 experiments performed on different experimental days.

rats (Fig. 6A). The colitis caused by dinitrobenzene sulfonic acid was also characterized by an increase in myeloperoxidase activity, an indicator of polymorphonuclear leucocyte accumulation, in the colon (Fig. 8). This finding is consistent with the observation made with light microscopy that the colon of vehicle-treated dinitrobenzene sulfonic acid-rats contained a large number of polymorphonuclear leucocytes. Treatment with GPI 15427 (Fig. 8.) resulted in a significant reduction in both the degree of polymorphonuclear neutrophil infiltration (determined as increase in myloperoxidase activity). To determine PARP activation during colitis, PAR formation was measured by immunohistochemical analysis in the distal colon. Colon sections obtained from vehicle-treated dinitrobenzene sulfonic acid-treated rats exhibited positive staining for PAR (Fig. 9B) localized in inflammatory cells and in disrupted epithelial cells (see particle Fig. 9B1). Sections from GPI 15427-treated rats did not reveal any positive staining for PAR (Fig. 9C). No positive staining for PAR was found in the colon section from sham-treated rats (Fig. 9A).

4. Discussion

This study provides evidence that post-injury treatment of rats with two PARP-1 inhibitors, GPI 15427 and GPI 16539, attenuates: (i) the development of splanchnic artery occlusion shock, (ii) the infiltration of the ileum with polymorphonuclear

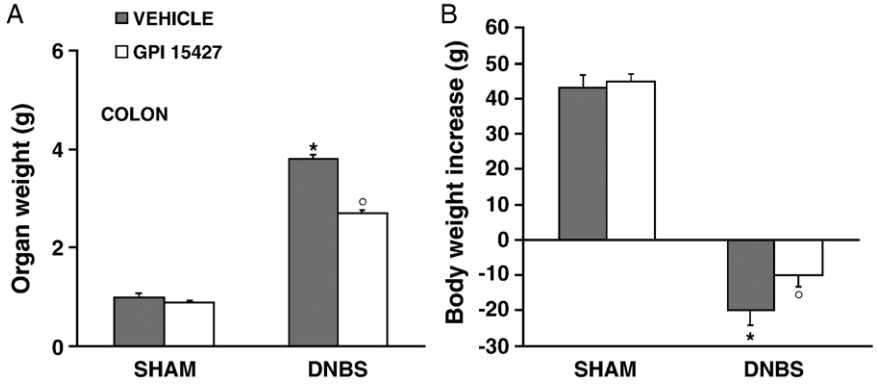

Fig. 7. Effect of GPI 15427 on organ weight (A) and body weight gain (B). A significant increase was consistently seen at 4 days after dinitrobenzene sulfonic acid injection in colon (A). The severe colitis caused by dinitrobenzene sulfonic acid was also associated with a significant loss in body weight (B). The weight of the colon as well as the body weight loss was significantly reduced in the rats which had been treated with GPI 15427. Values are means±s.e. means for 10 rats in each group. *$p<0.01$ vs. sham; °$p<0.01$ vs. dinitrobenzene sulfonic acid.

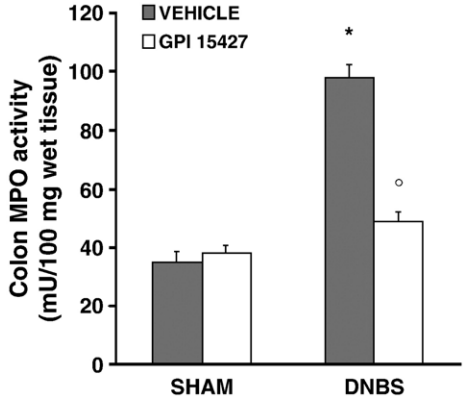

Fig. 8. Effect of GPI 15427 on neutrophil infiltration. Myeloperoxidase (MPO) activity was significantly increased in the colon from dinitrobenzene sulfonic acid-treated rats in comparison to sham. GPI 15427-treated rats show a significant reduction of myeloperoxidase activity. Values are means±s.e. means for 10 rats in each group. *$p<0.01$ vs. sham; °$p<0.01$ vs. dinitrobenzene sulfonic acid.

leucocytes, (histology and myeloperoxidase activity), (iii) the degree of PAR formation in the ileum, and (iv) the degree of ileum injury (histology) caused by ischemia and reperfusion, (v) the development of dinitrobenzene sulfonic acid-induced colitis, (vi) the infiltration of the colon by polymorphonuclear leucocytes (histology and myeloperoxidase activity), (vii) the degree of PAR formation in the colon, and (viii) the degree of colon injury (histology) in rats treated with dinitrobenzene sulfonic acid. All of these findings support the view that PARP-1 inhibition attenuates the degree of gut inflammation in the rat. There is now evidence that PARP inhibitors attenuate the tissue injury caused by ischemia-reperfusion and by inflammation. The conclusions derived from studies using PARP inhibitors have, in many cases, been substantiated by experiments using mice, in which the PARP gene (PARP-1) has been deleted (Eliasson et al., 1997; Szabo and Dawson, 1998). In these studies, the tissues and/or organs of PARP-1 knock-out mice were found to be more resistant to ischemia and reperfusion as well as inflammation (Liaudiet et al., 2000a,b). Together, the complementary results from studies of genetic deletion of PARP-1 and pharmacological inhibition of the enzyme have validated PARP as a novel target for potential therapeutic intervention to treat ischemia-reperfusion injury or inflammation. The development of PARP inhibitors for therapeutic purposes requires substantial improvement of the pharmacological profiles of compounds in terms of potency, specificity, solubility, bioavailability and toxicity.

We demonstrate here that ischemia and reperfusion of the intestine as well as experimental colitis result in a significant increase in PARP activity [measured as poly(ADP)-ribosylation of proteins by immunohistochemistry] in the ileum and in the colon, respectively.

Most notably, this increase in PARP activity was not seen in splanchnic artery occlusion-shocked rats or in dinitrobenzene

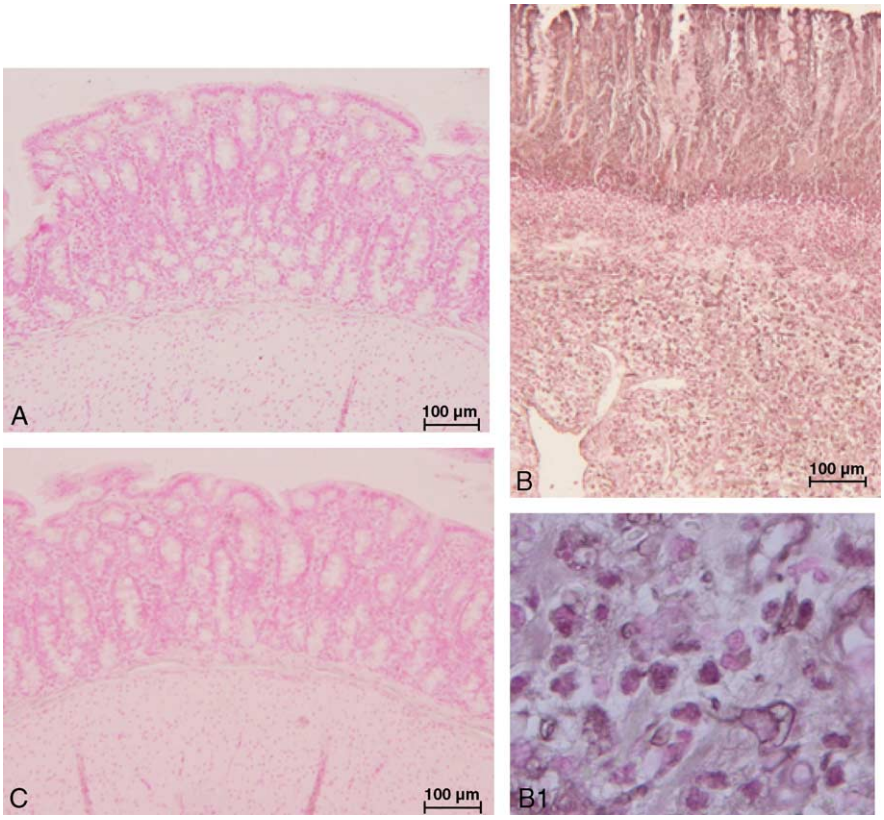

Fig. 9. Immunohistochemical localization for poly (ADP-ribose) in the colon. No staining for PAR was observed in the intestine section obtained from sham-operated rats (A). Immunohistochemical analysis for poly (ADP-ribose) (B) showed positive staining primarily localized in the infiltrated inflammatory cells and in disrupted epithelial cells (B1) from dinitrobenzene sulfonic acid-treated rats. The intensity of the positive staining for poly (ADP-ribose) (C) was significantly reduced in the colon from GPI 15427-treated rats. Figure is representative of at least 3 experiments performed on different experimental days.

sulfonic acid-treated rats treated with the water-soluble PARP-inhibitors, GPI 15427 and GPI 16539. This finding demonstrates that the dose of GPI 15427 and GPI 16539 used in this study was sufficient to abolish the increase in PARP activity caused by ischemia-reperfusion or by experimental colitis. In addition, we demonstrate that the degree of polymorphonuclear leukocyte infiltration into the ileum (at 1 h of reperfusion) as well as in the colon is significantly reduced in rats treated with GPI 15427 and GPI 16539. This result confirmed, as previously demonstrated (Mazzon et al., 2002a,b), that PARP inhibition can interrupt the interactions between neutrophils and endothelial cells. Taken together, the present data and those from another recent report (Cuzzocrea et al., 2002) demonstrate that PARP regulates the infiltration of neutrophils into inflamed tissues via a number of distinct mechanisms. The discovery that PARP regulates neutrophil trafficking may provide new insights in the interpretation of recent reports demonstrating the protective effect of PARP inhibition in experimental models of shock, ischemia-reperfusion injury and inflammation. For instance, there is good evidence that less-potent inhibitors of PARP activity (including 3-aminobenzamide: 10 mg/kg; nicotinamide: 10 mg/kg and 1,5-dihydroxyisoquinoline: 3 mg/kg) reduce by ~30–50% the degree of tissue injury associated with regional myocardial ischemia and reperfusion of the heart (Zingarelli et al., 1998), the brain (Eliasson et al., 1997), the gut (Cuzzocrea et al., 1997) and the kidney (Chatterjee et al., 1999).

Thus, as previously indicated (Mazzon et al., 2002a,b; Cuzzocrea et al., 1997), we propose that the inhibition of PARP would prevent endothelial injury, leading to an important inhibition of inflammatory cell infiltration and the consequent reactive oxygen species production. Our results demonstrate clearly that these two new PARP-1 inhibitors are protective in splanchnic artery occlusion-shock and in experimental colitis, and that regulation of neutrophil recruitment in the injured tissue probably accounts for its beneficial effects. We propose that these new classes of PARP-1 inhibitors may be useful for enhancing the tolerance of the intestine/colon to tissue injury, e.g. associated with ischemia/reperfusion or colitis. Furthermore, these data support the view that the local or systemic administration of PARP-1 inhibitors may be useful in a variety of conditions associated with inflammation.

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