5-Aminoisoquinolinone reduces colon injury by experimental colitis

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Abstract Poly (ADP-ribose) polymerase (PARP), a nuclear enzyme activated by strand breaks in DNA, plays an important role in the colon injury associated with experimental colitis. The aim of the present study was to examine the effects of 5-aminoisoquinolinone (5-AIQ), a novel and potent inhibitor of PARP activity, in the development of experimental colitis. To address this question, we used an experimental model of colitis, induced by dinitrobenzene sulfonic acid (DNBS). Compared with DNBS-treated mice, mice treated with 5-AIQ (3 mg/kg i.p.) or 3-aminobenzamide (3-AB; 10 mg/kg i.p. twice a day) and subjected to DNBS-induced colitis experienced a significantly lower rate in the extent and severity of the histological signs of colon injury. DNBS-treated mice experienced diarrhea and weight loss. Four 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 [MPO] activity in the mucosa) was associated with an up-regulation of intercellular adhesion molecule-1 (ICAM-1). Immunohistochemistry for PAR showed an intense staining in the inflamed colon. On the contrary, the treatment of DNBS-treated mice with 5-AIQ or with 3-AB significantly reduced the degree of hemorrhagic diarrhea and weight loss caused by administration of DNBS. 5-AIQ also caused a substantial reduction in the degree of colon injury, in the rise in MPO activity (mucosa), in the increase in staining (immunohistochemistry) for PAR, as well as in the up-regulation of ICAM-1 caused by DNBS in the colon. Thus, 5-AIQ treatment reduces the degree of colitis caused by DNBS. We propose that 5-AIQ treatment may be useful in the treatment of inflammatory bowel disease.

Keywords Colitis · 5-AIQ · PARP · Inflammatory infiltration · ICAM-1

Introduction

Inflammatory bowel disease (IBD) 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 species (ROS) or nitrogen species (RNS) importantly contribute to the pathophysiology of IBD. For instance, monocytes from patients with Crohn’s disease (Kitahora et al. 1988) and polymorphonuclear leukocytes (PMNs) from patients with ulcerative colitis (Shiratori et al. 1989) have an increased capacity to generate free oxygen radicals. Furthermore, advanced stages of bowel inflammation in humans (Middleton et al. 1993; Boughton-Smith et al. 1993; Lundberg et al. 1994; Nathan 1996) and animals (Morris et al. 1989; Aiko and Grisham 1995; Ribbons et al. 1995; Moureille et al. 1996; Ikeda et al. 1997) are associated with an enhanced (local) formation of nitric oxide (NO) by inducible nitric oxide synthase. Additionally, highly reactive molecules, radicals and oxidants may indiscriminately attack DNA, thus causing oxidative modification and strand breakage (Desreumaux et al. 1997; Fiocchi 1998; Takazoe et al. 2002). There is evidence, in fact, that DNA from colonic biopsy specimens of patients with ulcerative colitis have significantly increased levels of 8-hydroxyguanine, 2-hydroxyadenine,
8-hydroxyadenine and 2,6-diamino-5-formamido-pyrimidine (Desreumaux et al. 1997; Takazoe et al. 2002). Evidence from studies utilising cultured cells has demonstrated that ROS produce strand breaks in DNA which trigger the activation of the nuclear enzyme poly (adenosine 5’-diphosphate ribose) polymerase (PARP; Szabó et al. 1997).

Poly (ADP-ribose) polymerase is a ubiquitous, chromatin-bound enzyme, which is abundantly present in the nuclei of numerous cell types (Ikai and Ueda 1983). Continuous or excessive activation of PARP produces extended chains of ADP-ribose on nuclear proteins and results in a substantial depletion of intracellular NAD+ and subsequently, adenosine triphosphate (ATP), leading to cellular dysfunction and ultimately, cell death (Berger 1985; Chiarugi 2002). Chemically distinct inhibitors of PARP activity such as benzamides, e.g., 3-aminobenzamide (3-AB), nicotinamide, and isoquinoliones, e.g., 1,5-dihydroxyisoquinoline (5-hydroxyisoquinolin-1(2H)-one) (1,5-DHIQ), 3,4-dihydro-5-(4-(1-piperidinyl)butoxy)-1(2H)-isoquinolione (DPQ), can reduce the degree of injury associated with inflammation and these investigations have provided the basis for potential clinical applications of PARP inhibitors (Cuzzocrea et al. 2002). Furthermore, the degree of tissue injury caused by experimental colitis is attenuated in mice in which the gene for PARP has been disrupted by gene targeting (Zingarelli et al. 1999).

Therefore, several PARP inhibitors have been previously examined as potential novel therapeutic interventions against colon injury associated with experimental colitis. Specifically, these studies have demonstrated that the chemically distinct PARP inhibitors GPI6150, PJ34 and 3-AB can attenuate PARP activation and provide beneficial actions against colon injury and dysfunction in vivo (Jijon et al. 2000; Mabley et al. 2001; Mazzon et al. 2002). However, in contrast isoquinoliones derivatives such as 1,5-DHIQ and DPQ, 3-AB and nicotinamide are weak inhibitors of PARP activity that do not readily cross cell membranes (Banasik et al. 1992; Szabó and Dawson 1998). Furthermore, although the potency of recently developed PARP inhibitors has improved greatly, most lack good solubility in water, making it difficult to find a biocompatible vehicle for utilisation in vivo. Thus, there is still a great need for the development of potent, water-soluble inhibitors of PARP activity. Much effort has been made to develop new PARP inhibitors with better potency, selectivity and water solubility and there are now 13 chemical classes of PARP inhibitors (Cosi 2002). In (1991), Suto et al. used a cell-free preparation of PARP (purified 900-fold from calf thymus) to demonstrate that 5-aminoisoquinolione—5-aminoisoquinolin-1(2H)-one (5-AIQ)—is a water-soluble inhibitor of PARP activity. As previously published reports of the synthesis of 5-AIQ reported problems of low yield and unreliability (Wenkert et al. 1964; Suto et al. 1991), McDonald and colleagues have recently developed a novel and more efficient method for the synthesis of 5-AIQ (McDonald et al. 2000). We have previously demonstrated that 5-AIQ can reduce ischaemia/reperfusion injury of the heart, intestine and liver (Wayman et al. 2001; Mota-Filipe et al. 2002), and 5-AIQ has been shown to provide beneficial effects in rodent models of heart transplantation (Szabó et al. 2002) and lung injury (Cuzzocrea et al. 2002).

The present study was designed to evaluate the effects of 5-AIQ treatment in a rodent model of dinitrobenzene sulfonic acid (DNBS)-induced colitis. In order to gain a better insight into the mechanism(s) of action of the observed anti-inflammatory effects of 5-AIQ, we have also investigated the effects of 5-AIQ on:

1. The degree of colonic injury
2. The rise in myeloperoxidase (MPO) activity (mucosa)
3. The production of tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β; colon levels)
4. The increase in staining (immunohistochemistry) for poly (ADP-ribose) (PAR)
5. The increased expression of intercellular adhesion molecule (ICAM-1) caused by DNBS in the colon

**Materials and methods**

**Animals** The study was carried out in 6–8-week-old (20–25 g) CD1 male mice (Charles River, Calco, Italy). The animals were housed in a controlled environment and provided with standard rodent chow and water. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116/92) as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986).

**Experimental groups** Five groups of mice were used in the experiment. The first group (n=10) received an equal volume of vehicle (0.9% NaCl solution) instead of the PARP inhibitors (DNBS+vehicle group). The second and third groups (n=10 for each group) received 3-aminobenzamidine (10 mg/kg; i.p. twice a day) or 5-AIQ (3 mg/kg daily) by intraperitoneal injection. The fourth and fifth groups of animals (n=10,) the effect of PARP inhibitors was tested in groups of animals which received intracolonie administration of 50% ethanol alone. The dosage of PARP inhibitors regimen has been previously shown to exert anti-inflammatory effects (Zingarelli et al. 2003; Cuzzocrea et al. 2002). Drug treatment was started 24 h after DNBS intracolonie administration and continued until the end of the experimental period (4 days after DNBS administration).

**Induction of experimental colitis** Colitis was induced with a very low dose of DNBS (4 mg per mouse) by using a modification (Sturiale et al. 1999) of the method first described in rats (Lora et al. 1997). In preliminary experiments, this dose of DNBS was found to induce reproducible colitis without mortality. Mice were anaesthetised by enflurane. DNBS (4 mg in 100 μl of 50% ethanol) was injected into the rectum through a catheter inserted 4.5 cm proximally to the anus. Carrier alone
Myeloperoxidase activity, an indicator of PMN accumulation, was determined as previously described (Mullane et al. 1985). At 4 days after intra-colonic injection of DNBS, the colon was removed and weighed. The colon was 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 H₂O₂. The rate of change in absorbance was measured spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 μmol of peroxide per min at 37°C and was expressed in milliunits per gram weight of wet tissue.

Light microscopy After fixation for 1 week at room temperature in Dietrich solution (14.25% ethanol, 1.85% formaldehyde, 1% acetic acid), samples were dehydrated in graded ethanol and embedded in Paraplast (Sherwood Medical, Mahwah, NJ, USA). Thereafter, 7-μm sections were deparaffinised with xylene, stained with haematoxylin–eosin and trichromic van Giesson’s stain, and observed in a Zeiss microscope (Milan, Italy). In order to have a quantitative estimation of colon damage, colon sections (n=6 for each animal from each group) was scored using the criteria of Appleyard and Wallace (Appleyard and Wallace 1995) by two independent observers blinded to the experimental protocol. The following morphological criteria were considered: score 0, no damage; score 1 (mild), focal epithelial edema and necrosis; score 2 (moderate), diffuse swelling and necrosis of the villi; score 3 (severe), necrosis with presence of neutrophil infiltrate in the submucosa; score 4 (highly severe), widespread necrosis with massive neutrophil infiltrate and hemorrhage.

Myeloperoxidase activity Myeloperoxidase activity, an indicator of PMN accumulation, was administered in control experiments. Thereafter, the animals were kept for 15 min in the 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 anaesthetised with chloral hydrate and the abdomen was opened by a midline incision. The colon was removed, freed from surrounding tissues, opened along the antimesenteric border, rinsed, weighed and processed for histology and immunohistochemistry. Colon damage (macroscopic damage score) was evaluated and scored by two independent observers as described previously (Wallace et al. 1992; Zingarelli et al. 1993; Miller et al. 1995) according to the following criteria: 0, no damage; 1, localised hyperaemia without ulcers; 2, linear ulcers with no significant inflammation; 3, linear ulcers with inflammation at one site; 4, two or more major sites of inflammation and ulceration extending >1 cm along the length of the colon; and 5–8, one point is added for each centimetre of ulceration beyond an initial 2 cm.

Measurement of cytokines The levels of TNF-α and IL-1β were evaluated in the colon 4 days after intra-colonic injection of DNBS. The assay was carried out by using a commercial colorimetric kit (Calbiochem-Novabiochem, Milan, Italy).

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 intra-colonic 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, 1,500 μl of 20% acetic acid (pH 3.5), 1,500 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 3,000 g for 10 min. The absorbance of the supernatant was measured by spectrophotometry at 650 nm.

Localisation of PAR and ICAM-1 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 deparaffinisation, endogenous peroxidase was quenched with 0.3% H₂O₂ in 60% methanol for 30 min. The sections were permeabilised with 0.1% Triton X-100 in PBS for 20 min. Non-specific adsorption was minimised by incubating the section in 2% normal goat serum in PBS for 20 min. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin (DBA, Milan, Italy). Sections were incubated overnight with mouse anti-rat antibody directed at ICAM-1 polyclonal antibody (CD54; 1:500 in PBS, v/v; Pharmigen DBA, Milan, Italy) or with goat anti-poly(ADP-ribose), an indicator of PARP activation. PAR; 1:500 in PBS, v/v; Alexis; DBA, Milan, Italy). Specific labelling was detected with a biotin-conjugated goat anti-rabbit, donkey anti-goat or mouse IgG and avidin–bovitin peroxidase complex (DBA, Milan, Italy). To verify the binding specificity for ICAM-1 and 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.

Immunocytochemistry photographs (n=5) were assessed by densitometry as previously described (Cuzzocrea et al. 2000) by using Optilab Graftek software on a Macintosh personal computer.

Reagents Biotin blocking kit, biotin-conjugated goat anti-rabbit IgG and avidin–bovitin peroxidase complex were obtained from Vector Laboratories (Burlingame, CA, USA). Primary ICAM-1 (CD54) for immunohistochemistry was purchased from Pharmingen. Reagents, secondary and nonspecific IgG antibody for immunohistochemical analysis were from Vector Laboratories Inc. All other reagents and compounds used were obtained from Sigma.
Chemicals (Milan, Italy).

Statistical analysis All values in the figures and text are expressed as mean ± SEM of n observations, where n represents the number of animals studied. In the experiments involving histology or immunohistochemistry, the figures shown are representative of at least three experiments performed on different experimental days. Data sets were examined by one- and two-way analysis of variance and individual group means were then compared with Student’s unpaired t-test. Non-parametric data were analysed with the Fisher’s exact test. A P-value less than 0.05 was considered significant.

Results

Effect of PARP inhibitors treatment on the degree of colitis (histology and general assessment)

Four days after intra-colonic administration of DNBS, 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 damage score Fig. 1a). The histopathological features included a transmural necrosis and edema and a diffuse leukocyte cellular infiltrate in the submucosa of colon sections from DNBS-treated mice (Figs. 1b, 2b). The observed inflammatory changes of the large intestine were associated with an increase in the weight of the colon (Fig. 3a). Four days after colitis induced by DNBS treatment, all mice had diarrhea and a significant reduction in body weight (compared with the control group of mice; Fig. 3b). Treatment with 5-AIQ (3 mg/kg; i.p. daily) or with 3-AB (10 mg/kg; i.p. twice a daily) resulted in a significant decrease in the extent and severity of damage (Figs. 1, 2c, 3). Please note that the anti-inflammatory effect observed with the 5-AIQ treatment is significantly more potent in comparison with the 3-AB treatment. No histological alteration was observed in the colon tissue from vehicle-treated mice (Figs. 1, 2a, 3).

Effect of PARP inhibitor treatment on cytokine production

The levels of TNF-α and IL-1β were significantly elevated in the colon at 4 days after DNBS treatment. In contrast, the levels of these cytokines were significantly lower in mice treated with 5-AIQ or with 3-AB (Fig. 4). No significant increase in the levels of cytokines was observed in the colon of sham-treated mice.

Effect of PARP inhibitors on ICAM-1 expression, PMN infiltration and lipid peroxidation

The colitis caused by DNBS was also characterised by an increase in MPO activity, an indicator of PMN accumulation in the colon (Fig. 5a). This finding is consistent with the observation made with light microscopy that the colon of vehicle-treated DBNS-mice contained a large number of PMNs. 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. 5b). Treatment with 5-AIQ (3 mg/kg; i.p. daily) or with 3-AB (10 mg/kg; i.p. twice a daily) resulted in a significant reduction of both the degree of polymorphonuclear neutrophils infiltration (determined as increase in myeloperoxidase activity) and the associated lipid peroxidation (increase in tissue malondialdehyde levels) in the inflamed colon (Fig. 5). To further elucidate the effect of PARP inhibition on PMN accumulation in inflamed colon, we evaluated the intestinal expression of ICAM-1. Tissue sections obtained from sham-operated mice with anti-ICAM-1 antibody showed a specific staining along the vessels, demonstrating that ICAM-1 is expressed constitutively in endothelial cells (Figs. 6, 7a). After DNBS administration, the staining intensity substantially increased in the vessels of the lamina propria and submucosa. Immunohistochemical staining for ICAM-1 was also present in epithelial cells of injured colon and in infiltrated inflammatory cells in damaged tissues from DNBS-treated mice (Figs. 6, 7b). Sections from 5-AIQ-treated mice did not reveal any up-regulation of constitutive ICAM-1, which was normally expressed in the endothelium along the vascular wall (Figs. 6, 7c). Similarly, tissues from 3-AB-treated mice showed no upregulation of the constitutive ICAM-1 (Fig. 6).
Fig. 2 Effect of 5-aminoisoquinolinone (5-AIQ) on colon injury. a No histological alteration was observed in the colon section from sham-treated mice. b Mucosal injury was produced after DNBS administration characterised by the absence of epithelium and a massive mucosal and submucosal infiltration of inflammatory cells. c Treatment with 5-AIQ (3 mg/kg/day i.p.) corrected the disturbances in morphology associated with DNBS administration. The figure is representative of at least three experiments performed on different experimental days.

Fig. 3 Effect of PARP inhibitor treatment on a colon weight and b body weight changes after DNBS intracolonic administration. Four days after DNBS administration a significant increase in the weight of the colon was observed (a) and a significant reduction in body weight increase (b). Treatment with 5-AIQ (3 mg/kg daily i.p.) or with 3-AB (10 mg/kg i.p. twice a day) significantly reduced the colon weight and the increase in body weight loss. Data are means ± SEM of ten mice for each group. *P<0.01 vs. SHAM; †P<0.01 vs. DNBS; ‡P<0.01 vs. 3-AB.
Please note that the effect observed on ICAM-1 expression, PMN infiltration and lipid peroxidation with the 5-AIQ treatment is significantly more potent in comparison with the 3-AB treatment.

Effects of PARP inhibitor treatment on PAR formation

To determine PARP activation during colitis, PAR formation was measured by immunohistochemical analysis in the distal colon. Colon sections obtained from vehicle-treated DNBS-treated mice exhibited positive staining for PAR (Figs. 6, 8b) localised in inflammatory cells and in disrupted epithelial cells. Sections from 5-AIQ-treated mice did not reveal any positive staining for PAR (Figs. 6, 8c). Similarly, tissues from 3-AB-treated mice showed less positive staining for PAR (Fig. 6). No positive staining for PAR was found in the colon section from sham-treated mice (Figs. 6, 8a).

Please note that the inhibitory effect on PAR formation observed with the 5-AIQ treatment is significantly more potent in comparison with the 3-AB treatment.

Discussion

We demonstrate here that the treatment with 5-AIQ significantly reduced:

1. The degree of hemorrhagic diarrhea and weight loss
2. The degree of colonic injury
3. The infiltration of the colon PMNs
4. The positive staining (immunohistochemistry) for PAR
5. The increased expression of ICAM-1 caused by DNBS in the colon

All of these findings support the view that PARP activation plays an important role in the development of experimental colitis and those potent PARP inhibitors like 5-AIQ may be useful in the therapy of IBD. What, then, is the mechanism by which 5-AIQ inhibits colon inflammation caused by injection of DNBS?

Using a new and potent PAR inhibitor (5-AIQ), we confirm here as previously described (Zingarelli et al. 1999) that the activation of PAR mediates leukocyte-endothelial interactions by regulating the expression of ICAM-1 during experimental colitis. In 5-AIQ-treated mice subjected to DNBS-induced colitis, the up-regulation of ICAM-1 in the colon was largely attenuated. Endothelial cells appear to be major regulators of PMN trafficking, regulating the process of PMN chemoattraction, adhesion and emigration from the vasculature to the tissue. ICAM-1 is constitutively expressed on the surface of endothelial cells and is involved in PMN adhesion (Mulligan et al. 1992; Davenpeck et al. 1994; Farhood et al. 1995). Hypoxic or injured endothelial cells synthesise pro-inflammatory cytokines, which can up-regulate endothelial expression of ICAM-1 in an autocrine fashion (Shreeniwas et al. 1992; Luscinskas et al. 1995). Significant expression of ICAM-1 in microvessels of previously ischemic tissues occurs within 1 h after reperfusion (Clark et al. 1995; Horie et al. 1996) The upregulation in the expression of ICAM-1 corresponds with the induction of PMN recruitment within the first 4 days after DNBS administration (Zingarelli et al. 1999; Cuzzocrea et al. 2001). In accordance with these findings, we observed that DNBS (at day 4) induced an up-regulation of ICAM-1 on endothelial cells. The PAR inhibitor 5-AIQ abolished the up-regulation of ICAM-1 (Figs. 6, 7c), but did not affect the constitutive expression of ICAM-1 on endothelial cells (Fig. 5). These results confirm that inhibition of

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**Fig. 7** Immunohistochemical localisation of ICAM-1 in the colon of (a) sham-, (b) DNBS- and (c) 5-AIQ-treated mice. Section obtained from DNBS-treated mice showing intense positive staining for ICAM-1 on endothelial cells (b). No positive endothelial staining for ICAM-1 was observed in tissue sections obtained from mice treated with 5-AIQ (3 mg/kg/day i.p.)- (c) or in the tissue from sham-treated mice (a). The figure is representative of at least three experiments performed on different experimental days.
PARS activity may interfere with the interaction of PMNs and endothelial cells at the late firm adhesion phase mediated by ICAM-1. The absence of an increased expression of the adhesion molecules in the colon tissue of 5-AIQ-treated mice correlated with the reduction of leukocyte infiltration as assessed by the specific granulocyte enzyme MPO and with the moderation of the tissue damage as evaluated by histological examination. It is noteworthy, however, that tissue MPO activity was not completely abolished. This result is consistent with previous studies demonstrating that constitutive levels of ICAM-1 appear to be sufficient to support a lower degree of CD11/CD18-dependent trans-endothelial migration of activated PMNs (Furie et al. 1991; Kukiela et al. 1994).

Reduction of PMN infiltration was also paralleled with the inhibition of PAR immunoreactivity. Our finding (i.e., that PAR staining is reduced in 5-AIQ animals) coupled with the protective effects of PARP inhibition proves the existence of a self-amplifying suicide cycle in which early oxidant production by endothelium activates PARP; the consequent endothelium injury with activation of PMN-attractive factors (e.g., ICAM-1) and PMN infiltration leads to further production of oxidants, which ultimately are responsible for the colonic injury. Inhibition of PARP would intercept this cycle at the level of endothelial injury. This model would explain the reduction of malondialdehyde tissue levels during DNBS-induced colitis (see Fig. 4b) in the 5-AIQ or 3-AB-treated mice: reduced neutrophil infiltration leads to reduced reactive oxygen species. Therefore, to its effect on preserving the cellular energetic status and protecting against oxidant-induced cell necrosis, regulation of neutrophil recruitment may represent a novel important additional anti-inflammatory mode of action of this novel inhibitor of PARP activity.

Several in vitro and in vivo studies have demonstrated that the catalytic activity of the nuclear enzyme PARP, induced by single DNA strand breakage, is a direct result of oxidant injury in conjunction with a variety of immunological stimuli, including bacterial endotoxin and cytokines. More specifically, it has been previously

**Fig. 8** Immunohistochemical localisation of PAR in the colon of a sham-, b DNBS- and c 5-AIQ-treated mice. Immunohistochemical analysis for PAR (b) shows positive staining localised in the injured area from DNBS-treated mice. The intensity of the positive staining for PAR (c) was markedly reduced in tissue sections obtained from mice treated with 5-AIQ (3 mg/kg/day i.p.). No positive staining for PAR (a) was observed in tissue sections obtained from sham-treated mice. The figure is representative of at least three experiments performed on different experimental days.
reported that oxidant injury by NO, peroxynitrite and/or hydrogen peroxide induces metabolic changes and cytotoxicity in association with the intracellular elevation of PARP activity in macrophages and in pulmonary epithelial, smooth muscle and endothelial cells (Szabó and Dawson 1998). Recently, in vitro experiments with HUVEC cells, have demonstrated that oxidant injury by peroxynitrite or TNF-α stimulation induces an up-regulation of P-selectin and ICAM-1 surface expression, a process that is prevented by the PARP inhibitor 5-AIQ. It is possible that an enhanced adhesion molecule expression may be mediated by an increase in intracellular PARP activity. Thus, due to its water-solubility (as well as its potency), 5-AIQ currently represents a good pharmacological tool with which to elucidate the role of PARP in the pathophysiology of inflammation and other disease states (Thiemermann 2002). In contrast to “prototypical” or “classical” PARP inhibitors such as benzamidine analogues that have been available for over 20 years, there is currently a dearth of information available on the selectivity and potential side effects of more recently developed and potent PARP inhibitors such as the isoquinolinones (Szabó and Dawson 1998). Although it has been established that 5-AIQ displays 1000-fold selectivity for PARP over mono (ADP-ribose) transferase (McDonald et al. 2000) the effects of 5-AIQ on other enzymes has not been established. Furthermore, only limited pharmacodynamic and pharmacokinetic data exist for these novel PARP inhibitors and, therefore, appropriate caution should be used in experimental design and in the interpretation of data obtained using compounds such as 5-AIQ. In conclusion, the data presented here confirm that PARP is involved in the regulation of the expression of adhesion molecules and that, consequently, PARP plays a role in the tissue infiltration of PMNs.

The discovery of the concept that PARP regulates neutrophil trafficking may provide new insights in the interpretation of recent reports demonstrating the protective effect of PARS inhibition in experimental models of shock, ischaemia/reperfusion injury and inflammation.

of distinct mechanisms. The discovery of the concept that PARP regulates PMN trafficking may provide new insights in the interpretation of recent reports demonstrating the protective effect of PARS inhibition in experimental models of shock, ischaemia/reperfusion injury and inflammation.

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