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# Beneficial effects of 5-aminoisoquinolinone, a novel, potent, water-soluble, inhibitor of poly (ADP-ribose) polymerase, in a rat model of splanchnic artery occlusion and reperfusion

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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 tissue injury associated with ischemia–reperfusion and inflammation. Splanchnic artery occlusion and reperfusion causes an enhanced formation of reactive oxygen species which contribute to the pathophysiology of shock. The aim of the present study was to investigate the effects of 5-aminoisoquinolinone (5-AIQ), a potent water-soluble inhibitor of poly(ADP-ribose) polymerase (PARP), in the pathogenesis of splanchnic artery occlusion shock. Splanchnic artery occlusion shock was induced in rats by clamping both the superior mesenteric artery and the celiac artery for 45 min, followed thereafter by release of the clamp (reperfusion). At 60 min after reperfusion, all animals were sacrificed for histological examination and biochemical studies. Treatment of rats with 5-AIQ (3 mg/kg i.v.), attenuated the fall of mean arterial blood pressure caused by splanchnic artery occlusion shock. 5-AIQ also attenuated the ileum injury as well as the increase in the tissue levels of myeloperoxidase and malondialdehyde caused by splanchnic artery occlusion shock in the ileum. The immunohistochemical examination also demonstrated a marked increase in the immunoreactivity to PAR, nitrotyrosine, and intercellular adhesion molecule (ICAM-1) in the necrotic ileum from splanchnic artery occlusion-shocked rats. 5-AIQ treatment significantly reduced the increase of positive staining for PAR, nitrotyrosine and ICAM-I. In conclusion, these results show that 5-AIQ, a new water-soluble potent inhibitor of poly(ADP-ribose) polymerase, exerts multiple protective effects in splanchnic artery occlusion/reperfusion shock.


*Keywords:* 5-Aminoisoquinolinone; Reperfusion; Artery occlusion

## 1. Introduction

Poly(ADP-ribose) polymerase-1 (PARP-1) is a member of the PARP enzyme family consisting of PARP-1 and several recently identified novel poly(ADP-ribosylating) enzymes. PARP-1 is an abundant nuclear protein functioning as a DNA nick sensor enzyme. Upon binding of DNA breaks, activated PARP cleaves NAD+ into nicotinamide and ADP-ribose and polymerizes the latter onto nuclear acceptor proteins including histones, transcription factors, and PARP itself. Oxidative stress-induced overactivation of PARP consumes NAD+ and consequently ATP, culminating in cell dysfunction or necrosis (Du et al., 2003). PARP inhibitors, such as nicotinamide and 3-aminobenzamide have been used previously in both in vivo and in vitro studies investigating their beneficial role in various pathophysiological conditions ranging from ischemia–reperfusion injury to inflammation (Cuzzocrea et al., 1999).

There is good evidence that various chemically distinct inhibitors of PARP activity including 3-aminobenzamide, nicotinamide and 1,5-dihydroxyisoquinolinone 5-hydroxyisoquinolin-1-2*H*-one reduce the degree of tissue injury associated with regional ischemia and reperfusion of the

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heart (Thiemermann et al., 1997; Zingarelli et al., 1997a,b; Bowes et al., 1998b; Docherty et al., 1999 ), the brain (Eliasson et al., 1997), the gut (Cuzzocrea et al., 1997) and the kidney (Chatterjee et al., 2000). Most notably, the degree of tissue injury caused by ischemia and reperfusion of the heart (Grupp et al., 1999; Pieper et al., 2000), brain (Eliasson et al., 1997) and ileum (Liaudiet et al., 2000b) is attenuated in mice in which the gene for PARP has been disrupted by gene targeting PARP knock mice.

However, 3-aminobenzamide is a weak inhibitor of PARP activity that does not readily cross cell membranes (Bowes et al., 1998a, 1999). Although 1,5-dihydroxyisoquinoline and 3,4-dihydro-5-[4-(piperidin-1-yl)butoxy]isoquinolin-1(2*H*)-one (DPQ) are more potent inhibitors of PARP activity, these agents have to be dissolved in dimethylsulfoxide (DMSO). DMSO itself is a potent scavenger of hydroxyl radicals and inhibits PARP activity. Thus, there is still a great need for the development of potent, water-soluble inhibitors of PARP activity. In 1991, Suto et al. (1991) reported that 5-aminoiso-quinolin-1(2*H*)-one (5-AIQ) is a water-soluble inhibitor of PARP activity in a cell-free preparation (enzyme purified 900-fold from calf thymus). Recently, we have reported a novel route for the synthesis of 5-aminoisoquinoline (5-AIQ) and demonstrated that this compound is a water-soluble, potent inhibitor of PARP activity in human cells (Mc Donald et al., 2000). Most notably, recent study have clearly demonstrated that 5-AIQ reduced tissue injury in various experimental models including hemorrhagic shock, liver (Mota-Filipe et al., 2002) and heart (Mc Donald et al., 2000) ischemia and reperfusion and in acute inflammation. Based on these previous studies, we have investigated the effect of 5-AIQ in a model of gut ischemia and reperfusion injury (splanchnic artery occlusion shock). The results of the current study confirm the important role of PARP activation in the pathophysiology of splanchnic artery occlusion shock and support the previous suggestion that pharmacological inhibition of PARP may be a novel approach or therapeutic potential in ischemia–reperfusion injury.

## 2. Materials and methods

### 2.1. Animals

Male Sprague Dawley rats (250–300 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 the Italian Law relating to the protection of animals used for experimental and other scientific purposes (D.M. 116192) as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986). The experiments were performed in adherence to the National Institutes of Health Guidelines on the Use of Laboratory Animals, and the study was approved by the Institutional Animal Care and User Committee of the University of Messina.

### 2.2. Surgical procedures

Male Sprague–Dawley rats were anesthetized with pentobarbital (45 mg/kg intraperitoneally). After anesthesia, catheters were placed in the carotid artery and jugular vein as described previously (Caputi et al., 1980). Blood pressure was monitored continuously by 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. Rats were observed for 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 and the splanchnic circulation was allowed to reperfuse for 1 h at which time the experiment was terminated. After 1-h reperfusion, animals were sacrificed and an 8–10 cm length of ileum 30 cm distal to the stomach was isolated, washed in saline and used for histological examination of the bowel and for biochemical studies.

### 2.3. Experimental groups

Rats were randomly allocated into the following groups. In the treated group of animals, 5-AIQ, was given i.v. at 5 min before ischemia (3 mg/kg) (splanchnic artery occlusion + 5-AIQ group). In a vehicle-treated group of rats, vehicle (saline solution) was given instead of 5-AIQ (splanchnic artery occlusion group). In separate groups of rats, surgery was performed in its every aspect identical to the one in the splanchnic artery occlusion group, except that the blood vessels were not occluded (time-controlled sham group: Sham). In an additional group of animal, sham surgery was combined with the administration of 5-AIQ (dose as above) (Sham + 5-AIQ). The doses of 5-AIQ used here to reduce ischemia–reperfusion injury in the gut have previously been reported by us to reduce the tissue injury caused by ischemia–reperfusion in the liver (dose–response curve study) (Mota-Filipe et al., 2002). In another sets of studies, following reperfusion, the various groups of rats ($n = 20$ for each group) were observed for 24 h in order to determine survival differences.

### 2.4. Histological examination

For the histological examination, biopsies of the small intestine were taken 60 min after reperfusion. The tissue slices were fixed in 10% neutral-buffered formaldehyde for 5 days embedded in paraffin and sectioned. The sections were stained with hematoxylin and eosin.

### 2.5. Immunohistochemical localization of ICAM-1, PARP and nitrotyrosine

At the end of the experiment, the tissues were fixed in 10% (w/v) phosphate-buffered saline (PBS, 0.01 M, pH 7.4)-buffered formaldehyde and 8-μm sections were prepared from paraffin-embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% (v/v) hydrogen peroxide in 60% (v/v) methanol for 30 min. The sections were permeabilized with 0.1% (w/v) Triton X-100 in PBS for 20 min. Nonspecific adsorption was minimized by incubating the section in 2% (v/v) normal goat serum in PBS for 20 min. Endogenous biotin and avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin, respectively (DBA, Milan, Italy). Sections were incubated overnight with antinitrotyrosine rabbit polyclonal antibody (1:500 in PBS, v/v) or with mouse antirat antibody directed at ICAM-1 (CD54) (1:500 in PBS, v/v) (DBA). Specific labeling was detected with a biotin-conjugated goat antirabbit or goat antimouse IgG and avidin–biotin peroxidase complex (DBA). To verify the binding specificity for ICAM-1, some sections were also incubated with primary antibody only (no secondary antibody) or with secondary antibody only (no primary antibody). In these situations, no positive staining was found in the sections indicating that the immunoreactions were positive in all the experiments carried out. In order to confirm that the immunoreactions for the nitrotyrosine were specific, some sections were also incubated with the primary antibody (antinitrotyrosine) in the presence of excess nitrotyrosine (10 mM) to verify the binding specificity. Immunocytochemistry photographs ($N=5$ from each tested tissue section) were assessed by densitometry by using Optilab Graftek software on a Macintosh personal computer.

### 2.6. Myeloperoxidase activity

Myeloperoxidase activity, an index of polymorphonuclear leukocyte accumulation, was determined as previously described (Mullane et al., 1985). Intestinal tissues collected 60 min after reperfusion and homogenized in a solution containing 0.5% hexadecyl–trimethyl–ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at 20,000 × *g* al 4 °C. An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl-benzidine (1.6 mM) and 0.1 mM $H_20_2$. The rate of change in absorbance was measured by a spectrophotometer at 650 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmo1 of peroxide min-1 at 37 °C and was expressed in microunits per gram weight of wet tissue.

### 2.7. Reagents

Biotin blocking kit, biotin-conjugated goat antirabbit IgG and avidin–biotin peroxidase complex, were obtained from Vector Laboratories (DBA). Primary antinitrotyrosine antibody was purchased from Upstate Biotech (DBA). Primary anti-PAR was purchased from Alexis (DBA). Primary ICAM-l (CDS4) was purchased from Pharmingen (DBA). All other reagents and compounds used were obtained from Sigma (St. Louis, MO).

### 2.8. Statistics analysis

Data are expressed as mean ± standard error of the mean (S.E.M.) in all figures. 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 analysis of variance (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. Protective effects of 5-AIQ in splanchnic artery occlusion shock

Occlusion of the splanchnic arteries produced a continuous decline in mean arterial blood pressure (Fig. 1A). At histological examination of the small intestine at 60 min of reperfusion (see representative section at Fig. 2), we found the following pathologic changes. The ileum showed infiltration with neutrophils, lymphocytes and plasma cells, extending through the entire wall, with a number of cells being concentrated below the epithelial layer. We found some evidence of focal ulceration, sometimes extending through the muscularis mucosa (Fig. 2A). No histological alterations were found in tissue sections obtained from sham-operated rats (data not shown).

The PARP inhibitor 5-AIQ significantly reduced the fall in blood pressure (Fig. 1A) seen after reperfusion and also reduced the degree of tissue injury (Fig. 2B). In a separate set of experiments, we have evaluated the effects of the PARP inhibitor on survival. All sham-operated rats survived the entire 24 h observation period (Fig. 1B), while splanchnic artery occlusion produced a profound shock state characterized by a 100% lethality at the end of the 24 h reperfusion period (Fig. 1B). Administration of 5-AIQ significantly reduced the mortality caused by I/R (Fig. 1B).

### 3.2. ICAM-1 expression and neutrophil infiltration are reduced in 5-AIQ treated rats

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 lysosomes. Myeloperoxidase activity was significantly elevated after splanchnic ischemia/reperfusion in

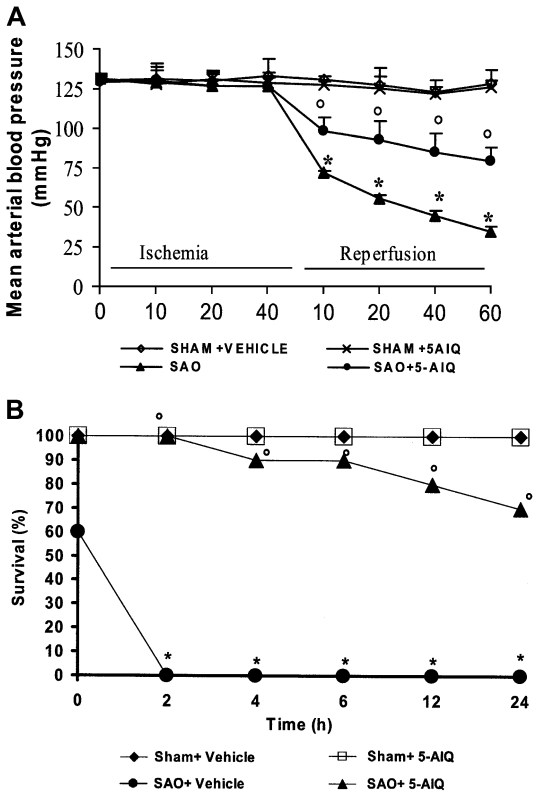


Fig. 1. Effect of 5-AIQ treatment on mean arterial blood pressure (A) and mortality (B). No significant alteration of mean arterial blood pressure was observed in sham-operated rats. Fall in mean arterial blood pressure and the mortality in splanchnic artery occlusion rats were significantly reduced by 5-AIQ treatment (3 mg/kg). Values are means ± S.E.M. *$P<0.01$ versus sham, °$P<0.01$ versus I/R.

splanchnic artery occlusion-shocked rats (Fig. 3A). In splanchnic artery occlusion-shocked rats treated with 5-AIQ, tissue myeloperoxidase activity (Fig. 3A) was markedly reduced in comparison to those treated with vehicle. The increase in myeloperoxidase activity was associated with the increase in immunonohistochemical staining for ICAM-1 (Figs. 4A and 5) in the injured splanchnic tissue. Significant less positive staining for ICAM-I was found in the intestine of splanchnic artery occlusion-shocked rats treated with 5-AIQ (Figs. 4B and 5). Staining of ileum 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 (Fig. 5).

### *3.3. 5-AIQ treatment reduced lipid peroxidation, nitrotyrosine and PAR formation*

The release of free radicals and oxidant molecules during the early period of reperfusion has been suggested to contribute significantly to the tissue necrosis and mucosal dysfunction (Cuzzocrea et al., 1997). Splanchnic ischemia/reperfusion injury of rats was characterized by an increase in tissue malondialdehyde, an indicator of lipid peroxidation (Fig. 3B). Furthermore, positive staining for nitrotyrosine, a marker of nitrosative stress, was found on epithelial and infiltrated inflammatory cells in the injured small intestine of rats subjected to splanchnic artery occlusion shock (Figs. 5 and 6A). In splanchnic artery occlusion-shocked rats treated with 5-AIQ, tissue malondialdehyde levels (Fig. 3B) were markedly reduced in comparison to those treated

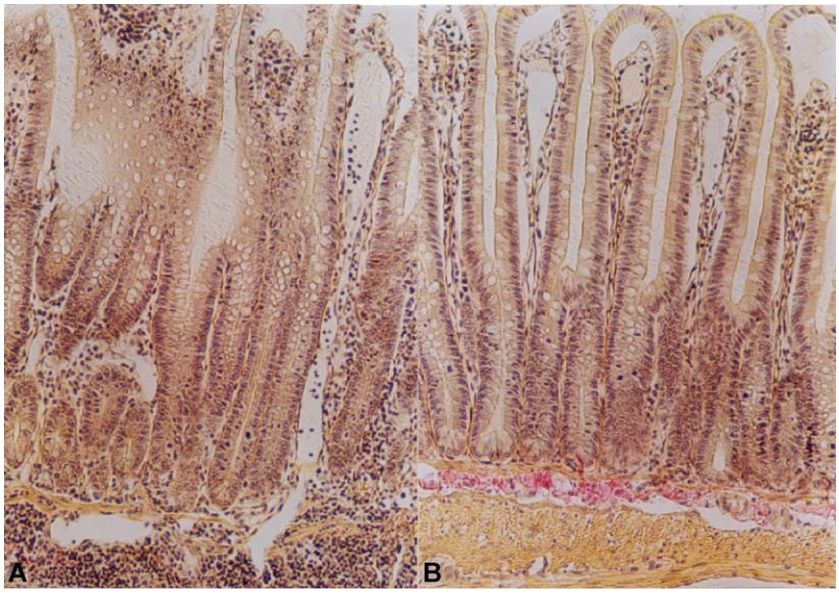


Fig. 2. Distal ileum section from splanchnic artery occlusion-shocked rats (A) showed inflammatory infiltration by polymorphonuclear leukocytes and lymphocytes extending through the wall and concentrated below the epithelial layer and demonstrating edema of the distal portion of the villi. Distal ileum from 5-aiq-treated rats (B) shows reduced splanchnic artery occlusion-induced organ injury. Original magnification: × 125. Figure is representative of at least three experiments performed on different experimental days.

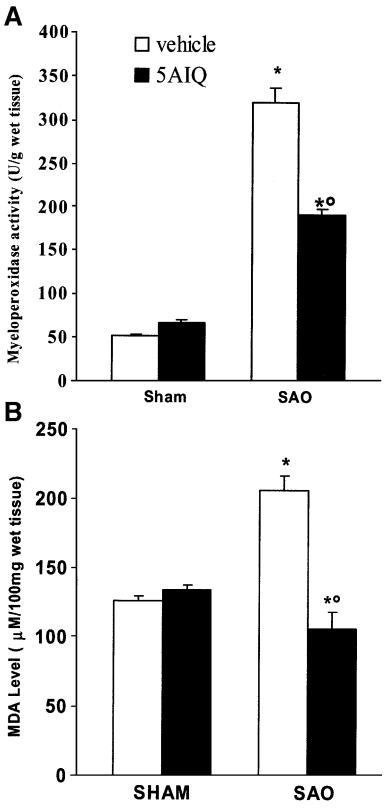


Fig. 3. Reperfusion of the ischemic splanchnic circulation leads to profound increase in myeloperoxidase (A) and in malondialdehyde (B) in ileum tissues which is inhibited by 5-AIQ treatment (3 mg/kg). Values are means ± S.E.M. *$P<0.01$ versus sham, °$P<0.01$ versus I/R.

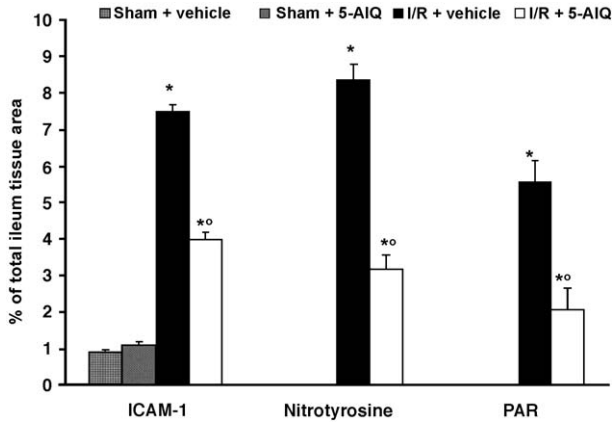


Fig. 5. Typical densitometry evaluation. Densitometry analysis of immunocytochemistry photographs ($n=5$) for ICAM-1, nitrotyrosine and PAR from ileum was assessed. The assay was carried out by using Optilab Graftek software on a Macintosh personal computer (CPU G3-266). Data are expressed as % of total tissue area. *$P<0.01$ versus sham vehicle, °$P<0.01$ versus IR.

with vehicle. No positive nitrotyrosine staining was found in the intestine of splanchnic artery occlusion-shocked rats treated with 5-AIQ (Figs. 5 and 6B).

Intestinal sections were also taken in order to determine the immunohistological staining for poly(ADP-ribosylated) (PAR) proteins (an indicator of PARP activation). Immunohistochemical analysis of intestinal sections obtained from rats subjected to splanchnic ischemia/reperfusion revealed a positive staining for PAR which was primarily localized in inflammatory cells (Figs. 5 and 6C). In contrast, significantly less positive PAR staining was found in the intestine of rats treated with 5-AIQ also subjected to splanchnic ischemia/reperfusion (Figs. 5 and 6D). It is important to underline that there was no staining for either nitrotyrosine or PAR in intestine obtained from sham-operated mice (Bittermann and Lefer, 1988; Fig. 5).

## 4. Discussion

Occlusion of the splanchnic arteries followed by reperfusion in anaesthetized rats results in an irreversible circulatory failure and shock. This model of shock is characterized by a marked decrease in systemic blood pressure and leucopenia as well as by disturbances in reticuloendothelial system activity, increased macrophage and plasma levels of thromboxane B2 and elevated plasma levels of platelet-activating factor (Lefer and Lefer, 1993; Zimmermann et al., 1993; Squadrito et al., 1994).

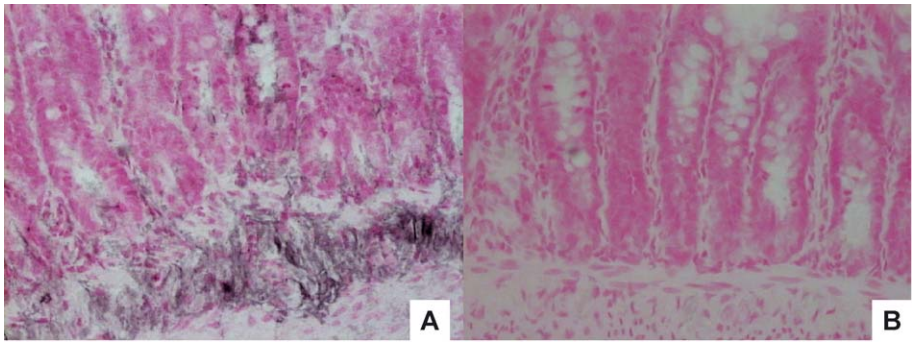


Fig. 4. I/R induced an increase of the positive staining for ICAM-1 along the endothelium wall (A). In 5-AIQ-treated rats (B) subjected to splanchnic artery occlusion shock, there was no increase of immunostaining for ICAM-1, which was present only along the endothelium wall. Original magnification: × 500. Figure is representative of at least three experiments performed on different experimental days.

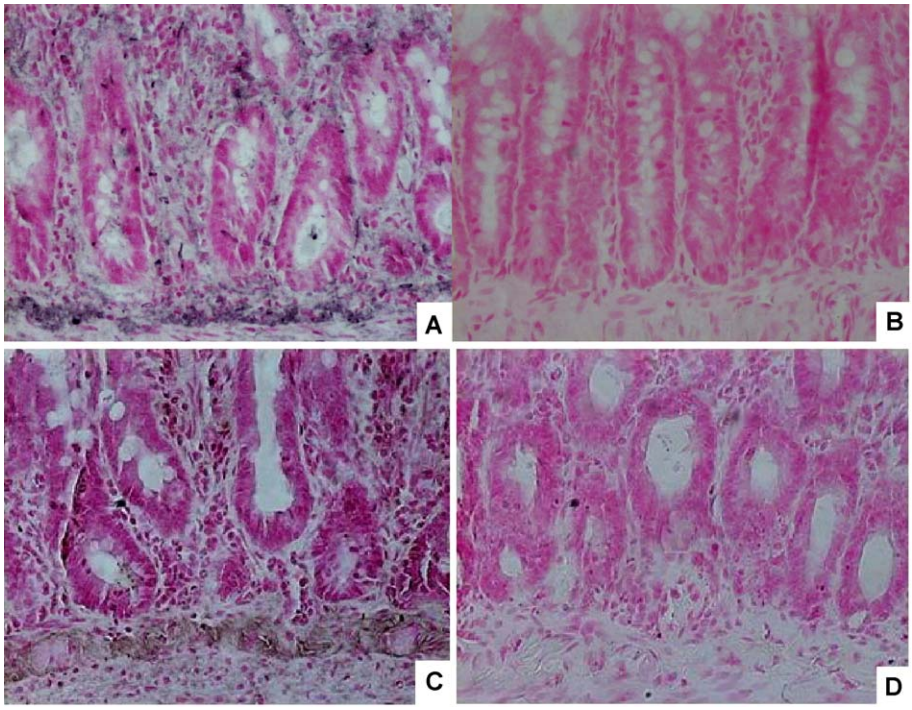


Fig. 6. Immunohistochemical staining of nitrotyrosine and PAR. After reperfusion nitrotyrosine (A) and PAR (C), staining was localized in the injured area from a splanchnic artery occlusion-shocked rat. There was no detectable immunostaining for nitrotyrosine (B) and PAR (D) in the ileum from 5-AIQ-treated rats. Original magnification: × 500. Figure is representative of at least three experiments performed on different experimental days.

There is now good evidence that PARP inhibitors, including 5-AIQ, attenuate the tissue injury caused by ischemia–reperfusion of the brain, heart, kidney, retina, intestine, liver. In addition, 5-AIQ attenuates the multiple organ injury and dysfunction associated with hemorrhagic shock in the rat, which is at least in part secondary to ischemia–reperfusion of the relevant target organs (Thiemermann et al., 1997; Zingarelli et al., 1997a; Szabo and Dawson, 1998). The conclusions derived from studies employing 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 (Liaudiet et al., 2000a,b). Together, the complementary results from studies of genetic deletion of PARP-1 and pharmacologic inhibition of the enzyme have validated PARP as a novel target for potential therapeutic intervention to treat ischemia–reperfusion injury.

The 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 members of the benzamide family of PARP inhibitors, these compounds (particularly at high concentrations) have been associated with side effects that were not due to inhibition of PARP activity (Milam and Cleaver, 1984). We are not aware of any nonspecific effects or side effects of 5-AIQ. There is now good evidence that PARP-1 facilitates DNA repair, especially base excision repair (Plaza et al., 1999). In a preliminary effort to look for impairment of DNA repair secondary to PARP inhibition by 5-AIQ, we found no evidence that 5-AIQ interferes with the repair and expression of exogenously damaged plasmid DNA in cells (unpublished observation).

The long-term effects of PARP inhibition by 5-AIQ and other potent PARP-inhibitors, however, remain to be established. As the physiological role(s) of PARP are not well defined, it is difficult to predict the consequences of chronic PARP inhibition. Although early experiments seem to suggest that PARP inhibition may adversely affect DNA repair, DNA replication, gene expression and other cellular functions, the apparent normal embryonic development, growth, reproduction and life span of PARP-1 −/− mice attests that PARP does not play a vital role in these processes. Alternatively, the functions of PARP-1 may be compensated for by other redundant mechanisms, or even other isoforms of PARP. Inhibition of PARP catalytic activity is likely to cause less side effect than eliminating PARP protein itself, which, in addition to poly(ADP-ribose) production may have effects, which are secondary to protein–protein interactions with regulators of gene transcription (Simbulan-Rosenthal et al., 1999).

Here we demonstrate for the first time that the novel, potent PARP inhibitor, 5-AIQ, exerts beneficial effects in a rat model of splanchnic artery occlusion shock. Specifically, we provide evidence that 5-AIQ (1) reduces fall in blood

pressure, (2) reduces the morphological signs of intestinal injury, (3) reduces the upregulation of ICAM-1 in the ileum and the subsequent accumulation of neutrophils, and (4) prolongs the survival time of rats subjected to splanchnic artery occlusion shock. What, then, is the mechanism by which 5-AIQ protects the ileum of the rat against injury and dysfunction caused by severe ischemia and reperfusion?

We demonstrate here that ischemia and reperfusion of the intestine results in a significant increase in PARP activity [measured as poly(ADP) ribosylation of proteins by immunohistochemistry] in the ileum. Most notably, this increase in PARP activity was not seen in rats subjected to splanchnic artery occlusion, but treated with the water-soluble PARP inhibitor 5-AIQ. This finding demonstrates that the dose of 5-AIQ used in this study was sufficient to abolish the increase in PARP activity caused by ischemia–reperfusion in the intestine. In addition, we demonstrate that the degree of polymorphonuclear leukocyte infiltration into the ileum (at 1 h of reperfusion) is significantly reduced in rats treated with 5-AIQ. The PARP inhibitor also reduced the upregulation of ICAM-1 (Figs. 5 and 6), but did not affect the constitutive expression of ICAM-1 in endothelial cells. These results demonstrate that 5-AIQ can interrupt the interactions between neutrophils and endothelial cells during the late firm adhesion phase mediated by ICAM-1. The absence of an increased expression of ICAM-1 in the ileum of 5-AIQ-treated rats indeed was associated with the reduction of leukocyte infiltration (as assessed by measuring tissue myeloperoxidase levels) and with the moderation of the postreperfusion tissue damage (as evaluated by histological examination).

Taken together, the data presented in the present study and in another recent report (Cuzzocrea et al., 2002) demonstrate that PARP regulates the infiltration of neutrophils into the inflamed tissues via a number of distinct mechanisms. 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, ischemia–reperfusion injury and inflammation. For instance, there is good evidence that less potent inhibitors of PARP activity (including 3-aminobenzamide: 10 $mg \cdot kg^{-1}$; nicotinamide: 10 mg $kg^{-1}$ and 1,5-dihydroxyisoquinoline: 3 mg/kg) reduce by $\sim$ 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). Interestingly, a much larger reduction in cerebral infarct size ( $\sim$ 80%) can be detected in mice in which the gene for PARP has been disrupted by gene-targeting (PARP knock out or $-/-$ mice) (Eliasson et al., 1997). Thus, it is possible that a much larger therapeutic benefit in conditions associated with inflammation, can be obtained with more potent, water-soluble inhibitors of PARP activity. Thus, as previously indicated (Cuzzocrea et al., 1997; Mazzon et al., 2002), we propose the following positive feedback cycle in early reactive oxygen species production $\gg$ PARP-related endothelial injury>>polymorphonuclear leukocyte infiltration>>more reactive oxygen species production. Inhibition of PARP would intercept this cycle at the level of endothelial injury. This model would explain the reduction of malondialdehyde tissue levels during splanchnic artery occlusion in the 5-aiq-treated rats; 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 antiinflammatory mode of action of this novel inhibitor of PARP activity.

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