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PARP inhibition reduces acute colonic inflammation in rats

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Abstract

Poly(ADP-ribose) polymerases (PARP) comprise a family of enzymes which catalyse poly(ADP-ribosyl)ation of DNA-binding proteins. Multiple researches indicate the importance of PARP in promoting cell recruitment and thereby inducing organ injury in various forms of inflammation, such as colitis. We have evaluated the effects of two PARP inhibitors, nicotinamide and 1,5-dihydroxyisoquinoline, in acute colitis induced by trinitrobenzensulfonic acid (TNBS) in rats. Nicotinamide (20–40 mg/kg) and 1,5-dihydroxyisoquinoline (4–8 mg/kg) were administered 48, 24 and 1 h prior to the induction of colitis as well as 24 h later. 48 h after colitis induction the lesions were blindly scored and quantified as ulcer index. Histological study and colonic inflammation were assessed by gross appearance and myeloperoxidase (MPO) activity. Prostaglandin E_2 (PGE_2) synthesis and, cyclooxygenase-1 and cyclooxygenase-2 expressions by Western blotting and immunohistochemistry were also performed. Inflammation following TNBS induction was characterized by increased colonic wall thickness, oedema, diffuse inflammatory cells infiltration in the mucosa and necrosis. Furthermore, increased MPO activity, cyclooxygenase-2 expression and PGE_2 synthesis were significantly augmented after TNBS instillation. On the contrary, treatment with 1,5-dihydroxyisoquinoline significantly reduced the degree of colon injury and also caused a substantial reduction in the rise in MPO activity, in the increase of staining for cyclooxygenase-2, as well as in the up-regulation of PGE_2 caused by TNBS in the colon. Although nicotinamide significantly did not reduce macroscopic damage, it decreased both MPO activity and PGE_2 colonic levels. In conclusion, we demonstrated that PARP inhibition can exert beneficial effects in experimental colitis and may, therefore, be useful in the treatment of ulcerative colitis.

Keywords: PARP; Nicotinamide; 1,5-dihydroxyisoquinoline; Cyclooxygenase-2; PGE_2

1. Introduction

Ulcerative colitis is an idiopathic chronic inflammatory disease of multifactorial aetiology. Its pathogenesis and molecular biological mechanisms are not yet elucidated (Fiocchi, 2002). This disease affects principally the mucosa of the rectum and left colon. Its clinical course is unpredictable and presents remissions and exacerbation, characterizing by rectal bleeding and diarrhoea (Fiocchi, 2005).

Activated immune cells, primarily represented by neutrophils, macrophages, and cytotoxic T cells play the role of aggressors that attack and destroy the intestinal barrier either directly through physical contact or indirectly through the release of reactive oxygen and nitrogen metabolites, cytotoxic proteins and lytic enzymes (Abreu, 2002).

Cyclooxygenase-2 is expressed as an early response to proinflammatory mediators and mitogen stimuli. Cyclooxygenase-2 is induced in colonic epithelial cells in inflammatory bowel disease (Singer et al., 1998), indeed, large amounts of cyclooxygenase-2 mRNA and protein have been found in inflamed areas, producing most of the prostaglandins (PGs) (Dong et al., 2003; Singh et al., 2003). It is well known that the production of PGs is greatly increased in affected areas of the colon in ulcerative colitis (Cuzzocrea et al., 2001a,b; Kruidenier and Verspaget, 2002), contributing to the formation of inflammatory oedema, hyperaemia and dismotility. In our previous studies we have observed that the increased PGs production during acute colitis is dependent upon the activity of cyclooxygenase-2 (Martín et al., 2003, 2004; Sanchez-Hidalgo et al., 2005). Therefore, suppression of the inflammatory

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response may be reached through the inhibition of prostaglandin E_2 (PGE_2) production and cyclooxygenase-2 activation.

Poly(ADP-ribose) polymerases (PARP) comprise a family of enzymes which catalyse poly(ADP-ribosyl)ation of DNA-binding proteins. The generation of free radicals, reactive oxygen species, and peroxynitrite causes overactivation of PARP resulting in the depletion of NAD^+ and ATP and consequently resulting in depletion of intracellular energetic, intestinal barrier dysfunction and cellular death (Zingarelli et al., 2003, 2004). A number of recent studies indicate the importance of PARP in promoting cell recruitment and thereby inducing organ injury in various forms of inflammation (Cuzzocrea et al., 1998; Zingarelli et al., 1999). PARP have also been involved in the up-regulation of numerous pro-inflammatory genes through the activation of several transcription nuclear factors.

Over the last years, a series of cell culture system and animal experiments have been performed to prove that PARP inhibitors therapy represents an effective approach to treating a variety of diseases (Virag and Szabo, 2002). In fact, in many models of inflammation, PARP inhibition and PARP deficiency (Zingarelli et al., 1999) have been shown to be associated with reduced infiltration of neutrophils as well as with a reduction in associated oxidative stress (Szabo et al., 1997; Cuzzocrea et al., 1998; Szabo and Dawson, 1998; Zingarelli et al., 1998, 1999; Kaplan et al., 2005). Therefore, several PARP inhibitors have been previously investigated as potential novel anti-inflammatory agents against colon injury associated with experimental colitis such as GPI6150, PJ34, 3-aminobenzamide and 5-aminoisoquinolinone (Jijon et al., 2000; Mabley et al., 2001; Mazzon et al., 2002).

Consistent with these findings, the aim of the present study was to evaluate the effects of two PARP inhibitors, the benzamide, nicotinamide and, the isoquinolinone, 1,5-dihydroxyisoquinoline (5-dihydroxyisoquinolin-11(2*H*)-one), on the extent and severity of colitis induced by trinitrobenzensulfonic acid (TNBS) in rats. The inflammatory response was assessed by histology and MPO activity, as an index of quantitative inflammation and neutrophil infiltration in the mucosa. Histological and histochemical analyses of the lesions were also carried out. In order to gain a better insight into the action mechanism(s) of the observed protective effects of PARP inhibitors, PGE_2 generation and the expression of cyclooxygenase-1 and -2 by Western blotting and immunohistochemistry have been also investigated.

2. Materials and methods

2.1. Experimental animals

Male and female Wistar rats supplied by Animal Services of the University of Seville, Spain, weighing 180–200 g, were placed singled in cages with wire-net floors in a controlled room (temperature 24–25 °C, humidity 70–75%, lighting regimen of 12 L/12 D) and were fed a normal laboratory diet (Panlab, Barcelona, Spain). Rats were deprived of food for 24 h prior to the induction of colitis, but were allowed free access to tap water throughout. They were randomly assigned to groups of 12 animals. Experiments followed a protocol observed by the Animal Ethics Committee of the University of Seville and all experiments were in accordance with the recommendations of the European Union regarding animal experimentation (Directive of the European Counsel 86/609/EC).

2.2. Induction of colitis and treatments

Colitis was induced according to the procedure described by Morris et al. (1989). Briefly, rats were slightly anesthetized with chloral hydrate following a 24 h fast, and then a medical-grade polyurethane cannula for enteral feeding (external diameter 2 mm) was inserted into the anus and the tip was advanced to 8 cm proximal to the anus verge. TNBS (Sigma-Chemical Co, St Louis, MO, USA) dissolved in ethanol (50% v/v) was instilled into the colon (10 mg) to induce acute colitis in a total volume of 0.25 ml. Following the instillation of the hapten, the animals were maintained in a head-down position for a few minutes (2–3 min) to prevent leakage of the intracolonic instillate. Two PARP inhibitors were used, nicotinamide (20–40 mg/kg) and 1,5-dihydroxyisoquinoline (4–8 mg/kg) (Sigma, St Louis, USA). They were suspended in 0.9% saline solution and were administered in a volume of 1 ml/kg body weight by intraperitoneal route (i.p.) 48, 24 and 1 h prior to the induction of colitis and also 24 h later. The animals were sacrificed, using an overdose of anaesthetic, 48 h after induction of colitis. Different reference control groups were used for comparison with the TNBS/ethanol colon instillation group: rats in the sham group received physiological saline instead of the TNBS solution in a comparable volume, and the ethanol group that received an enema of 0.25 ml 50% (v/v) ethanol. These reference groups and TNBS group also received the vehicle (0.9% saline solution in a volume of 1 ml/kg body weight) by i.p. route. The rats were checked daily for behaviour, body weight, and stool consistency.

2.3. Assessment of colitis

An independent observer who was blinded to the treatment evaluated the severity of colitis. For each animal, the distal 10 cm portion of the colon was removed and cut longitudinally, slightly cleaned in physiological saline to remove faecal residues and weighed. Macroscopic damage was quantified measuring the extent of the lesions in the distal colon by planimetry. It was expressed as ulcer index (cm^2), which is the area of colon damage calculated as the product of length and width of the erosion, and the ratio weight/length of the rat colon as indicator of inflammation was also determined. The presence of adhesions (score 0–2), and/or stool consistency (score 0–1) was evaluated according to the criteria of Bobin-Dubigeon et al. (2001) with slight modifications. Photographs taken from colon samples were digitized using Kodak D290 Zoom camera (Eastman Kodak Co, Rochester, NY, USA). Pieces of damaged colon were collected and frozen in liquid nitrogen for measurement of biochemical parameters.

2.4. Histological studies

For examination with the light microscope we used tissue samples from the distal inflamed colon of each animal and fixed

in 4% buffered formaldehyde, dehydrated by increasing concentrations of ethanol, and embedded in paraffin. Thereafter, sections of tissue were cut at 5 μm with a Leica Ultracut (Leica Microsystems, Wetzlar, Germany), mounted on clean glass slides and dried overnight at 37 °C. Sections were cleared, hydrated, and stained with haematoxylin and eosin and with Alcian blue for histological evaluation of colonic damage and mucus content, respectively, according to standard protocols. The slides were coded to prevent observer bias during evaluation. All tissue sections were examined under an Olympus BH-2 microscope (GMI, Minnesota, USA) and Motic Images 2000 release 1.1, (MicroOptic Industrial Group B1 Series System Microscopes) was used for characterization of histopathological changes. Analysis of the imagines was carried out by Adobe Photoshop® Version 6.0 image-analysis program.

2.5. Assessment of leukocyte involvement

Myeloperoxidase (MPO) activity was assessed as an index of neutrophil infiltration according to the method of Grisham et al. (1990). Samples were obtained from all animals and rapidly rinsed with ice-cold saline, blotted dry, and frozen at −70 °C. The tissue was thawed, weighed and homogenized in 10 volumes 50 mM phosphate-buffered saline (PBS), pH=7.4. The homogenate was centrifuged at 20,000 ×*g*, 20 min, 4 °C. The pellet was again homogenized in 10 volumes 50 mM PBS, pH=6.0, containing 0.5% hexadecyl-trimethylammonium bromide (HETAB) and 10 mM ethylenediaminetetraacetic acid (EDTA). This homogenate was subjected to one cycle of freezing/thawing and a brief period of sonication. A sample of homogenate (0.5 μl) was added to a 0.5 ml reaction volume containing 80 mM PBS, pH 5.4, 0.5% HETAB and 1.6 mM 3,3′,5,5′-tetramethylbenzidine (TMB). The mixture was incubated at 37 °C for 5 min and the reaction started by the addition of 0.3 mM H_2O_2. Each tube containing the complete reaction mixture was incubated for exactly 3 min at 37 °C. The reaction was terminated by the sequential addition of catalase (20 μg/ml) and 2 ml 0.2 M sodium acetate, pH=3.0. The changes in absorbance at 655 nm were measured with a microplate reader (Labysistem Multiskan EX, Helsinki, Finland). One unit of MPO activity was defined as the amount of enzyme present that produced a change in 1.0-absorbance units per min at 37 °C in the final reaction volume containing the acetate. Results were quantified as U/mg prot.

2.6. Immunohistochemical localisation of COX-1 and COX-2-like immunoreactivity

Colonic tissues were fixed in 4% buffered paraformaldehyde, dehydrated through graded concentrations of ethanol, embedded in paraffin, and sectioned. Sections (5 μm) were mounted on slides, cleared, and hydrated. They were treated with a buffered blocking solution (3% bovine serum albumin) for 15 min. Then, sections were incubated with primary antibodies for cyclooxygenase-1 and cyclooxygenase-2 (goat polyclonal, M-19 and M-20 of Santa Cruz Biotechnologies, CA, California, USA) at a dilution of 1:400 at room temperature for 1 and 24 h, respectively. Sections were washed with PBS and incubated with secondary antibody anti-goat immunoglobulin G (IgG), peroxidase conjugated (Santa Cruz Biotechnologies, CA California, USA) (1:500 in phosphate-buffered saline (PBS), v/v) at room temperature for 1 h. Thereafter, sections were washed as before and with Tris–HCl 0.05 M, pH 7.66, and then co-incubated with 3,3-diaminobenzidine solution in the dark at room temperature for 10 min. Sections were washed with Tris–HCl, stained with haematoxylin according to standard protocols (Torres et al., 1999) and observed under an Olympus BH-2 microscope.

2.7. Isolation of cytoplasmic proteins and Western blot assay

Frozen colonic tissues were weighed and homogenized in ice-cold buffer (50 mM Tris HCl, pH 7.5, 8 mM $MgCl_2$, 5 mM ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*′*N*′-tetraacetic acid (EGTA), 0.5 mM EDTA, 0.01 mg/ml leupeptin, 0.01 mg/ml pepstatin, 0.01 mg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 250 mM NaCl). Homogenates were centrifuged (12,000 ×*g*, 15 min, 4 °C) and the supernatants were collected and stored at −80 °C.

Protein concentration of the homogenate was determined following Bradford's colorimetric method. Aliquots of supernatants containing equal amounts of protein (50 μg) were separated on 10% acrilamide gel by sodium dodecyl sulfate-polyacryamide gel electrophoresis. In the next step, the proteins were electrophoretically transferred onto a nitrocellulose membrane and incubated with specific primary antibodies: cyclooxygenase-1 and cyclooxygenase-2 (Santa Cruz Biotechnology, CA, California, USA). Each filter was washed three times for 15 min and incubated with the secondary horseradish peroxidase-linked donkey anti-goat immunoglobulin G antibody (Santa Cruz Biotechnology, CA, California, USA). To prove equal loading, the blots were analysed for B-actin expression using an anti-B-actin antibody (Santa Cruz Biotechnology, CA, California, USA). Immunodetection was performed using enhanced chemiluminiscence light-detecting kit (SuperSignal® West Pico Chemiluminescent Substrate, Pierce, IL, USA). Densitometric data were studied following normalisation to the control (house-keeping gene). The signals were analyzed and quantified by a Scientific Imaging Systems (KODAK 1D, Image Analysis Software).

2.8. Measurement of PGE_2 content

The tissue was weighed and homogenized in 6 ml TEAP buffer (pH 3.24) that contained a cyclooxygenase inhibitor, Inyesprin®. The homogenate was centrifuged (1500 ×*g*, 10 min, 4 °C) and the supernatant was removed and passed through a reverse-phase octadecylsilica C18 Sep Pak cartridge which was washed with 10 ml distilled water, 10 ml 15% ethanol, 10 ml hexane and 10 ml ethylacetate, and the eluate collected. Each fraction was evaporated with ethylacetate, and the dry residue re-dissolved in ethanol. PGE_2 was determined by a competitive enzyme immunoassay kit (Assay Desingns, Inc, Michigan, USA), and results were expressed as percentage respect to control group.

Table 1
Parameters quantified after administration of PARP inhibitors in rats with acute colitis induced by TNBS intracolonic instillation (10 mg/animal)

Group	*n*	Body weight changes (g)	Adhesions (score 0–2)	Diarrhoea (score 0–2)
Sham	12	14.0±1.4	0.0	0.0
TNBS	12	−17.5±2.0[a]	1.57±0.12[a]	0.60±0.01[a]
Nicotinamide 20	12	−15.3±2.0[a]	1.30±0.21[a]	0.50±0.01[a]
Nicotinamide 40	12	−14. 7±2.2[a]	1.48±0.18[a]	0.50±0.01[a]
1,5-dihydroxyisoquinoline 4	12	−8.5±1.2[b]	1.25±0.15[a]	0.40±0.02[b]
1,5-dihydroxyisoquinoline 8	12	−9.3±1.5[b]	1.15±0.18[a]	0.35±0.01[b]

Colonic parameters were quantified in the sham group, which received saline instillation. TNBS group received the hapten intracolonically in a vehicle of 50% (v/v) ethanol. Data are expressed as mean±S.E.M.
[a]$P<0.001$ and [b]$P<0.05$ vs. sham group.

2.9. Statistical analysis

All values in the figures and text are expressed as arithmetic means±standard error of the mean (S.E.M.). Data were evaluated with Graph Pad Prism® Version 2.01 software. The statistical significance of any difference in each parameter among the groups was evaluated by one-way analysis of variance (ANOVA) followed by Tukey test. P values of <0.05 were considered statistically significant. In the experiment involving histology, the figures shown are representative of at least six experiments performed on different days.

3. Results

In this acute model of colitis, the animals which received intracolonic TNBS administration showed prostration, pilorection and hypomotility. Body weight loss was significantly elevated compared with sham animals ($P<0.001$) and presence of adhesions to adjacent organs ($P<0.001$) and diarrhoea ($P<0.001$) were frequently observed in TNBS-treated rats (Table 1). 48 h after intracolonic administration of TNBS, the colon appeared flaccid and filled with liquid stool. A significant increase of weight/length of the rat colon, as indicator of inflammation, was observed in TNBS-treated rats ($P<0.001$ vs. sham group) (Fig. 1). The macroscopic inspection of colon and rectum showed presence of mucosal congestion, erosion and haemorrhagic ulcerations. Lesions in the distal colon were quantified using an ulcer index. TNBS group showed the highest ulcer index (7.8±0.8 cm^2, $P<0.001$ vs. sham group) (Fig. 1). The histological study of the colon from sham rats showed a histological normal structure (Fig. 2A). However, slides of TNBS-treated rats presented transmural necrosis,

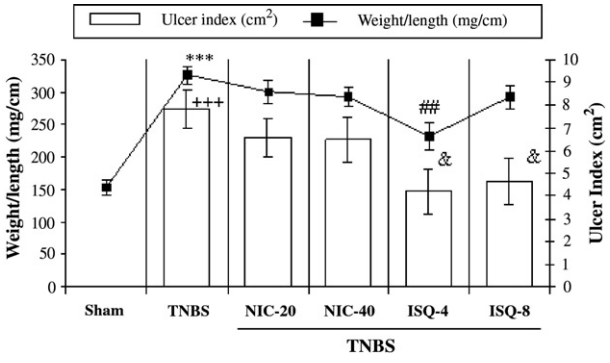

Fig. 1. Effects of acute administration of PARP inhibitors on the colonic parameter and macroscopic damage. Colonic macroscopic damage resulting from trinitrobenzene sulphonic acid (TNBS, 10 mg/animal) instilled into rat colon was scored, as indicated in Materials and methods. Weight/length of the rat colon and ulcer index were quantified in the absence of treatment, but with daily administration of the vehicle saline solution (sham and TNBS groups), or in the presence of nicotinamide (NIC, 20–40 mg/kg) and 1,5-dihydroxyisoquinoline (ISQ, 4–8 mg/kg). Data are expressed as means±S.E.M. *** $P<0.001$ and $^{+++}P<0.001$ vs. sham group; & $P<0.05$ and ## $P<0.01$ vs. TNBS group.

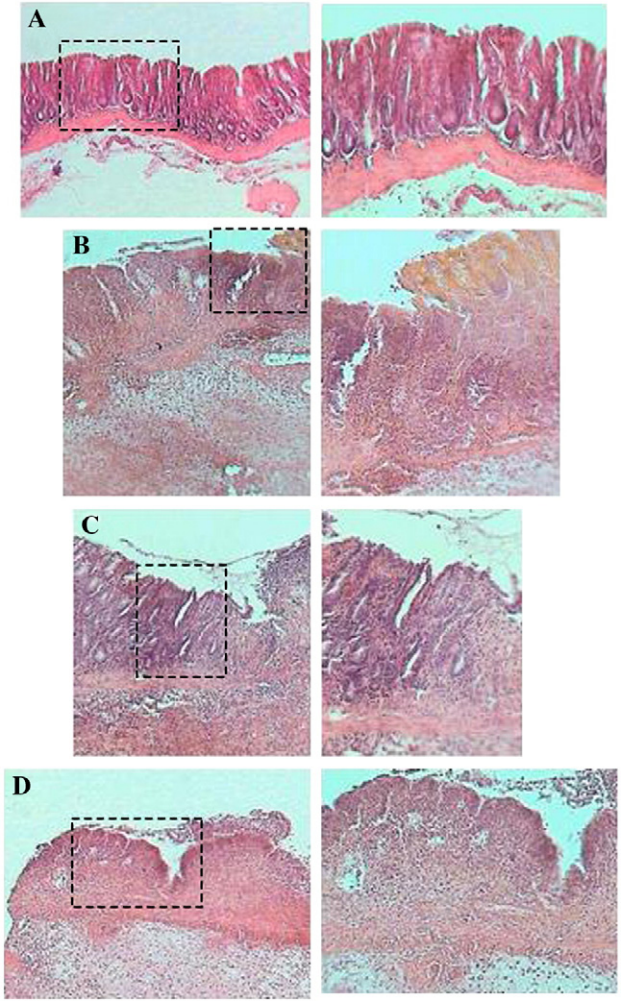

Fig. 2. Acute colitis model induced by TNBS: Effect of PARP inhibitors on colon injury. Representative histological appearance of rat colonic mucosa in 1,5-dihydroxyisoquinoline (8 mg/kg) and nicotinamide (40 mg/kg) treated animals (C and D, respectively) and compared with reference control sham group (A) and TNBS group (10 mg/kg) (B). Mucosal injuries after TNBS administration was characterized by necrosis of epithelium, focal ulceration of the mucosa and diffuse infiltration of inflammatory cells in the mucosa and submucosa. Treatment with nicotinamide (C) showed exfoliation of epithelial cells in the ulcerative areas and in 1,5-dihydroxyisoquinoline (D) samples some areas of the epithelium remained intact. H–E: Hematoxylin and eosin staining. Original missing (B). Respect to 1,5-ihydroxyisoquinoline and nicotinamide administration the mucin layer was clearly visible (C,D). Original magnification 200×.

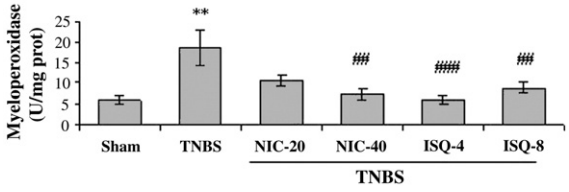

Fig. 3. Effects of PARP inhibitors administration on myeloperoxidase activity (MPO, U/mg tissue) in trinitrobenzene sulphonic acid colitis rats (TNBS, 10 mg/animal). MPO activity was quantified in the absence of treatment (Sham and TNBS group) but with daily administration of the vehicle saline solution or in the presence of or in the presence of nicotinamide (NIC, 20–40 mg/kg) and 1,5-dihydroxyisoquinoline (ISQ, 4–8 mg/kg). The sham group received physiological saline instead of the TNBS solution in an equal volume. Data are expressed as the means ± S.E.M. ** $P<0.01$ vs. sham group; ## $P<0.01$ and ### $P<0.001$ vs. TNBS group.

oedema and diffuse inflammatory cells infiltrating the mucosa. In sections of ulcerated areas necrotic tissue adjacent to surface cells could be observed. Extensive granulation tissue with the presence of fibroblasts was also apparent. There was focal ulceration of the colonic mucosa extending through the muscularis mucosae, desquamated areas and loss of the epithelium and partial destruction of the glands (Fig. 2B). Goblet cells were totally absent at the surface epithelium respect to sham group. Some areas showed accumulation of mucus and cell remnants, however, Alcian blue-positive cells were less numerous. In addition, the mucin layer of the epithelium was missing (data not show).

Treatment with the PARP inhibitor 1,5-dihydroxyisoquinoline presented significant variations in the weight/length respect to the TNBS group with the lower dose ($P<0.01$ vs. TNBS group) and significantly decreased the extend and severity of damage ($P<0.05$ vs. TNBS control group). However, no significant changes were observed in nicotinamide-treated-rats. Respect to loss in body weight, the presence of adhesions to adjacent organs and the diarrhoea no significant changes were observed between them (Table 1). After administration of 1,5-dihydroxyisoquinoline, there was an attenuation of histological morphological signs of cell damage, some areas of the epithelium remained intact and the mucin layer was clearly visible (Fig. 2C). After nicotinamide treatment, exfoliation of epithelial cells in the ulcerative areas, descent of inflammatory cells in the lamina propria (Fig. 2D) and glandular hypertrophy accompanied by mucus secretion could be observed (data not show).

As shown in Fig. 3, a significant increase in MPO activity ($P<0.01$), an indicator of polymorphonuclear (PMN) leukocyte infiltration into the colon, also characterized the colitis caused by TNBS, being this result consistent with the histological findings. In contrast, treatment of TNBS-rats with 1,5-dihydroxyisoquinoline and the highest dose of nicotinamide (40 mg/kg) significantly reduced the neutrophil infiltration degree, which is in accordance with the histological results.

The levels of cyclooxygenase-1 and cyclooxygenase-2 expression were measured by Western blotting of cytosolic extracts from colonic mucosa. The levels of cyclooxygenase-1 protein remained unchanged in all groups, indicating that cyclooxygenase-1 protein was constitutively expressed in the colonic tissue and was not significantly changed after TNBS-enema or in the presence of PARP inhibitors (data not shown). On the other hand, cyclooxygenase-2 protein was significantly increased by TNBS ($P<0.01$), indicating that the inducible isoenzyme expression could be induced at the early stage of colonic lesion caused by TNBS. Nevertheless, oral administration of both PARP inhibitors tended to diminish the up-regulation of cyclooxygenase-2 expression although it was not statistically significant (Fig. 4A).

In normal colons, specific immunosignals for cyclooxygenase-1 were obtained in surface epithelium as well as in the upper half of the crypts. Mononuclear cells of the lamina propria and the regional lymphatic nodules as well as cells of the muscularis mucosae of slides of TNBS group showed cyclooxygenase-1 specific immunosignals (data not shown). Respect to the localization of the enzyme cyclooxygenase-2, in animals treated with TNBS, prominent cyclooxygenase-2 expression was found in cells of surface epithelium and in cells of the inflammatory infiltrate, while crypt epithelial cells were negative (Fig. 5B). At this time, PARP inhibitors- treated rats showed an apparent decrease in the level of expression of the inducible isoform in apical epithelial cells of inflamed colon (Fig. 5C and D).

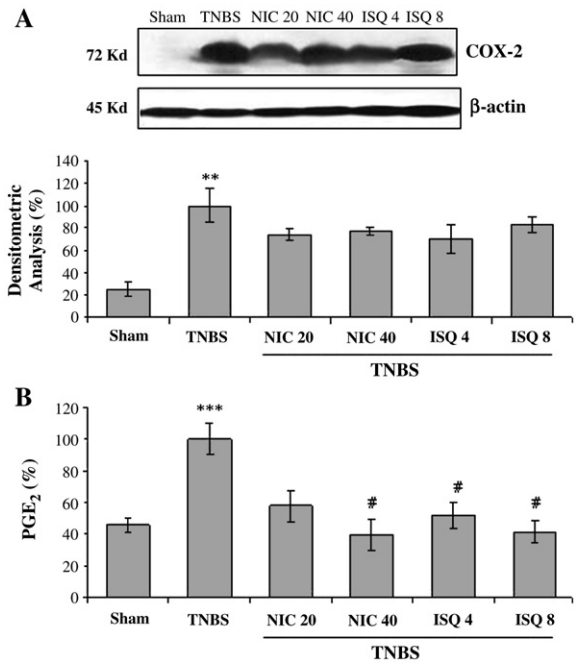

Fig. 4. Western blot representative analysis of cyclooxigenase-2 protein (A) and prostaglandin E_2 (PGE_2, pg/mg tissue) after PARP inhibitors administration, nicotinamide (NIC, 20–40 mg/kg) and 1,5-dihydroxyisoquinoline (4–8 mg/kg) in rats with acute colitis produced by trinitrobenzene sulphonic acid intracolonic instillation (TNBS, 10 mg/animal). (A) Cyclooxigenase-2 expression was higher in TNBS-rats in relation to sham animals. The PARP inhibitors attenuated cyclooxigenase-2 expression. Densitometric data were studied following normalization to the control (house-keeping gene). The results are representative of three experiments performed on different samples. (B) The synthesis of prostanoid was quantified as described in Materials and methods and it is expressed as percentage. Data are expressed as means ± S.E.M. ** $P<0.01$ and *** $P<0.001$ vs. sham group; # $P<0.05$ vs. TNBS group.

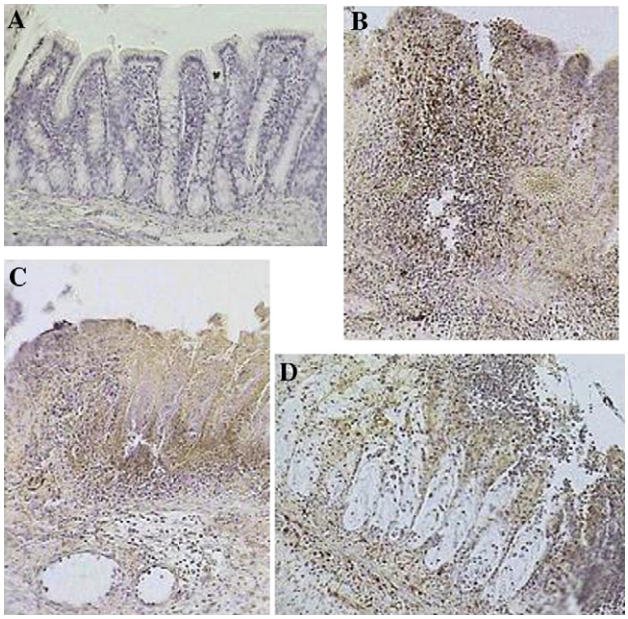

Fig. 5. Immunohistochemical localization of ciclo-oxygenase-2 isoenzyme in sections of colon. Control section was incubated by omitting the primary antibody: negative control (Data not show). Cyclooxygenase-2 expression in normal colonic mucosa (A). Cyclooxygenase-2 is strongly expressed in the colon of TNBS-control rats (B). Cyclooxygenase-2 expression was decreased in apical epithelial cells of inflamed colon treated with nicotinamide (40 mg/kg) and 1,5-dihydroxyisoquinoline (8 mg/kg) (C and D, respectively). Original magnification 200×.

The PGE_2 determination demonstrated a significant increase in colonic mucosa of TNBS group respect to sham group ($P<0.001$). In addition, under our experimental conditions, treatment with 1,5-dihydroxyisoquinoline and the highest dose of nicotinamide (40 mg/kg) diminished significantly PGE_2 production ($P<0.05$ vs. TNBS group) (Fig. 4B).

4. Discussion

Acute TNBS administration is a model that involves a localized inflammatory reaction in the distal colon (Morris et al., 1989) and also is of particular interest in relation ship with inflammatory bowel disease (Elson et al., 1995). Our study confirms that intracolonic TNBS administration induces mucosal erosion and ulceration and a degree of inflammation in the rat colon, which is associated with an infiltration of neutrophils into the mucosa. Histologically, neutrophils appear to be the effector cell causing tissue necrosis and mucosal dysfunction associated with colitis (Guo et al., 1999) as they represent a major source of reactive oxygen radicals in the inflamed colon mucosa (Grisham, 1994; Cuzzocrea et al., 2001a,b). Indeed, neutrophils play a crucial role in inflamed tissue in the destruction of foreign antigens and in the breakdown and remodelling of injured tissue (Koh et al., 2004; Martin et al., 2006; Talero et al., 2006).

Our data also show that the treatment with 1,5-dihydroxyisoquinoline significantly reduced the degree of colonic injury although both PARP inhibitors reduced significantly MPO activity (an index of tissue-associated neutrophil accumulation). These findings are in agreement with previous studies using different PARP inhibitors and experimental models of colitis (Jijon et al., 2000; Mabley et al., 2001; Mazzon et al., 2002; Cuzzocrea et al., 2004; Zingarelli et al., 2004; Di Paola et al., 2004, 2005).

The interpretation of data obtained with both PARP inhibitors could be explained in part, as they present different potencies demonstrated by the values of concentration required to inhibit activity by 50% (Curtin, 2005). Although nicotinamide is a product of the PARP-mediated catalysis of NAD^+, it is itself a weak feedback inhibitor of the enzyme (Li and Zhang, 2001). Moreover, the majority of PARP inhibitors contain the nicotinamide pharmacopore. However, 1,5-dihydroxyisoquinoline is a PARP inhibitor from 3-aminobenzamide belonging to the second-generation of PARP inhibitors, which have increasing potency to inhibit nuclear polymerase enzyme activity (Banasik et al., 1992; Virag and Szabo, 2002).

Recently, it has been demonstrated that PARP regulates the infiltration of neutrophils into the inflamed tissues via a number of distinct mechanisms (Di Paola et al., 2004; Cuzzocrea et al., 2004). For instance, the existence of a self-amplifying suicide cycle in which early production by endothelium activates PARP has been proposed; 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 (Cuzzocrea et al., 2004). PARP inhibition would intercept this cycle at the level of endothelial injury, which is consistent with our results, so that nicotinamide and 1,5-dihydroxyisoquinoline reduced leukocyte infiltration, leading to reduced reactive oxygen species.

Although the role of nuclear transcription factor NF-κB activation was not evaluated in this study, Zingarelli et al. (2003) demonstrated that in a model of TNBS-induced colitis the anti-inflammatory properties of 1,5-dihydroxyisoquinoline could be ascribed, at least in part, to its ability to modulate the signal transduction mediated by NF-κB and AP-1. NF-κB has been shown to activate, via transcription the genes encoding the expression of enzymes (e.g. inducible nitric oxide synthase and cyclooxygenase-2). Thus, it is possible that one mechanism underlying the protective effects of PARP inhibitors involves a reduction of neutrophil infiltration into the colonic mucosa, possibly via inhibition of NF-κB activation.

Cyclooxygenase-1 and cyclooxygenase-2-derived prostanoids play an important role in the development of colitis and should be considered as possible targets of treatment. We have recently demonstrated that colitis is strongly correlated with intestinal cyclooxygenase-2 expression and moreover, that the increased PG production during TNBS-induced acute colitis is dependent upon the activity of cyclooxygenase-2 (Martín et al., 2003; Sanchez-Hidalgo et al., 2005; Talero et al., 2006). In our study, treatment with PARP inhibitors tended to diminish the positive staining for cyclooxygenase-2. In addition, both PARP inhibitors also reduced the increase of colonic PGE_2 levels. Analyzing the literature published in this line, there is a previous finding demonstrating that abrogation of PARP activity leads to down-regulation of cyclooxygenase-2 expression in a model of ischaemic stroke (Koh et al., 2004), but it is

the first time that these reduction has been observed in TNBS-induced colitis in rats.

In conclusion, we demonstrated that inhibition of PARP exerts therapeutic effects in acute experimental colitis. The anti-inflammatory effect observed with these inhibitors, according to our results, may be mediated at least in part, by their ability to reduced neutrophil infiltration and cyclooxygenase-2 immunosignals, as well as PGE_2 production. Further studies are needed to deepen in the molecular mechanism of these molecules for potential therapeutic application in inflammatory bowel disease.

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