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European Journal of Pharmacology 554 (2007) 69–77
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Ameliorative effect of pyrrolidinedithiocarbamate on acetic acid-induced colitis in rats

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Received 15 February 2006; received in revised form 14 September 2006; accepted 19 September 2006
Available online 13 October 2006

Abstract

Ulcerative colitis is a chronically recurrent inflammatory bowel disease of unknown origin. The present study examined the effect of NF-κB inhibitor and antioxidant, pyrrolidinedithiocarbamate (PDTC) on experimental ulcerative colitis in rats. Animals were randomly divided into 4 groups, each consisting of 6 animals; normal control group, acetic acid group, PDTC-treated group and sulfasalazine-treated group as a positive control group. Induction of colitis by intracolonic administration of 3% acetic acid produced severe macroscopic inflammation in the colon 24 h after acetic acid administration as assessed by the colonic damage score. Microscopically, colonic tissues showed ulceration, oedema and inflammatory cells infiltration. Biochemical studies revealed increased serum levels of lactate dehydrogenase (LDH), and nitrite/nitrate and colonic concentrations of tumor necrosis factor-α (TNF-α) and the neutrophil infiltration index, myeloperoxidase (MPO). Oxidative stress was indicated by elevated lipid peroxides formation and depleted reduced glutathione concentrations (GSH) in colonic tissues. Immunohistochemical studies of colonic sections revealed upregulation of inducible nitric oxide synthase (iNOS). Pretreatment with PDTC at a dose of (200 mg/kg/day, i.p.), three days before induction of colitis decreased serum LDH, nitrite/nitrate and TNF-α levels, colonic concentrations of MPO and lipid peroxides while increased colonic GSH concentration. Moreover, PDTC pretreatment attenuated colonic iNOS expression. Finally, histopathological changes were nearly restored by PDTC pretreatment. The findings of the present study provide evidence that PDTC may be beneficial in patients with inflammatory bowel disease.

Keywords: Pyrrolidinedithiocarbamate; Inflammatory cytokine; Oxidative stress; Nitrite/nitrate; Inducible nitric oxide synthase; Ulcerative colitis

1. Introduction

Inflammatory bowel diseases including ulcerative colitis and Crohn's disease are among the most challenging human illness. Although ulcerative colitis etiology is largely unknown but the current literature suggests that multiple immune, genetic, and environmental factors influence both the initiation and progression of colitis (Strober et al., 1998). There is evidence for an intense local immune response associated with recruitment of lymphocytes and macrophages followed by release of soluble cytokines and other inflammatory mediators. Subsequent activation of these cells causes a self-augmenting cycle of cytokine production, cell recruitment and inflammation (Sartor, 1997; Shanahan, 2001). This uncontrolled immune system activation results in a sustained massive production of cytokines such as tumor necrosis factor (TNF)-α and interleukins (IL-1β and IL-8) (Inoue et al., 1999; Ogata and Hibi, 2003). In addition to cytokines, leukotrienes, thromboxane, platelet-activating factor, nitric oxide and reactive oxygen species are also released from activated mucosal cells (Podolsky, 1991; Woywodt et al., 1999; MacDonald et al., 2000).

Most of the current therapies for inflammatory bowel diseases involve treatment with glucocorticosteroids and 5-aminosalicylic acid (Podolsky, 1991; Strober et al., 1998). Immunosuppressive drugs have also been used to control severe illness, regardless of the more serious complications and toxic side effects associated

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doi:10.1016/j.ejphar.2006.09.066

with them (Shanahan, 2001). Although many types of treatment have been proposed and clinically proven, additional therapeutic approaches are needed because many patients either do not respond to the currently available options or demonstrate significant side effects, thereby precluding their prolonged use.

The dithiocarbamates represent a class of antioxidants reported to be strong inhibitors of nuclear factor-κB (NF-κB) in vivo and in vitro (Schreck et al., 1992; Cuzzocrea et al., 2002). The metal-chelating properties of the diethyl derivative of dithiocarbamate (diethyldithiocarbamate, DDTC) have been exploited for decades for the treatment of metal poisoning in humans (Sunderman, 1981). More recently, DDTC has been used to retard the onset of acquired immune deficiency syndrome in human immunodeficiency virus (HIV)-infected individuals, a phenomenon thought to be related to its effect on NF-κB activation (Hersh et al., 1991; Sunderman, 1991). In this regard, the most effective NF-κB inhibitor appears to be the pyrrolidine derivative of dithiocarbamate (pyrrolidinedithiocarbamate, PDTC) because of its ability to traverse the cell membrane and its prolonged stability in solution at physiological pH (Schreck et al., 1992). PDTC bears a number of beneficial properties including antioxidation, anti-inflammation and immunoregulation (Cuzzocrea et al., 2002). The ability of dithiocarbamates to modulate the effects of oxidants and NF-κB activation suggests that these agents may offer therapeutic benefit in inflammatory conditions in which activation of NF-κB plays a major role (Schreck et al., 1992; Frode-Saleh and Calixto, 2000). Moreover, we have recently shown that PDTC protected against NF-κB activation during ischemia/reperfusion-induced gastric injury in rats (El Eter et al., 2005). The aim of the current investigation is to evaluate the effects of PDTC on acetic acid-induced ulcerative colitis in rats and its possible mechanism of action.

2. Materials and methods

2.1. Materials

Pyyrolidinedithiocarbamate (ammonium salt), reduced glutathione, sulfasalazine, 2-thiobarbituric acid, and 5, 5-dithio-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma Chemical Co. (St. Louis, Mo, USA). A primary antibody for iNOS was obtained from Santa Cruz Biotechnologies Inc. (California, U.S.A.). Anti-sheep IgG peroxidase conjugated was purchased from Sigma-Aldrich Company Ltd. LDH kit was obtained from Human GmbH company (Wiesbaden, Germany). TNF-α ELISA kit was purchased from R&D Systems Inc. (Minneapolis, USA).

2.2. Animals

Adult male Wistar rats obtained from the Animal Care Center, College of Pharmacy, King Saud University and weighing 220–250 g, were placed singly in cages with wire–net floors in a controlled room (temperature 24–25°, humidity 70–75%, lighting regimen of 12-h light:12-h dark) and were fed a normal laboratory diet. Rats were deprived of food for 24 h prior to the induction of colitis, but were allowed free access to tap water throughout. The animals used in this study were handled and treated in accordance with the strict guiding principles of the National Institution of Health for experimental care and use of animals. The experimental design and procedures were approved by the Institutional Ethical Committee for Animal Care and Use at the King Saud University, Riyadh, Kingdom of Saudi Arabia.

2.3. Experimental design

Rats were randomized into 4 groups, each consisting of 6 animals. Group (1) Normal control group, received saline at a dose of 0.5 ml/kg, orally. Group (II) Acetic acid group, colitis was induced by intracolonic injection of 2 ml of 3% acetic acid. Group (III) PDTC-treated group, rats were given PDTC at a dose of (200 mg/kg/day, i.p.). Group (IV) Sulfasalazine-treated group, rats were treated with sulfasalazine (500 mg/kg/day, orally), and used as a positive control group. PDTC was dissolved in saline while sulfasalazine was suspended in 0.5% carboxymethyl cellulose. The drugs were given once daily starting 72 h before induction of colitis. In previous work, there was no effect of 0.5% carboxymethyl cellulose on the severity of acetic acid-induced ulcerative colitis (Mustafa et al., 2006).

2.4. Induction of experimental colitis

Colitis was induced according to the method previously described (Millar et al., 1996). Briefly, rats were slightly anaesthetized with ether following a 24 h fast, and then a medical-grade polyurethane canal 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. 2 ml of acetic acid (3% v/v in 0.9% saline) or saline alone (control animals) was instilled into the colon through the cannula for 30 s, after which fluid was withdrawn. 24 h later, animals were sacrificed; blood and colons were collected. Blood samples were centrifuged; sera were separated and stored at −80 °C until assayed for lactate dehydrogenase (LDH) and nitrite/nitrate levels. Portions of colonic specimens were kept in 10% formalin for microscopic, histopathological and immunostaining studies. The remaining portions of colonic specimens were snap-frozen in liquid nitrogen and stored at −80 °C until assayed for biochemical studies.

2.5. Assessment of colitis

2.5.1. Macroscopic scoring

For each animal, the distal 10 cm portion of the colon was removed and cut longitudinally, and slightly cleaned in physiological saline to remove faecal residues. Macroscopic inflammation scores were assigned based on clinical features of the colon using an arbitrary scale ranging from 0–4 as follows: 0 (no macroscopic changes), 1 (mucosal erythema only), 2 (mild mucosal oedema, slight bleeding or small erosions), 3 (moderate oedema, slight bleeding ulcers or erosions), 4 (severe ulceration, oedema and tissue necrosis) (Millar et al., 1996).

2.5.2. Histopathological studies

Colonic specimens were fixed in 10% formalin in phosphate buffered saline, embedded in paraffin and cut into 4 μm

sections. Paraffin sections were deparaffinized with xylene, hydrated and stained with hematoxylin and eosin for mucosal damage assessment.

2.6. Biochemical assays

Samples from the colon were stored immediately at −80 °C till analysis. Tissue samples were homogenized in 10 mmol Tris–HCl buffer (pH 7.1) and the homogenate was used for the measurement of myeloperoxidase (MPO), lipid peroxidation, reduced glutathione (GSH) and TNF-α.

2.7. Measurement of serum LDH

Serum LDH was measured using the commercially available kit.

2.8. Determination of serum nitrite/nitrate level

Serum NO level was estimated as nitrite and nitrate by the acidic Griess reaction after reduction of nitrate to nitrite by vanadium trichloride according to the method described by Miranda et al. (2001). The Griess reaction relies on a simple colorimetric reaction between nitrite, sulfonamide and *N*-(1-naphthyl) ethylenediamine to produce a pink azo-product with maximum absorbance at 543 nm. The concentrations were determined using a standard curve of sodium nitrate and the results were expressed as μmol/l.

2.9. Determination of colonic TNF-α levels

Colonic TNF-α was assayed according to the method described by Reinecker et al. (1993). Colonic samples were immediately weighed, minced on an ice-cold plate, suspended in a tube with 10 mmol/l sodium phosphate buffer (pH 7.4) (1:5 w/v). The tubes were placed in a shaking water bath (37 °C) for 20 min and centrifuged at 9000 ×*g* for 30 s at 4 °C; the supernatant was frozen at −80 °C until assay. TNF-α was quantified by enzyme-linked immunoabsorbent assay and the results were expressed as picograms per gram of wet tissue.

2.10. Measurement of colonic lipid peroxides concentration

Lipid peroxidation, an indicator of mucosal injury induced by reactive oxygen species was measured as thiobarbituric acid reactive substance. The amount of colonic lipid peroxides was measured by the thiobarbituric acid assay (TBA) as previously described by Buege and Aust (1978). Briefly, 0.5 ml of colonic tissue homogenates prepared were reacted with 2 ml of TBA reagent containing 0.375% TBA, 15% trichloroacetic acid and 0.25 N HCl. Samples were boiled for 15 min, cooled and centrifuged. Absorbance of the supernatants was spectrophotometrically measured at 532 nm. TBARS concentrations were calculated by the use of 1,3,3,3 tetra-ethoxypropane as a standard. The results were expressed as μmol/g wet tissue weight.

2.11. Determination of colonic GSH contents

Colonic GSH was determined as previously described by Ellman (1959) and modified by Nagi et al. (1992). Briefly, GSH in tissue homogenate was reacted with Ellman's reagent (5, 5-dithio-2-nitrobenzoic acid) in phosphate buffer saline (PBS, pH 8.0) and the absorbance was measured at 412 nm. GSH concentration was calculated using a standard solution of GSH. The results were expressed as nmol/g wet tissue weight.

2.12. Assessment of colonic MPO activity

MPO activity was assessed as a marker of neutrophil infiltration according to the method described by Mullane et al. (1985). In brief, colonic tissues were homogenized in a solution containing 0.5% (w/v) hexadecyltrimethyl ammonium bromide dissolved in 10 mM sodium phosphate buffer (pH 7.4) in an ice bath using polytron homogenizer (50 mg tissue/ml). The homogenates were centrifuged for 30 min at 20,000 ×*g* at 4 °C. An aliquot of the supernatant was allowed to react with a solution of tetramethyl benzidine (1.6 mM) and 0.1 mM hydrogen peroxide. The rate of change in absorbance was measured spectrophotometrically at 650 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmol of peroxide/min at 37 °C and was expressed in units/mg wet tissue.

2.13. Immunohistochemical study

Colonic tissues were fixed in 4% buffered paraformaldehyde, dehydrated through graded concentrations of ethanol, embedded in paraffin, and sectioned. Sections (5 μm thick) were mounted on slides, cleared, and hydrated. All of them were treated with a buffered blocking solution (3% BSA) for 15 min. Then, sections were co-incubated with primary antibody for iNOS at a dilution of 1:500 at room temperature for 1 h. Sections were washed with PBS and co-incubated with secondary antibody anti-sheep IgG peroxidase conjugated (1:500 in 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 and stained with haematoxylin according to standard protocols.

2.14. Statistical analysis

Results were expressed as means ± S.E.M. The statistical significance of any difference in each parameter among the groups was evaluated by one-way ANOVA, using Tukey–Kramer multiple comparisons test as *post hoc* test. P values of < 0.05 were considered statistically significant.

3. Results

3.1. Effect of PDTC on macroscopic scores

24 h after induction of colitis, there was macroscopic evidence of extensive colonic mucosal injury along the 1–3 cm segment at

Table 1
Effect of pyrrolidinedithiocarbamate (PDTC) on macroscopic scoring of acetic acid-induced ulcerative colitis in rats

Treatment	Macroscopic scoring
Control	0.0±0.0
3% Acetic acid (2 ml/rat, rectally)	3.8±0.17[a]
PDTC (200 mg/kg/day, I.P.)	1.33±0.21[b]
Sulfasalazine (500 mg/kg/day, orally)	1.5±0.34[b]

Results are expressed as mean±SEM, $n=6$/group.
[a]$P<0.001$ *vs.* control group, [b]$P<0.001$ *vs.* acetic acid group.

the site of instillation as assessed by the colonic damage score. The mucosa appeared ulcerated, oedematous and haemorrhagic compared to normal control group (Table 1). PDTC treatment reduced the severity of gross lesion score (Table 1).

3.2. Histopathological results

The histopathological features of untreated animals included transmural necrosis, oedema and diffuse inflammatory cell infiltration in the mucosa, desquamated areas and loss of the epithelium. An infiltrate consisted of mixed inflammatory cells was observed (Fig. 1B). Pretreatment of rats with PDTC (Fig. 1C) or sulfasalazine (Fig. 1D) significantly attenuated the extent and severity of the histological signs of cell damage. There were no inflammatory cells in the lamina propria and the epithelium remained intact (Fig. 1 C and D).

3.3. Effect of PDTC on serum LDH levels

As illustrated in Fig. 2, serum LDH level was significantly elevated following acetic acid instillation compared to control animals (2172±182.2 U/l *vs.* 659.8±45.9 U/l, respectively, $P<0.001$). PDTC and sulfasalazine treatments reduced the increase in serum LDH level (1023±39.34 U/l and 1063.8±52.46 U/l, respectively, $P<0.001$) but serum LDH levels were still higher than the control value (659.8±45.9 U/l, $P<0.05$).

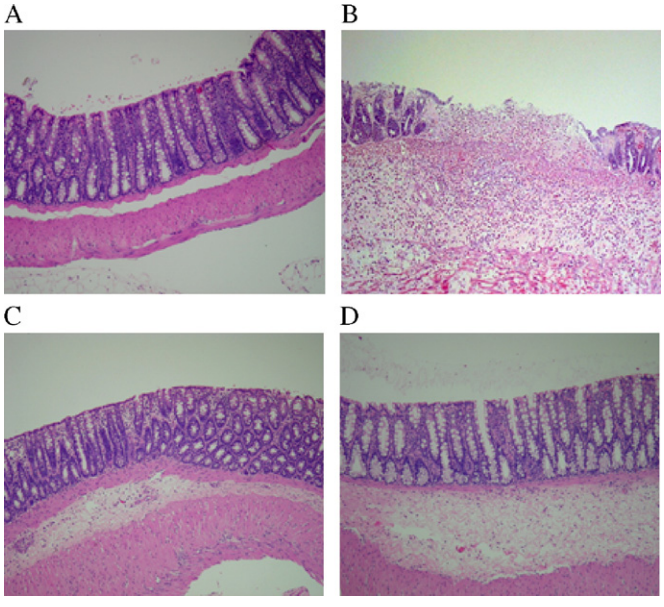

Fig. 1. Photomicrographs of haematoxylin and eosin stained paraffin sections of rat colonic tissues. (A) Normal intact mucosa from normal control animals showed intact epithelial surface. (B) Acetic acid-induced colitis showing massive necrotic destruction of epithelium. (C and D) Pre-treatment with pyrrolidinedithiocarbamate (PDTC, 200 mg/kg/day, i.p.) and sulfasalazine (500 mg/kg/day, orally), respectively, attenuated the extent and severity of the histological signs of cell damage. Magnification X100.

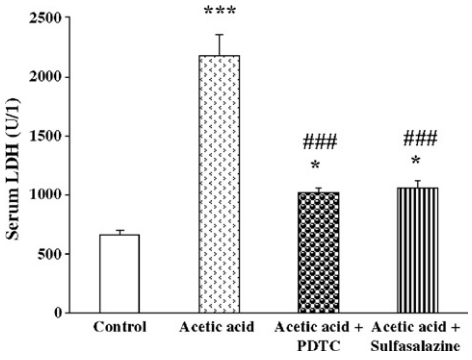

Fig. 2. Effect of pyrrolidinedithiocarbamate (PDTC, 200 mg/kg/day, i.p.) pretreatment for three consecutive days on serum lactate dehydrogenase (LDH) levels (U/l) in acetic acid-induced ulcerative colitis in rats. Results are expressed as mean±SEM, $n=6$/group. *$P<0.05$; ***$P<0.001$ *vs.* control group, ###$P<0.001$ *vs* acetic acid group.

3.4. Effect of PDTC on serum nitrite/nitrate level

Acetic acid-induced colitis resulted in increased serum nitrite/nitrate level in comparison with control animals (51±3.4 μmol/l *vs.* 20±3 μmol/l, $P<0.001$, Fig. 3). Administration of PDTC or sulfasalazine produced a significant reduction in serum nitrite/nitrate level compared to acetic acid-induced colitis group (27±2.2 μmol/l and 30±2.5 μmol/l *vs.* 51±3.4 μmol/l, respectively, $P<0.001$).

3.5. Effect of PDTC on colonic TNF-α levels

Colonic TNF-α level in acetic acid group was significantly higher than the corresponding value in the control group (180±5.2 pg/mg *vs.* 108±2.3 pg/mg, $P<0.001$, Fig. 4). This increase in TNF-α was significantly attenuated by the pretreatment with either PDTC or sulfasalazine compared to acetic acid-induced colitis group (127±2.2 pg/mg and 130±3 pg/mg *vs.* 180±5.2 pg/

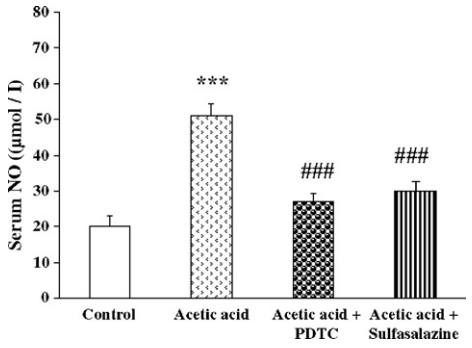

Fig. 3. Effect of pyrrolidinedithiocarbamate (PDTC, 200 mg/kg/day, i.p.) pretreatment for three consecutive days on serum nitrite/nitrate (NO) level (μM/l) in acetic acid-induced ulcerative colitis in rats. Results are expressed as mean±SEM, $n=6$/group. ***$P<0.001$ *vs.* control group, ###$P<0.001$ *vs.* acetic acid group.

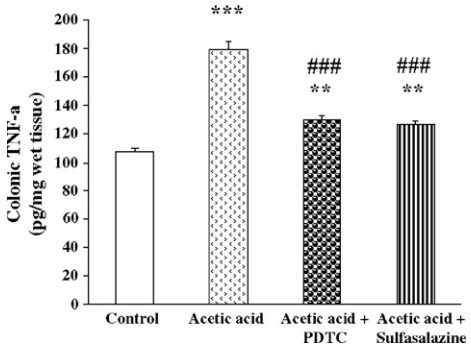

Fig. 4. Effect of pyrrolidinedithiocarbamate (PDTC, 200 mg/kg/day, i.p.) pretreatment for three consecutive days on colonic tumor necrosis factor-α (TNF-α) levels (pg/mg wet tissue) in acetic acid-induced ulcerative colitis in rats. Results are expressed as mean±SEM, $n=6$/group. $^{**}P<0.01$; $^{***}P<0.001$ *vs.* control group, $^{###}P<0.001$ *vs.* acetic acid group.

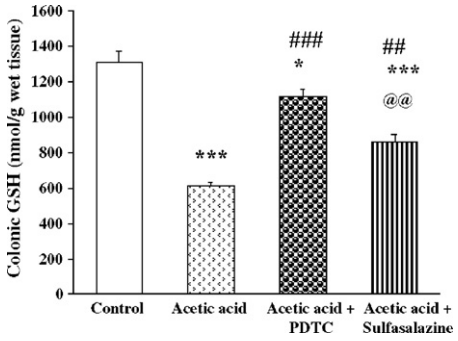

Fig. 6. Effects of pyrrolidinedithiocarbamate (PDTC, 200 mg/kg/day, i.p.) pretreatment for three consecutive days on colonic glutathione (GSH) content (nmol/g wet tissue) in acetic acid-induced ulcerative colitis in rats. Results are expressed as mean±SEM, $n=6$/group. $^{*}P<0.05$, $^{***}P<0.001$ *vs.* control group, $^{##}P<0.01$, $^{###}P<0.01$ *vs.* acetic acid group, $^{@@}P<0.01$ *vs.* PDTC-treated group.

mg, respectively, $P<0.001$) but the colonic TNF-α concentrations did not return to normal control value (108±2.3 pg/mg, $P<0.01$).

3.6. Effect of PDTC on colonic lipid peroxides concentration

Colonic lipid peroxides concentration in acetic acid group increased in comparison to the control group (0.443±0.035 μmol/g *vs.* 0.177±0.03 μmol/g, $P<0.001$, respectively, Fig. 5). Treatment of rats with PDTC produced a marked significant decrease in lipid peroxides concentration that reaches to the control value (0.19±0.01 μmol/g, $P<0.001$). Sulfasalazine also provided protection against the elevation in lipid peroxides concentration induced by acetic acid treatment (0.23± 0.02 μmol/g *vs.* 0.443±0.035 μmol/g, respectively, $P<0.001$).

3.7. Effect of PDTC on colonic GSH concentrations

As depicted in Fig. 6, induction of colitis produced a significant decrease in colonic GSH content compared with the control group (612±23 nmol/g *vs.* 1307±64 nmol/g respectively, $P<0.001$). PDTC treatment significantly increased GSH content as compared with acetic acid group (1120±39 nmol/g *vs.* 612±23 nmol/g, $P<0.001$). Sulfasalazine also protected against GSH depletion induced by acetic acid (863±38 nmol/g, *vs.* 612±23 nmol/g, $P<0.01$) but to a lower degree than PDTC (863±38 nmol/g *vs.* 1120±39 nmol/g, $P<0.01$).

3.8. Effect of PDTC on colonic MPO activity

Fig. 7 demonstrates the increased mucosal MPO concentration in colonic mucosa of rats following intrarectal administration of acetic acid (1.1±0.04 units/mg *vs.* 0.59±0.02 units/mg respectively, $P<0.001$). Pre-treatment with either PDTC or sulfasalazine produced a significant ($P<0.05$) reduction in MPO activity as compared to acetic acid group (0.92±0.03 units/mg, 0.95± 0.04 units/mg *vs.* 1.1±0.04 units/mg, respectively) but was still higher than the control value (0.59±0.02 units/mg, $P<0.001$).

3.9. iNOS immunostaining

In normal controls, colonic iNOS expression was mainly observed on neutrophils and smooth muscle cells with a sparse distribution in the epithelial cells (Fig. 8A). Immunohistochemical examination revealed that iNOS was upregulated after

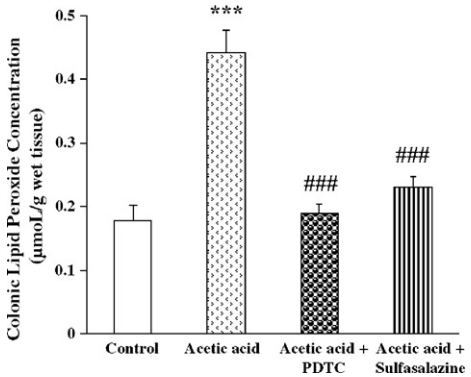

Fig. 5. Effect of pyrrolidinedithiocarbamate (PDTC, 200 mg/kg/day, i.p.) pretreatment for three consecutive days on colonic lipid peroxides concentration (μmol/g wet tissue) in acetic acid-induced ulcerative colitis in rats. Results are expressed as mean±SEM, $n=6$/group. $^{***}P<0.001$ *vs.* control group, $^{###}P<0.001$ *vs.* acetic acid group.

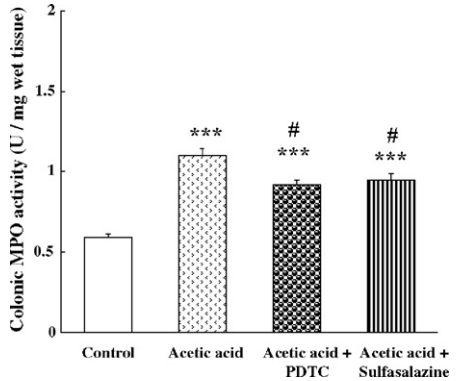

Fig. 7. Effects of pyrrolidinedithiocarbamate (PDTC, 200 mg/kg/day, i.p.) pretreatment for three consecutive days on colonic myeloperoxidase (MPO) activity (U/mg wet tissue) in acetic acid-induced ulcerative colitis in rats. Results are expressed as mean±SEM, $n=6$/group. $^{***}P<0.001$ *vs.* control group, $^{#}P<0.001$ *vs.* acetic acid group.

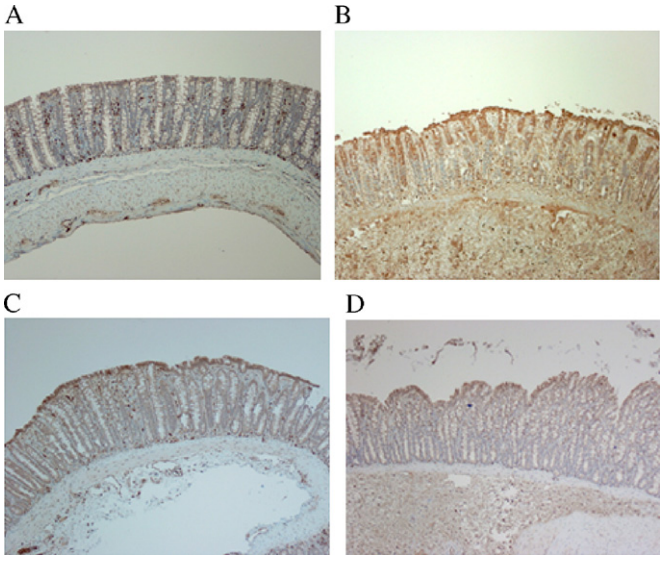

Fig. 8. A. Immunohistochemical localization of iNOS in normal control, which was manifested as fine brown granules distributed mainly in neutrophils and smooth muscle cells. (B) Positively stained granules for iNOS were significantly increased in both number and intensity in colonic tissue of acetic acid treated rats. (C and D) Pre-treatment with pyrrolidinedithiocarbamate (PDTC, 200 mg/kg/day, i.p.) and sulfasalazine (500 mg/kg/day, orally), respectively reduced colonic iNOS expression.

induction of colitis and was localized in the infiltrated inflammatory cells and in disrupted epithelial cells (Fig. 8B). Pretreatment with PDTC or sulfasalazine attenuated iNOS expression-induced by acetic acid treatment (Fig. 8C and D respectively).

4. Discussion

Induction of colitis by acetic acid in rats is one of standardized methods to produce an experimental model of inflammatory bowel disease. Several major causative factors in the initiation of human colitis such as enhanced vasopermeability, prolonged neutrophils infiltration and increased production of inflammatory mediators are involved in the induction of this animal model (Elson et al., 1995).

The present study has shown that acetic acid-induced ulcerative colitis was associated with macroscopic, microscopic and biochemical changes. Pretreatment with NF-κB inhibitor and antioxidant PDTC attenuated colitis as shown by the lower serum levels of LDH, and nitrite/nitrate, the reduced colonic tissue contents of lipid peroxides, MPO and TNF-α, the down regulation of iNOS expression in colonic sections, the increase in GSH concentration and the marked improvement in the histopathological results.

Ulcerative colitis is a chronically recurrent inflammatory bowel disease of unknown origin. Oxidative stress has been implicated in the pathogenesis of ulcerative colitis in experimental animals (Keshavarzian et al., 1990) and in humans (Kitahora et al., 1998). In the present study, there was elevated lipid peroxides concentration in colonic tissue that was in parallel with depleted reduced glutathione content, which is indicative of oxidative stress. Excess production of reactive oxygen metabolites e.g., superoxide, hydroxyl radical, hydrogen peroxide, hypochlorous acid and oxidant derivatives, such as *N*-chloramines, are detected in the inflamed mucosa and may be pathogenic in inflammatory bowel disease (Keshavarzian et al., 1992). Sustained production of reactive oxygen metabolites during colonic inflammation may overwhelm the endogenous antioxidant defense system that regulate their production leading to oxidative injury (Blau et al., 1999). Decreased endogenous antioxidant levels in patients with ulcerative colitis have been reported (D' Odorico et al., 2001). The main sources of reactive oxygen metabolites in the inflamed mucosa are activated phagocytic leukocytes, capable of producing superoxide and a cascade of various species leading to a very reactive hydroxyl radical and peroxide. The xanthine oxidase pathway in colonocytes also produces superoxide anion by conversion of xanthine/hypoxanthine to uric acid. A third possible source is the oxidation of arachidonic acid either through the lipooxygenase reaction, producing leukotrienes, or the prostaglandin generating cyclooxygenase reaction (Loguercio et al., 1996).

Pretreatment with PDTC in this study protected against colonic GSH depletion and restored the levels toward the normal value suggesting an antioxidant action. Glutathione plays a key role in controlling the redox state of the cell through several mechanisms, including scavenging of ROM and keeping the enzyme GSH peroxidase in a reduced state (Sies, 1999). The antioxidant activity of PDTC has been demonstrated in organ injury in renal ischemia/reperfusion, myocardial injury, lung injury, brain injury, gastric injury and in a model of collagen-induced arthritis (Ross et al., 2000; Cuzzocrea et al., 2002; Chatterjee et al., 2003; Nurmi et al., 2004; El Eter et al., 2005). PDTC functions as an antioxidant due to two structural features: direct scavenging of ROM by the dithiocarboxy group, and chelating activity for heavy metal ions that may catalyze formation of ROM. Other antioxidant substances, such as curcumin (Ukil1 et al., 2003), melatonin (Wei-Guo et al., 2003) and mesna (Shusterman et al., 2003) have been shown to be effective in experimental colitis in rats. In the present study, although PDTC was more effective ($P<0.01$) in increasing colonic GSH content than sulfasalazine but there was no difference between the two treatments regarding the microscopic aspects of colitis. This may be explained by the fact that the increase in GSH concentration by PDTC was not enough to completely overcome the damage inflicted by acetic acid in the colon (GSH concentration was not normalized by PDTC compared to normal control group, $P<0.05$). In addition, endogenous antioxidants other than GSH may be affected by acetic acid.

In our experiments, the colonic MPO activity, an index of neutrophil activation and inflammation was increased in acetic acid-treated animals. This increase in MPO activity was substantially reduced in rats treated with PDTC. Activated neutrophils pass out of the circulation and enter the inflamed mucosa and submucosa of the large intestine during acute inflammation, leading to overproduction of reactive oxygen and nitrogen species, proteases, lactoferrin and lipid mediators that can contribute to intestinal injury (Bobin-Dubigeon et al., 2001, Abreu, 2002; Kruidenier and Verspaget, 2002). The principal free radical in tissues is superoxide anion (O_2^-) which is converted to the

secondary oxidant H_2O_2 by superoxide dismutase. O_2^- can be produced by both endothelial cells through xanthine oxidase and activated neutrophils through NADPH oxidase, which reduces molecular oxygen to the O_2^- radical, and through the enzyme MPO. This enzyme catalyzes the formation of such potent cytotoxic oxidants as hypochlorous acid from H_2O_2 and chloride ions and *N*-chloramines. The reduction in colonic MPO activity as well as the histological finding of the absence of cellular infiltration following treatment with PDTC may indicate its antioxidant and anti-inflammatory effects in the prevention of acetic acid-induced colitis. PDTC was shown to protect against NF-κB-mediated pathological effects induced by various stimuli including lipopolysaccharide and cytokines (Rahman et al., 1998; Liu et al., 1999). Furthermore, it has been effective in reducing carrageenan-induced inflammation in rats (Cuzzocrea et al., 2002). The anti-inflammatory properties of PDTC might be exerted through a biochemical mechanism related to NF-κB inhibition in ulcerative colitis. In a recent study (El Eter et al., 2005), PDTC protected against ischemia/reperfusion-induced gastric injury via NF-κB inhibition. NF-κB is a key player in the regulation of inflammatory gene expression (Baeuerle and Baltimore, 1996). Activation of NF-κB increases the expression of genes encoding proinflammatory mediators such as cytokines (TNF-α and IL-1, -6, and -12), cell adhesion molecules (VCAM-1 and ICAM-1), inducible nitric oxide synthase, and cyclooxygenase-2 (Pahl, 1999; Woywodt et al., 1999). In addition, NF-κB is activated in patients with inflammatory bowel disease (Neurath et al., 1998; Segain et al., 2000) and in rats with trinitrobenzene sulfonic acid-induced colitis (Jun-Hua et al., 2005).

This study also shows that the proinflammatory cytokine TNF-α production was increased in colonic mucosa after acetic acid instillation. Cytokines are important to gastrointestinal host defense, but their overproduction may cause excessive gut inflammation and intestinal motility disorders (Bossone et al., 2001). TNF-α is one of the most significant factors participating in the inflammatory process of patients with inflammatory bowel disease (Derkz et al., 1993; Rogler and Andus, 1998). It induces the production of other cytokines including adhesion molecules, arachidonic acid metabolites, and activation of immune and non-immune cells. Antibodies of avian tumor necrosis factor effectively treated inflammatory bowel diseases in rats (Bobin-Dubigeon et al., 2001) and in humans (Brown and Abreu, 2005; Sandborn, 2005). There is good evidence that TNFα and IL-1 cause the activation and translocation of NF-αB into the nucleus (Bauerle and Henkel, 1994). In the current investigation, PDTC significantly reduced colonic TNF-α indicating that PDTC has an anti-inflammatory effect probably due to its powerful antioxidant properties and to NF-κB inhibition.

Many studies have shown that nitric oxide (NO) takes part in the pathogenesis of inflammatory bowel disease (Perner and Rask-Madsen, 1999; Wei-Guo et al., 2003). Altered regulation of NO has been implicated in many gastrointestinal disease states. More specifically, NO production was shown to be increased in ulcerative colitis, Crohn's disease, toxic megacolon, and diverticulitis (Boughten-Smith et al., 1993; Grisham et al., 2002). As an important inflammatory mediator, NO could react with superoxide anion to form more poisonous nitrite anion, which then disturbs the function of inflammatory cells and further impairs the colonic mucosa (Dijkstra et al., 1998). In the present study, the mucosal NO content in the inflamed colon was significantly increased with enhanced expression of iNOS. These results are in accordance with the previous reports in other animal models (Southey et al., 1997; Kankuri et al., 1999; Perner and Rask-Madsen, 1999, Wei-Guo et al., 2003). Inflammatory cells such as phagocytic leukocytes express inducible nitric oxide synthase (iNOS) when appropriately stimulated by cytokines (e.g. IL-1 and TNFα) or bacterial products such as lipopolysaccharide (Rachmilewitz et al., 1995). The expression of iNOS results in the synthesis of micromolar quantities of nitric oxide, which can be deleterious to cells through the formation of nitric oxide-reactive products (Beckman et al., 1990). In the present study, pretreatment with PDTC resulted in a significant reduction in iNOS expression with subsequent decrease in serum NO level. Inhibition of iNOS seems to ameliorate the inflammatory response and tissue injury in experimental colitis (Hogaboam et al., 1995). In contrast to these observations, a deleterious effect of iNOS deficiency was reported on the ability to resolve a colonic injury in experimental ulcerative colitis (Mccafferty et al., 1997). The discrepancy in these reports may relate to the difference in the stimuli used to induce the injury. However, similar controversial roles of iNOS-derived NO have been ascribed in a variety of pathophysiological conditions. In the current study, TNF-α upregulation is associated with colonic iNOS induction, in this regard, it should be noted that TNF-α may be one of the cytokines responsible for the induction of iNOS (Grisham et al., 1999).

Pharmacotherapy of ulcerative colitis is principally aimed at inhibiting the production of inflammatory mediators and at modulating the immune system. The multitude of reactions in which ROS participate provides a new area of research in intestinal inflammation. The current study tried to reduce pharmacologically the excessive ROS production and/or action in the inflamed colonic mucosa. Using acetic acid-induced colitis model, the present work supports a possible role for NF-κB inhibitor and antioxidant therapy in inflammatory bowel disease patients. This appears to be a promising approach that may be considered as a complementary treatment of ulcerative colitis.

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