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Delineation of the protective action of zinc sulfate on ulcerative colitis in rats

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Abstract

The protective action of zinc compounds in Crohn's disease-like inflammatory bowel disease in animals has been shown. A similar action of zinc sulfate on ulcerative colitis has not been defined. The present study aimed to delineate the protective action of zinc sulfate and the pathogenic mechanisms of 2,4-dinitrobenzene sulfonic acid (DNBS)-induced ulcerative colitis in rats. Zinc sulfate at different concentrations was given either orally (p.o.) or rectally (p.r.) to rats at 42, 48, 66 and 72 h following the induction of colonic inflammation by DNBS. Rats were killed 96 h after instillation of DNBS rectally to assess the severity of colonic damage, myeloperoxidase and xanthine oxidase activities. The involvement of mast cell degranulation and histamine release in the pathogenesis of DNBS-induced colitis was determined by using a mast cell stabilizer (ketotifen) and histamine receptor blockers (terfenadine and ranitidine). DNBS given rectally produced inflammation and ulceration in rats with a pathology resembling ulcerative colitis. Myeloperoxidase activity but not xanthine oxidase activity was sharply increased by this agent. Intrarectal administration of zinc solution and parenteral injection of histamine blockers significantly reduced tissue damage and myeloperoxidase but not xanthine oxidase activity. Ketotifen, a mast cell stabilizer, also significantly decreased mucosal injury and myeloperoxidase activity in the colon. In conclusion, mast cell degranulation followed by histamine release plays an important role in the pathogenesis of DNBS-induced ulcerative colitis. Zinc given rectally has a therapeutic effect against this colitis model, perhaps through the reduction of inflammation and inhibition of the above pathogenic mechanisms. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: 2,4-dinitrobenzene sulfonic acid (DNBS); Ulcerative colitis; Zinc sulfate; Histamine; Mast cell; Terfenadine; Ranitidine; Ketotifen; Reactive oxygen metabolite; Neutrophil

1. Introduction

Inflammatory bowel disease is a chronic inflammatory condition affecting the gastrointestinal tract. It is divided into two major categories: ulcerative colitis and Crohn's disease. Ulcerative colitis is a disease involving mainly the colon while Crohn's disease is a condition that affects every part of the gastrointestinal tract. The cause of inflammatory bowel disease is unknown but several factors have been implicated. These include environmental factors, genetic factors, microbial pathogens, defects in immunoregulation and altered levels of inflammatory mediators (Fiocchi, 1998; Nielsen and Rask-Madsen, 1996; Sartor, 1995).

Concerning inflammatory mediators, reactive oxygen metabolites have been proposed to be associated with inflammatory bowel disease. Data from animal models indicate that reactive oxygen metabolites are involved in gut inflammation. Several studies had shown large quantities of reactive oxygen metabolites in the mucosa of patients with inflammatory bowel disease that were correlated with disease severity (Keshavarzian et al., 1992; Simmonds et al., 1992). The reactive oxygen metabolites are mainly produced by activated phagocytic leukocytes in the inflamed mucosa via NADPH oxidase and xanthine oxidase. There is also evidence of mast cell activation resulting in an elevated level of histamine in the mucosa of inflammatory bowel disease patients (Knutson et al., 1990).

Zinc, one of the essential trace elements, has also been linked to inflammatory bowel disease. Zinc deficiency has been observed in patients with Crohn's disease (Solomons et al., 1977; McClain et al., 1980). Two recent studies had demonstrated beneficial effects of zinc enema against 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, a Crohn's disease-like animal model (Yoshikawa et al., 1997; Chen et al., 1999). There is no report concerning the action of zinc compounds on ulcerative colitis, a more

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common inflammatory disorder in the gastrointestinal tract. Since zinc is an antioxidant and constituent of several free radical scavengers and is also a mast cell stabilizer (Chen et al., 1999; Cho and Ogle, 1997, 1978), its anti-inflammatory action, if any, on both inflammatory bowel diseases is probably mediated through the reduction of oxygen free radicals and histamine release from mast cells. In this study, we used 2,4-dinitrobenzene sulfonic acid (DNBS) to induce colonic inflammation. This compound produces acute and chronic inflammation and ulceration in the colon with a pathology resembling ulcerative colitis rather than Crohn's disease but the exact etiology is not yet known (Hawkins et al., 1997). With this colitis model, we studied the pathogenesis of ulcerative colitis and the potential therapeutic application and the mechanisms of zinc action in the treatment of inflammatory bowel disease in rats.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 200–400 g, were obtained from the Laboratory Animal Unit of the University of Hong Kong. The animals were housed in cages with wide mesh wire bottoms to prevent coprophagy and were fed a standard laboratory diet and given free access to tap water. The cages were kept in a room with controlled temperature (22 ± 1 °C), relative humidity (65–70%) and day/night cycle (12:12 light/dark). The following experimental protocol had been approved by the Committee on the Use of Live Animals in the University of Hong Kong.

2.2. Induction of experimental colitis

DNBS-induced colitis was produced according to the method of Hawkins et al. (1997). Rats were lightly anesthetized by inhalation of ether. A polyethylene catheter was inserted rectally into the colon so that the tip was 8 cm proximal to the anus. DNBS (Sigma, USA) dissolved in 50% ethanol (BDH, UK) in the concentration of 120 mg/ml was instilled into the lumen of the colon in a volume of 0.25 ml through a polyethylene catheter.

2.3. Macroscopic assessment of colonic inflammation and damage

The rats were killed 96 h after the induction of inflammation. The distal colon was removed, opened longitudinally and the mucosal damage was scored on a 0–10 scale according to Wallace et al. (1989) (Table 1). The total surface area of colonic lesions was measured and expressed as the lesion area (mm^2). A 6–8-mm sample block of the inflamed colonic tissue was excised from a region with visible damage, or at 2–4 cm proximal to the anus for tissues without visible damage and fixed in 10% buffered formalin for further histological examination. The colonic tissue, which was 5 mm above and 5 mm below the area with visible damage, was removed. The remaining length of the colonic tissue was minced with scissors for 1 min and then stored at −80 °C for subsequent biochemical assays.

Table 1
Criteria for scoring of colonic damage

Score	Criteria
0	No damage
1	Hyperaemia; no ulcers
2	Hyperaemia and thickening of the bowel wall; no ulcers
3	One ulcer without thickening of the bowel wall
4	Two or more sites of ulceration/inflammation
5	Two or more major sites of ulceration and inflammation or one site of ulceration/inflammation extended >1 cm along the length of the colon
6–10	If damage covered >2 cm along the length of the colon, the score was increased by 1 for each additional cm of involvement

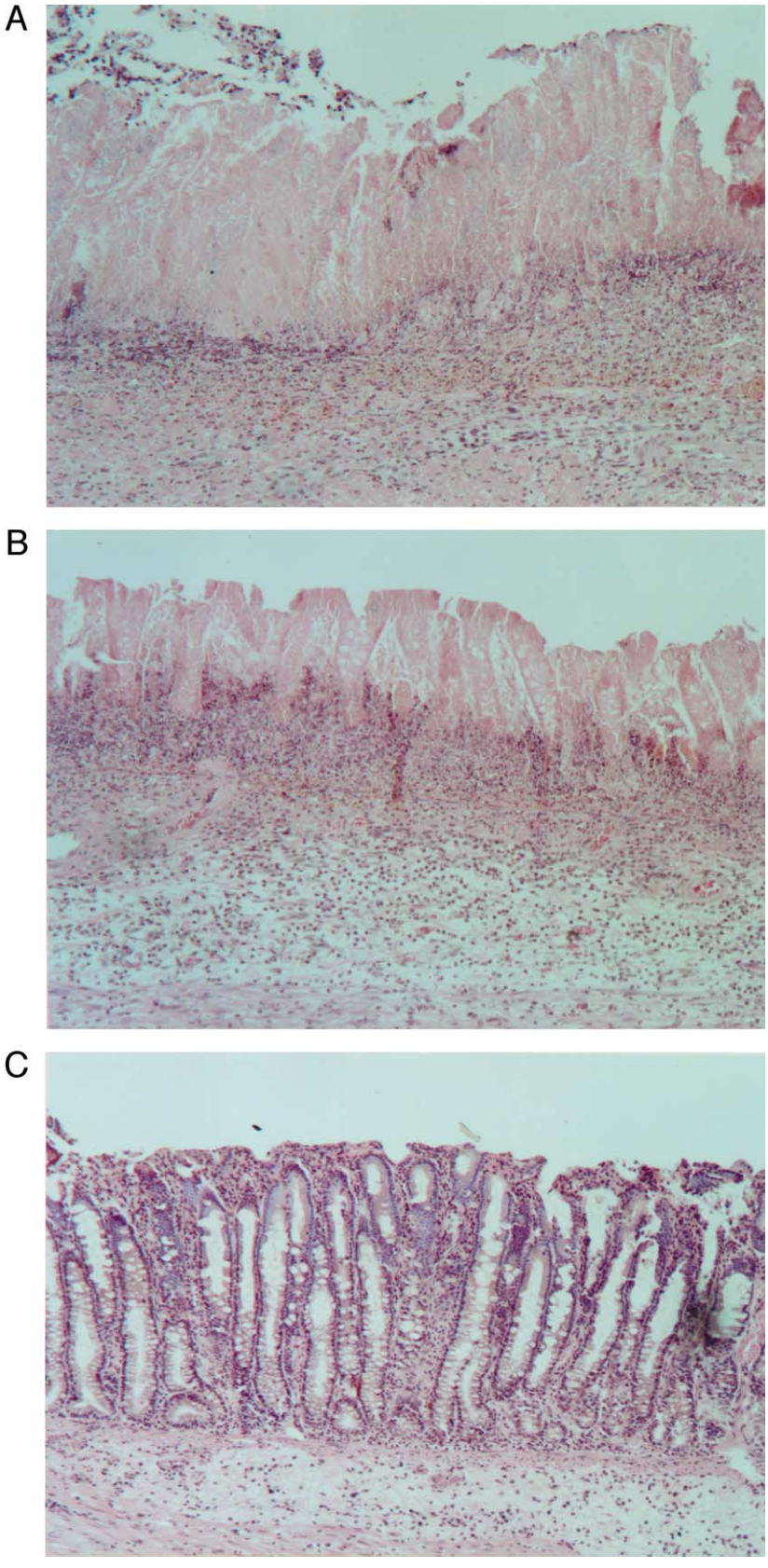
2.4. Procedures for tissue preparation and staining

After formalin fixation, the excised sample block was processed for histological evaluation. The sample block was first dehydrated by immersion in progressively increasing concentrations of ethanol (Merck, Germany) and then xylene (Merck). Following this, the dehydrated tissue was immersed in melted paraffin (Fisher Scientific, USA) at 60 °C for 3 h before being embedded in a paraffin block. Sections 5 μm thick were cut by using an 82-spence microtome (American Optical, USA). The sections were then deparaffinized by treatment with xylene, ethanol and water. Tissues were stained with haematoxylin and eosin (H&E) and then left in the fume cupboard overnight.

2.5. Microscopic assessment of colonic inflammation and damage

The histological damage was evaluated by light microscopy (CX-40, Olympus, Japan) and the extent of mucosal damage was scored (Wallace et al., 1989): 0, normal histological appearance; 1, histological damage limited to surface epithelium; 2, focal ulceration and cell disruption limited to mucosa; 3, focal, transmural inflammation and ulceration; 4, extensive transmural ulceration and inflammation bordered by areas of normal mucosa; 5, extensive

Fig. 1. Rats were given 2,4-dinitrobenzene sulfonic acid (DNBS) 30 mg in 0.25 ml of 50% ethanol intrarectally and killed 96 h later. They were given either distilled water as a control (A) or zinc sulfate, 30 mg/kg (B), or 120 mg/kg (C), intrarectally at 42, 48, 66 and 72 h after inflammation induction. Colonic mucosae were stained with H and E and observed under the microscope (100X). The crypts were extensively distorted with marked infiltration of inflammatory cells at the basal layer of the mucosa. All these were improved by zinc sulfate treatment.


A
B
C

transmural ulceration and inflammation involving entire section from epithelium to serosa.

2.6. Zinc treatment for DNBS-induced colitis

Zinc sulfate solution or enema was prepared by dissolving zinc sulfate (Sigma) in distilled water. All rats were divided into oral and rectal treatments groups. Each treatment was administered either orally (p.o.) or rectally (p.r.) to rats at 42, 48, 66 and 72 h following the induction of inflammation. In the rectal treatment groups, either distilled water (1 ml/kg) or zinc sulfate (30, 60 or 120 mg/kg) was given at the appointed times by the same method as used for DNBS administration. Oral treatment groups also received distilled water (1 ml/kg) as a control or zinc sulfate (60 mg/kg) as a drug treatment group. Ninety-six hours following the induction of inflammation, the rats were killed and assessed for damage according to procedures described above.

2.7. Histamine receptor antagonists on DNBS-induced colitis

Terfenadine (Sigma) and ranitidine (Glaxo Research and Development, UK) were used to block histamine type 1 and type 2 receptors, respectively. Ranitidine was dissolved in normal saline and terfenadine was suspended in normal saline with 5% ethanol (v/v) as a co-solvent and ultrasonicated for 1 min. Ranitidine was given intraperitoneally (i.p.) and terfenadine was injected subsutaneously (s.c.) at the doses of 20 mg/kg (5 ml/kg) and 12.5 mg/kg (2 ml/kg), respectively, to rats. One hour after administration of the drugs, DNBS was given p.r. to the rats via a polyethylene catheter. Ranitidine and terfenadine were further administered at the same doses at 24, 48 and 72 h following the induction of inflammation. Control animals received 5% ethanol in normal saline solution in a volume of 2 ml/kg. After 96 h following rectal administration of DNBS, the rats were killed and the distal colon was removed for damage assessment.

2.8. Ketotifen on DNBS-induced colitis

Ketotifen (Sigma) was dissolved in distilled water and shaken ultrasonically for 1 min. All rats were divided into control and treatment groups. Treated rats were given ketotifen (1 g/kg) p.o. at 42, 48, 66 and 72 h following the induction of damage. Distilled water was administered to control animals at the same times. After 96 h following induction of colitis, all rats were killed. The distal colon was removed, opened by a longitudinal incision and the degree of damage was evaluated.

2.9. Measurement of myeloperoxidase activity

Myeloperoxidase activity was determined by the tetramethylbenzidine method with modifications. Colonic tissue was weighed and placed in a plastic tube with hexadecyltrimethylammonium bromide (Sigma) buffer (0.5% hexadecyltrimethylammonium bromide in 50 mM sodium phosphate buffer (BDH), 9 μl/mg tissue). The tissue was homogenized with a homogenizer (IKA T-25, Ultra-Turraz, USA) three times for 10 s on ice. After homogenization, the homogenate was freeze-thawed, sonicated for 10 s three times and centrifuged at 30,000 × *g* for 20 min. The supernatant was assayed for myeloperoxidase activity, which was measured spectrophotometrically: 50 μl of supernatant was combined with 250 μl of 80 mM sodium phosphate buffer, 150 μl of 2 mM tetramethylbenzidine (Sigma) and 50 μl of 0.3 M H_2O_2 (Sigma). The mixture was incubated at 25 °C for 25 min. The reaction was then stopped by addition of 2.5 ml of 0.5 M sulphuric acid (BDH). The change in absorbance at 450 nm was measured with a spectrophotometer (Beckman DU650, Fullerton, CA, USA). One unit of myeloperoxidase activity was defined as that oxidizing 1 μmol of peroxide per min at 25 °C at pH 5.0.

2.10. Measurement of xanthine oxidase activity

The colonic sample was weighed and placed in a plastic tube containing homogenizing buffer (9 μl/mg) consisting of 50 mM potassium phosphate (BDH) buffer (pH 7.0) with 10 mM dithioerythritol (Sigma), 1 mM phenylmethyl sulfonyl fluoride (Sigma) and 0.1 mM EDTA (Sigma). The tissue was then homogenized for 30 s on ice and centrifuged at 20,000 × *g* for 10 min. Thirty microliters of the resulting supernatant was mixed with 150 μl of 1 mM xanthine (Sigma), 150 μl of 2 mM EDTA and 200 μl of bovine serum albumin (Sigma) and 2.47 ml of 50 mM potassium phosphate buffer. The mixture was incubated at 25 °C for 5 min followed by 1 ml of 0.5 M HCl to stop the reaction. The change in absorbance at 295 nm was measured with a spectrophotometer. One unit of xanthine oxidase activity was defined as that which could convert 1.0 μmol of xanthine to uric acid per min at pH 7.5 at 25 °C.

2.11. Statistical analysis

The results were expressed as the means ± standard error of mean (S.E.M.). Statistical significances were assessed by Turkeys' test following one-way analysis of variance (ANOVA). Lesion score and histological score were expressed as medians and compared using Krustal–Wallis nonparametric ANOVA followed by Dunn's multiple comparison test. Differences with a P value less than 0.05 were considered significant.

3. Results

3.1. Effects of zinc treatment on DNBS-induced colitis

There was no visible damage in the normal control group and histological examination showed normal architecture.

Table 2
Effects of zinc enema on DNBS-induced colitis

	Rectal control group	Experimental group 1 (zinc 30 mg/kg)	Experimental group 2 (zinc 60 mg/kg)	Experimental group 3 (zinc 120 mg/kg)
Lesion area (mm^2)	234.47 ± 28.55	166.85 ± 15.60	63.55 ± 12.31[a]	103.67 ± 13.07[b]
Lesion score	5	5	3[a]	3[c]
Histological score	3	3	2	2.25
Myeloperoxidase activity (U/g)	1.32 ± 0.14	0.87 ± 0.12	0.60 ± 0.07[b]	1.06 ± 0.13
Xanthine oxidase activity (U/g)	3.04 ± 0.25	2.74 ± 0.31	3.17 ± 0.22	2.68 ± 0.42
Number of rats	15	13	11	6

Zinc enemas were given at 42, 48, 66 and 72 h after induction of inflammation; rats were killed 96 h later.
Each value represents mean ± S.E.M. and lesion and histological scores were expressed as medians.
[a] $P<0.001$ when compared with the control group.
[b] $P<0.01$ when compared with the control group.
[c] $P<0.05$ when compared with the control group.

At autopsy, the part of colon exposed to DNBS was distended and adhering to surrounding tissues. The distal colon was then removed and opened longitudinally. In the control rats, DNBS enema produced severe colitis with hyperemia, edema, thickening and ulceration of the mucosa. The ulcerated mucosa was distended transversely and shortened longitudinally and covered with a grayish brown mud-like substance, and these lesions were usually continuous but sometimes segmental with intervening uninvolved area. The light microscopic study of H&E-stained colonic specimens of control rats showed marked epithelium destruction and crypt distortion with the infiltration of numerous inflammatory cells. These changes usually extended transmurally (Fig. 1A). All the glandular cells were lost. Zinc sulfate-treated animals showed a relatively normal mucosa with a gradual increase of intact crypts in the colonic mucosa with increasing doses of zinc sulfate from 30 to 120 mg/kg (Fig. 1B and C). The activity of myeloperoxidase was sharply increased by DNBS enema from 0.18 ± 0.02 to 1.32 ± 0.14 U/g ($P<0.001$). In contrast, the level of xanthine oxidase was not significantly affected by DNBS enema, i.e. 3.04 ± 0.25 U/g in the DNBS control group compared with 3.63 ± 0.29 U/g in the normal control group.

Zinc sulfate enema at concentrations ranging from 30 to 120 mg/kg reduced colonic lesions (Table 2). The therapeutic effect of zinc enema was observed at a dose of 30 mg/kg (though it did not achieve a statistically significant level) and increased with the concentration of zinc, reaching a maximum at 60 mg/kg. Increasing the dose of zinc did not show additional benefit. Sections from the colon of the rectal experimental groups showed smaller ulceration and milder inflammation as compared to the control group, though the mucosa did not appear normal. Myeloperoxidase activity per unit tissue weight was significantly decreased by zinc enema but xanthine oxidase activity per unit tissue weight was unaffected (Table 2). The drug also dose dependently improved the body weight loss in the DNBS-treated rats. Oral administration of zinc solution at the dose of 60 mg/kg did not affect the changes observed in the colon 96 h after the administration of DNBS.

3.2. Effects of histamine receptor antagonists on DNBS-induced colitis

Terfenadine and ranitidine at doses of 12.5 and 20 mg/kg, respectively, significantly reduced the lesion area but did not affect the dilatation and adhesion of the colon observed 96 h after DNBS administration (Table 3). The two drugs significantly inhibited myeloperoxidase activity. The xanthine oxidase activity per unit weight was slightly decreased but the decrease did not reach statistical significance. The light microscopic study of H&E-stained colon specimens from the terfenadine and ranitidine groups showed less ulceration and milder inflammation as compared with the respective control group. However, the effect was not statistically significant.

3.3. Effect of ketotifen on DNBS-induced colitis

Ketotifen (1 g/kg) significantly inhibited adhesion of the colon but did not affect the dilatation of the colon

Table 3
Effects of histamine blockers on DNBS-induced colitis

	Control	Terfenadine (12.5 mg/kg, s.c.)	Ranitidine (20 mg/kg, i.p.)
Lesion area (mm^2)	305.00 ± 54.22	76.25 ± 41.60[a]	118.25 ± 8.76[b]
Lesion score	6.5	3[b]	5
Histological score	3.5	3	2.25
Myeloperoxidase activity (U/g)	1.05 ± 0.12	0.56 ± 0.06[b]	0.63 ± 0.11[b]
Xantine oxidase activity (U/g)	2.89 ± 0.36	1.94 ± 0.14	2.56 ± 0.63
Number of rats	4	4	4

Terfenadine and ranitidine were given at 1 h prior to the rectal administration of DNBS. The drugs were further administered at the same doses at 24, 48 and 72 h following the induction of inflammation; the rats were killed 96 h later.
Each value represents mean ± S.E.M. and lesion and histological scores were expressed as medians.
[a] $P<0.01$ when compared with the control groups.
[b] $P<0.05$ when compared with the control groups.

Table 4
Effects of ketotifen on DNBS-induced colitis

	Control	Ketotifen (100 μg/100 mg)
Lesion area (mm^2)	263.78 ± 32.54	46.33 ± 11.37[a]
Lesion score	6	3[a]
Histological score	4	2[b]
Myeloperoxidase activity (U/g)	0.98 ± 0.07	0.69 ± 0.09[c]
Number of rats	9	9

Ketotifen solutions were given orally at 42 h, 48 h, 66 h and 72 h following the induction of inflammation; the rats were killed 96 h later.
Each value represents mean ± S.E.M. and lesion and histological scores were expressed as medians.
[a] $P < 0.001$ when compared with the control groups.
[b] $P < 0.01$ when compared with the control groups.
[c] $P < 0.05$ when compared with the control groups.

observed 96 h after DNBS administration. The drug markedly reduced the lesion area induced by DNBS (Table 4). Myeloperoxidase activity per unit tissue weight was also significantly inhibited by ketotifen. Sections from the colon of control rats showed severe ulceration, crypt distortion and extensive inflammatory cell infiltration. Sections from the colon of rats treated with ketotifen had much smaller ulceration and milder inflammation as compared with the control.

4. Discussion

Animal models have been utilized to study the etiology of inflammatory bowel disease. DNBS-induced colitis is a new type of experimental model developed recently (Hawkins et al., 1997; Ko et al., 2001). As indicated in these studies, DNBS enema produced acute and chronic inflammation and ulceration in the colon with a pathology resembling ulcerative colitis. We used this animal model to examine whether a zinc compound could protect against inflammation in addition to its therapeutic effect on Crohn's disease-like colitis in animals (Yoshikawa et al., 1997; Chen et al., 1999). Furthermore, the pathogenic mechanisms of DNBS-induced colitis were investigated.

Reactive oxygen metabolites have been implicated in a variety of diseases. There is now increasing evidence that reactive oxygen metabolites may play an important role in the pathogenesis of inflammatory bowel disease. Several laboratories had demonstrated enhanced reactive oxygen metabolism in phagocytes from patients with active inflammatory bowel disease (Grisham, 1993). By using ultraweak chemiluminescence, Keshavarzian et al. (1992) had found that the inflamed colon from two experimental models of colitis produce much larger quantities of reactive oxygen species than do control colons. In addition, Oshitani et al. (1993) reported that vascular endothelial cells and invading monocytes in patients with ulcerative colitis produced greater amounts of superoxide than did control colons. These findings suggest that there is an increased oxidative stress in the inflamed colon during chronic gut inflammation.

In the present study, rectal administration of DNBS increased myeloperoxidase activity nearly eight-fold. Since myeloperoxidase is a membrane-bound enzyme found almost exclusively in neutrophils and to a lesser extent in monocytes, its level may provide an estimate of neutrophil infiltration in tissues (Krawisz et al., 1984). Our results, therefore, indicated a dramatic increase in neutrophil infiltration into the intestinal mucosa after inflammation induction. The neutrophils may contribute to the production of reactive oxygen metabolites via activation of their NADPH oxidase and secretion of myeloperoxidase into extracellular space. Although we did not measure NADPH oxidase activity in our experiment, it was speculated that this enzyme could also be activated by DNBS enema since NADPH oxidase and myeloperoxidase are two enzymes that are activated almost simultaneously (Weiss, 1989). Previous findings indicate that using the same animal model to induce colitis can provoke luminal-amplified chemiluminescence, an assay for free radicals production in the tissue (Sham, 2001). The reactive oxygen metabolites generated by the two enzymes may be involved in mediating cellular injury and lesion formation.

The effect of DNBS enema on the other reactive oxygen metabolite-producing enzyme xanthine oxidase was also evaluated. It was found that DNBS enema did not have a significant effect on the xanthine system, suggesting that this enzyme may not be involved in the pathogenesis of DNBS-induced colitis. The protective effect of zinc sulfate on colitis formation along with the reduction of myeloperoxidase but not xanthine oxidase would be fully consistent with this phenomenon.

In addition to oxygen-derived metabolites, the possible role of inflammatory mediators such as histamine and mast cells in the pathogenic mechanisms of DNBS-induced colitis was also investigated. Our preliminary study indicated that colon inflammation markedly increased the number of mast cells, at the site of ulceration (more than 20-fold increase), suggesting that inflammation itself can induce mast cell proliferation, which in turn would further worsen ulceration in the colonic tissue. Zinc sulfate treatment was able to reduce the severity of inflammation and lower the mast cell number by about 25% in colonic tissue. Nevertheless, the larger pool of mast cells in the tissue would imply more histamine, which contributes in part to aggravate the formation of mucosal injury in the colon. To substantiate further the importance of histamine in mucosal injury, terfenadine and ranitidine were used to block histamine type 1 (H_1) and type 2 (H_2) receptors, respectively. Both histamine receptor antagonists significantly reduced the lesion area observed after instillation of DNBS enema implicating that both types of histamine receptors play a contributory role in the pathogenesis of DNBS-induced colitis. The two drugs also significantly reduced colonic myeloperoxidase activity, indicating that histamine receptor

activation is closely associated with neutrophil infiltration in the inflamed mucosa. In agreement with other reports, our results had shown that blockade of histamine receptors did not have significant effects on the xanthine oxidase system (Tarnok and Tarnok, 1987; Akamatsu et al., 1991), suggesting that ranitidine and terfenadine probably act via mechanisms other than inhibition of the xanthine oxidase pathway, which is probably through inhibition of neutrophil infiltration.

The underlying mechanism by which histamine receptor blockade reduced neutrophil infiltration seems to be related to the effect of histamine on leukocyte recruitment. Migration of leukocytes from the circulation to the sites of inflammation is a complex process that requires several steps (Roitt et al., 1998). Leukocytes are first slowed as they roll down the endothelium by interaction with selectins. Specifically, P-selectin moved to the cell surface from Weibel–Palade bodies of endothelial cells is a likely candidate to mediate leukocyte rolling. P-selectin can be mobilized to the endothelial cell surface by many different pro-inflammatory mediators, including histamine, leukotriene C_4 and oxidants. Gaboury et al. (1995) demonstrated that the histamine H_1 receptor antagonist, diphenhydramine, but not the leukotriene synthesis inhibitor nor the platelet-activating factor (PAF)-receptor antagonist prevents the mast cell degranulation-mediated increase in flux of rolling leukocytes. They proposed that recruitment of circulating leukocytes is promoted by histamine-induced, P-selectin-dependent, leukocyte rolling via the histamine H_1 receptor. Thorlacius et al. (1995) found that topical administration of histamine caused a four-fold potentiation of leukotriene B_4-induced leukocyte adhesion. Moreover, histamine significantly increased the rolling leukocyte flux and this effect was blood flow-dependent. These findings showed that histamine could promote leukocyte recruitment through a combination of microvascular actions, in addition to P-selectin-dependent upregulation of leukocyte rolling.

In addition to histamine H_1 receptors, H_2 receptors also play a contributory role in mediating leukocyte recruitment. Farber et al. (1986) demonstrated that endothelial cells could generate lipid products which enhance neutrophil migration, and these chemoattractant products are elaborated by histamine via histamine H_2 receptors on endothelial cells. These findings make it not surprising that terfenadine and ranitidine could reduce DNBS-induced damage by blocking histamine-stimulated leukocyte recruitment via both histamine H_1 and H_2 receptors.

The involvement of histamine in the pathogenesis of DNBS-induced colitis was further confirmed by the effect of ketotifen on this model. Ketotifen is a mast cell stabilizer, which prevents degranulation of mast cells (Eliakim et al., 1992). Histamine is a naturally occurring amine stored primarily in mast cells. Release of histamine from mast cells can be triggered by a number of stimuli ranging from anaphylaxis to physical and chemical insults. Inhibition of mast cell degranulation can thereby block histamine release from mast cells. As indicated in our study, oral administration of ketotifen significantly reduced both lesion area and colonic myeloperoxidase level. The reduced myeloperoxidase activity suggested a decreased neutrophil infiltration. Since histamine is the major mediator released from mast cells, it is reasonable to speculate that the therapeutic effect of ketotifen is mediated through inhibition of mast cell degranulation and its subsequent histamine-induced neutrophil infiltration in colonic tissues.

Similarly, the therapeutic action of another known mast cell stabilizer, zinc sulfate, on ulcerative colitis was also demonstrated in the present study. In fact, several studies had demonstrated that histamine release from mast cells was inhibited by zinc (Cho et al., 1977; Escolar and Bulbena, 1989; Kazimierczak and Maslinski, 1974a,b). This effect was proposed to be related to its membrane-stabilizing effect. Marone et al. (1981) found that zinc caused a dose-dependent inhibition of histamine release from basophils induced by various stimuli and suggested that this was due to the microtubule-stabilizing effect of zinc. The state of aggregation of microtubules plays a significant role in the control of histamine release. It is possible that zinc displaces calcium from the microtubules, resulting in deterioration of the structure and related function of microtubules and the subsequent release of histamine.

Besides its mast cell-stabilizing effects, zinc has been reported to reduce prostaglandin E_2 and leukotriene B_4 levels in TNBS-induced colitis in rats (Chen et al., 1999). Leukotriene B_4 is one of the most potent stimuli for polymorphonuclear neutrophil migration and secretion. By inhibiting this eicosanoid, zinc may down-regulate the recruitment of polymorphonuclear neutrophils and reduce the severity of inflammation. This action may reduce the number of mast cells at the injury site and lessen the severity of colonic damage as suggested previously.

Oral administration of zinc solution was not as effective as that of zinc enema, suggesting that zinc may not have a systemic effect at the dosage now used and could act more effectively when the drug is given rectally, where it can act directly on the inflammatory tissue and provide protection. As zinc sulfate was given at the stage after inflammation, the therapeutic potential of zinc compound is recognized as an alternative for the treatment of ulcerative colitis.

In summary, histamine released from mast cells plays an important role in the pathogenesis of ulcerative colitis in animals. Histamine can induce neutrophil recruitment via H_1 and H_2 receptor stimulation. Oxygen-derived free radicals released from neutrophils and myeloperoxidase activation may contribute to colonic injury. The xanthine oxidase pathway is not involved in mediating tissue damage in the colitis model we now used. Zinc enema has a therapeutic implication for DNBS-induced colitis through the reduction of neutrophil infiltration and also mast cell degranulation. All these effects are largely responsible for the beneficial effect of zinc sulfate in ulcerative colitis.

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