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ORIGINAL PAPER

The Role of Zinc and Metallothionein in the Dextran Sulfate Sodium-Induced Colitis Mouse Model

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Abstract Zinc (Zn) and its binding protein metallothionien (MT) have been proposed to suppress the disease activity in ulcerative colitis. To determine the role of Zn and MT in the dextran sulfate sodium (DSS)-induced model of colitis in mice, a DSS dose-response study was conducted in male C57BL/6 wild-type (MT+/+) and MT-null (MT−/−) mice by supplementing 2%, 3%, and 4% DSS in the drinking water for 6 days. In the intervention study, colitis was induced with 2% DSS, Zn (24 mg/ml as ZnO) was gavaged (0.1 ml) daily, concurrent with DSS administration, and the disease activity index (DAI) was scored daily. Histology, MT levels, and myeloperoxidase (MPO) activity were determined. DAI was increased ($P < 0.05$) by 16% and 21% with 3% and 4% concentrations of DSS, respectively, compared to 2%, evident after 5 days of DSS administration. MPO activity was increased in MT+/+ compared to MT−/− mice and those receiving DSS. Zn administration had a 50% ($P < 0.05$) lower DAI compared to DSS alone. Zn partially prevented the distal colon of MT+/+ by 47% from DSS-induced damage compared to MT−/− mice. MT did not prevent DSS-induced colitis and Zn was partially effective in amelioration of DSS-induced colitis.

Keywords Inflammatory bowel disease · DSS-induced colitis · Zinc · Metallothionein (MT) · MT knockout

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Introduction

Inflammatory bowel disease (IBD) is a chronic condition affecting between 2 and 5 per 1000 individuals in Western civilization [1]. There are two distinct forms of IBD, ulcerative colitis (UC) and Crohn's disease. The aetiologies of the diseases are believed to differ slightly, with UC affecting mainly the distal colon, while Crohn's disease can affect every part of the gastrointestinal (GI) tract [2]. Although many studies have been undertaken, the cause of the disease remains unknown, however, several factors have been implicated. These include environmental [3] and genetic [4] factors, microbial pathogens, autoimmune mechanisms, vascular impairment, and infectious factors [5]. There is increasing evidence that reactive oxygen species (ROS) play a fundamental role in the pathogenesis of IBD [6–8] and large quantities of ROS have been described in the mucosa of patients with IBD, correlating with severity of disease [9].

Zinc (Zn), one of the essential trace elements, has been proposed to have beneficial effects in IBD [10]. Several studies [10–12] have demonstrated that Zn enemas were able to reduce damage to the intestinal mucosa induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) in rats. Polaprezinc (*N*-[3-aminopropionyl]-L-histidinato zinc), a chelate compound consisting of Zn ion, L-carnosine, dipeptide of β-alanine, and L-histidine, has been shown to ameliorate

dextran sulfate sodium (DSS)-induced colitis in mice [13]. Furthermore, Zn has been reported to regulate tight-junction permeability in experimental colitis [14]. The mechanism of action of Zn in UC is unclear, however, Zn has been proposed as an antioxidant and also a mast cell stabilizer [10] as well as a constituent of superoxide dismutase [6, 7] and metallothionien (MT) [15], the latter presumably mediating its anti-inflammatory action on IBD through the scavenging of ROS.

MT is a low molecular weight metal binding protein with a high cysteine content [16] that regulates metal ion concentrations, as well as participating in cell differentiation, proliferation, and apoptosis. MT directly interacts with ROS and acts as a scavenger of toxic radicals. It protects against ionizing radiation and intracellular oxidative stress [17]. MT expression can be induced by diverse factors such as stress, steroids, and metals, in particular Zn [16]. It is proposed that Zn administration may influence the expression of MT and complement Zn in the processes of protection and repair. The putative role of MT in colitis may also be determined utilizing mice lacking gene expression for MT-I and MT-II, the only isoforms capable of incorporating Zn in the liver and GI tract of rodents [18].

DSS is a reliable inducer of UC in many experimental rodent models [19–21]. Here we investigate the influence of MT and of Zn administration on DSS-induced colonic inflammation in wild-type mice and mice lacking MT expression.

Methods

Animals

Wild-type C57BL/6 male mice (MT+/+) were obtained from the University of Adelaide (Adelaide, South Australia) at 8 weeks of age. MT-I- and MT-II-null (MT−/−) mice (mixed genetic background of OLA129 and C57BL/6 strains) were obtained from a breeding colony at the Children, Youth and Women's Health Service Animal Care Facility (North Adelaide, South Australia). The generation of MT-null mice has been described previously [18]. Briefly, the MT-I and MT-II genes located on mouse chromosome 8 were prevented from replication by performing a 20-base-pair frame shift and inserting a 1.2-kilobase selection marker, respectively. All animals were group-housed in a temperature-controlled room (adjusted for a 10/14-hr light/dark period) until the commencement of the study, when they were placed in individual housing. All animals were acclimatised for 1 week prior to commencement of the trial. The present study complied with the Australian Code of Practice for the Care and Use of Animals, and ethical approval was obtained from the Children, Youth and Women's Health Service Animal Ethics Committee.

DSS dose-response study

Twenty-four MT+/+ and 24 MT−/− male mice were randomly allocated to one of three treatment groups ($n = 8$)—2%, 3%, or 4% DSS (MP Biomedicals, Eschwege, Germany)—to induce colitis. Tap water was supplemented with DSS (w/v) and mice had free access to water for the next 6 days. DSS-induced colitis was assessed by a qualitative disease activity index (DAI) scoring system (see below for description).

Zn intervention study

Twenty four MT+/+ and 24 MT−/− male mice had colitis induced by supplementing 2% (w/v) DSS in the drinking water for 6 days. The DSS concentration resulted in an appropriate degree of colitis and was chosen based on the dose-response study, consistent with previous studies in mice [22]. The MT+/+ ($n = 8$/group) and MT−/− ($n = 8$/group) mice were randomly allocated to one of the three groups following concurrent interventions with DSS treatment, (i) control (no DSS), (ii) DSS alone, and (iii) DSS + 24 mg/ml Zn as ZnO (Sigma Chemicals, St. Louis, MO, USA). Treatments were administered via orogastric gavage of 0.1 ml daily. The control group consuming water without DSS and the DSS-alone group were also gavaged with 0.1 ml of water daily. During the 6-day experimental period, DAI was assessed daily. At the end of the experimental period the animals were sacrificed, and the colons collected, fixed, and embedded in paraffin wax for histological assessment, as well as assaying for MPO activity and MT levels.

Disease activity index assessment

The qualitative DAI scoring system has been described previously by Howarth et al. [23]. Briefly, the scoring system comprised examination of stool consistency, rectal bleeding, weight loss, and general well-being of the animal. Each of these factors was scored on a 0–3 scale, with 0 representing no disease symptom and 3 representing severe disease symptom. Weight loss was scored as 0 representing no weight loss compared to the original weight, 1 representing a weight loss of less than 5%, 2 representing a weight loss of between 5% and 10%, and 3 representing a weight loss of more than 10% of the original weight. The severity of each variable was scored from 0 to 3. Data are the sum of scores for four independent variables.

Tissue collection

At the end of the 6-day experimental period, all mice were killed by CO_2 asphyxiation, followed by cervical dislocation. The colon was removed, measured, and weighed and a 2-cm portion was fixed in 10% formalin for 24 hrs and embedded in paraffin wax for histological assessment. The remainder of the colon was weighed and snap-frozen in liquid nitrogen for myeloperoxidase (MPO) activity and analysis of MT levels.

Histological assessment

Colon sections (5 μm) were stained with hematoxylin and eosin for histological examination by assessing parameters including enterocyte disruption, goblet cell loss, crypt loss, crypt disruption, polymorphonuclear cell infiltration, submucosal thickening, and muscularis thickening as described by Howarth et al. [24]. This is based on a 0–3 scoring scale in which 0 represents no damage; 1, mild; 2, moderate; and 3, severe colonic damage. The severity scores for each parameter were scored from 0 to 3. Data are the sum of scores for seven independent variables. Microscopy was carried out using an Olympus BH-2 light microscope (Olympus, Tokyo) with a Sony Digital camera (Olympus) and the Image Pro Plus package V4.5.1.27 (Media Cybernetics, USA). Crypt depth was also measured using the Image Pro Plus Package as described by Howarth et al. [24].

Myeloperoxidase activity

MPO activity was determined in the colon as described previously [25]. MPO is an intracellular enzyme localized in the granules of neutrophils and acts as an indicator of neutrophil infiltration into the damaged colon. Briefly, MPO was released from the sample during a 1-min period of homogenization (Ultra Turrex homogenizer; Janke and Kunkel, Germany) in 1 ml saline. This sample was then centrifuged for 10 min at 14,000 *g* (Mikro Benchtop Centrifuge; Hettich GmbH and Co., Tuttlingen, Germany), the supernatant removed, and the pellet resuspended in hexadecyltrimethylammonium bromide (HTAB), a detergent (Sigma Chemicals, Sydney). This was centrifuged for 2 min at 5000*g*, and the supernatant removed and added to a hydrogen donor 30%, w/v, hydrogen peroxide (Merck Pty., Victoria, Australia). This was then analyzed with a spectrophotometer (Dynatech MR7000; Guernsey, Channel Islands), which measured the activity at 1-min intervals over a 15-min period. Results are expressed as units per milligram of tissue.

Metallothionein analysis

Tris-HCl, pH 8.2 (10 μl, 300 mM), was added to 300 μl of colonic homogenate. The sample was boiled and centrifuged at 14,000 *g* for 3 min and the supernatant was used for analysis of MT using a ^{109}Cd/heme affinity assay.[16] Results are expressed as nanomoles of Cd bound per gram wet weight.

Statistical analysis

Qualitative DAI and semi quantitative histological severity scores were normalized with a natural log transformation and then compared using multiple-pairwise comparison three-way ANOVA. A Tukey's post hoc test was used to determine statistical significance among different doses of DSS, genotypes, and time for the dose-response study and among treatment, genotype, and tissue region for the Zn intervention study. However, for the DAI of the intervention study a two-way ANOVA followed by a Tukey's post hoc test was used to determine statistical significance between treatment and genotype. MPO data were log transformed, and together with the MT, crypt depth and colon length data were assessed using a multiple-pairwise comparison two-way ANOVA followed by a Tukey's post hoc test for statistical significance. Post hoc tests were performed if a significant F value ($P < 0.05$) was attained from the ANOVA. All statistical analysis was performed using SigmaStat Statistical Software, version 2.03 (SPSS Inc., Chicago, IL, USA). Statistical significance is given at $P < 0.05$ unless otherwise stated in the text. DAI and histological severity scores results are expressed as the geometric means $\pm$ SD, and the others are expressed as mean $\pm$ SE.

Results

DSS dose-response study

DAI

MT $+/+$ mice (2.2 ± 0.3 units; geometric mean $\pm$ SD) had a significantly ($P = 0.02$) higher DAI compared to MT $-/-$ animals (2.0 ± 0.3 units) irrespective of DSS dose and time (Table 1). Administration of increasing doses of DSS had a marked effect on the DAI, with animals receiving 3% and 4% DSS having a significantly ($P < 0.05$) elevated DAI, by 16% and 21%, respectively, compared to those receiving 2% DSS (Table 1), irrespective of genotype and time. Furthermore, after day 3, the severity of the DAI increased in proportion to time after administration irrespective of genotype and DSS dose (Table 1).

Histological severity score assessment

There was no difference in histological severity scores between MT $+/+$ and MT $-/-$ mice. Increasing doses of DSS did not alter the severity score assessment (2%,

Table 1 Disease activity index (DAI) of metallothionein wild-type (MT+/+) and null (MT−/−) mice after consuming increasing concentrations of dextran sulfate sodium (DSS) for 6 days

	DAI (U)							
	MT+/+			MT−/−				
	2.2 ± 0.3			2.0 ± 0.3*				
	2% DSS		3% DSS		4% DSS			
	1.9 ± 0.3**		2.2 ± 0.3		2.3 ± 0.3			
Day	MT+/+	MT−/−	MT+/+	MT−/−	MT+/+	MT−/−	Mean[a]	3-way ANOVA *P* value
								0.02, genotype
1	1.0 ± 0.0	1.5 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	0.001, DSS dose
2	1.2 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	1.3 ± 0.2	0.001, time
3	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	0.06, genotype × DSS dose
4	1.7 ± 0.2^{x}	1.5 ± 0.2^{x}	2.2 ± 0.2^{x}	2.0 ± 0.2^{x}	3.1 ± 0.2^{x}	1.8 ± 0.2^{x}	2.0 ± 0.2^{x}	0.002, genotype × time
5	3.2 ± 0.2^{y}	2.8 ± 0.3^{y}	4.6 ± 0.1^{y}	3.8 ± 0.2^{y}	5.2 ± 0.2^{y}	3.2 ± 0.3^{y}	3.7 ± 0.2^{y}	0.45, DSS dose × time
6	6.2 ± 0.1^{z}	4.5 ± 0.2^{z}	7.0 ± 0.1^{z}	6.0 ± 0.1^{z}	7.8 ± 0.1^{z}	4.4 ± 0.2^{z}	5.9 ± 0.2^{z}	0.75, genotype × DSS dose × time

Note. The DAI of each variable was scored from 0 to 3 and values are the sum of scores of four independent variables as detailed under Materials and Methods. *Significantly ($P < 0.05$) different compared to MT+/+ mice.**Significantly ($P < 0.05$) different compared to 3% and 4% DSS. Means within a column not sharing a common superscript letter (x, y, z) are significantly different at $P < 0.05$.

[a]Geometric means ± SD of row.

14.2 ± 0.1; 3%, 14.4 ± 0.1; 4%, 13.2 ± 0.1 units; geometric mean ± SD), although the P value ($P = 0.03$) from the three-way ANOVA suggested that there was a significant difference (Table 2). The disease severity score in the proximal colon of MT+/+ mice receiving 2% DSS was 30% lower than in the distal colon and 23% lower than in the proximal colon of MT−/− mice receiving the same dose of DSS (Table 2). Although the histological severity scores were higher in the distal compared to the proximal regions, this reached significance only at the 4% concentration of DSS (proximal colon, 11.0 ± 0.09 units; distal colon, 15.8 ± 0.07 units), irrespective of genotype (Table 2).

Colon length and crypt depth

Increasing doses of DSS (2%, 3%, and 4%) did not have an effect on colon length in either MT+/+ (mean ± SE: 51 ± 2, 52 ± 2, and 49 ± 2 mm, respectively) or MT−/− (59 ± 4, 53 ± 3, and 52 ± 3 mm, respectively) mice. MT+/+ mice receiving 3% (82 ± 9 μm; mean ± SE) and 4% (89 ± 8 μm) DSS had significantly shorter crypts in the proximal colon compared to those receiving 2% DSS (142 ± 34 μm) (Fig. 1A). However, there were no differences in crypt depth between MT+/+ and MT−/− mice in the proximal colon for a given dose of DSS. There are

Table 2 Histological severity score assessment of the proximal and distal colon of metallothionein wild-type (MT+/+) and null (MT−/−) mice after consuming increasing concentrations of dextran sulfate sodium (DSS) for 6 days

	Histological severity score (U)						
	MT+/+			MT−/−			
	14.0 ± 0.1			13.8 ± 0.1			
	2% DSS		3% DSS		4% DSS		
	14.2 ± 0.1		14.4 ± 0.1		13.2 ± 0.1		
	Proximal	Distal	Proximal	Distal	Proximal	Distal	3-way ANOVA *P* value
MT+/+	11.3 ± 0.04*	16.3 ± 0.03	14.2 ± 0.03	15.1 ± 0.02	11.6 ± 0.03	16.6 ± 0.06	0.57, genotype
MT−/−	14.7 ± 0.04	14.9 ± 0.05	14.2 ± 0.04	13.9 ± 0.08	10.5 ± 0.12	15.1 ± 0.07	0.03, DSS dose
							0.001, colon region
							0.02, genotype × DSS dose
Mean[a]	12.9 ± 0.07	15.6 ± 0.05	14.2 ± 0.04	14.5 ± 0.06	11.0 ± 0.09	15.8 ± 0.07**	0.02, genotype × colon region
							0.001, DSS dose × colon region
							0.03, genotype × DSS dose × colon region

Note. Values are the sum of scores of seven independent histologic criteria on colonic segments as detailed under Materials and Methods. The severity of each histologic parameter was scored from 0 to 3. *Significantly ($P < 0.05$) different compared to the proximal colon of MT−/− mice receiving 2% DSS and the distal colon of MT+/+ mice receiving 2% DSS.**Significantly ($P < 0.05$) different compared to the colon of mice receiving 4% DSS.

[a]Geometric mean ± SD of column (proximal or distal colon).

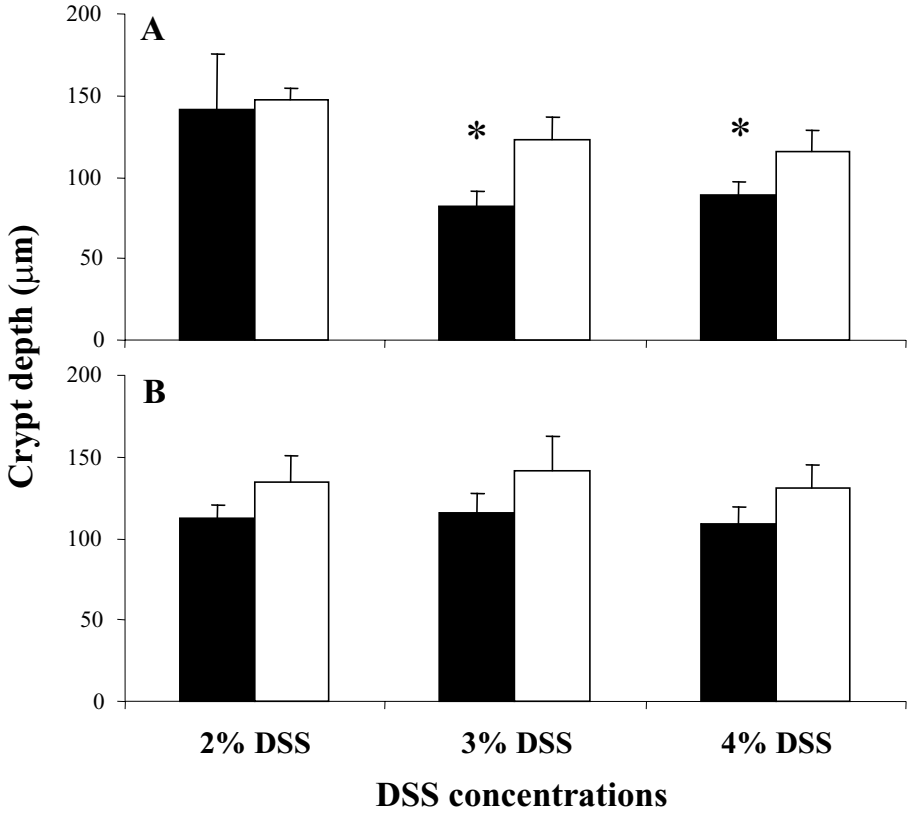

Fig. 1 Crypt depth (μm) in the proximal colon (A) and distal colon (B) of metallothionein wild-type (MT + / + ; ■) and null (MT − / − ; □) mice after consuming increasing concentrations of dextran sulfate sodium (DSS) for 6 days. Data are expressed as mean ± SE. *Significantly ($P < 0.05$) different compared to MT + / + mice treated with 2% DSS

no differences in crypt depth in the distal colon between genotypes and dose of DSS (Fig. 1B).

Metallothionein levels

MT levels did not change with administration of 2% (3.4 ± 0.7 nmol of Cd bound/g wet weight; mean ± SE), 3% (4.4 ± 1.6 nmol of Cd bound/g wet weight) or 4% (4.8 ± 1.2 nmol of Cd bound/g wet weight) DSS in MT + / + mice. MT levels in MT − / − mice were < 0.5 nmol of Cd bound/g wet weight.

As the DAI and histological severity scores were similar in the colon irrespective of dose of DSS by day 6, a study was conducted to determine the effect of Zn treatment and of MT on the severity of colitis. In this study, 2% DSS was administered to MT + / + and MT − / − mice and the DAI was assessed on day 6.

Zn intervention study

Disease activity index

MT − / − mice had a 46% lower DAI after administration of 2% DSS compared to MT + / + animals, irrespective of treatment. This was also apparent in the DSS group, where MT − / − mice had a DAI of 2.0 ± 0.2 units (geometric mean ± SD), compared to 4.9 ± 0.1 units for MT + / + animals (Table 3). MT + / + mice receiving DSS + Zn (2.1 ± 0.2 units) had a 57% lower DAI compared to the DSS (4.9 ± 0.1 units) group; this was not evident in the MT − / − mice (Table 3).

Histological severity score assessment

Mice receiving DSS alone (12.3 ± 0.1 units; geometric mean ± SD) and those receiving DSS + Zn (13.2 ± 0.2 units) had significantly ($P < 0.05$) higher severity scores compared to control (2.1 ± 0.2 units) mice (Table 4). There were no differences in severity scores between MT + / + and MT − / − mice. The severity of colitis in the colon of MT + / + mice receiving DSS + Zn (7.0 ± 0.3 units) was less damaged compared to the proximal region MT + / + (15 ± 0.1 units) and also compared to the distal colon of MT − / − mice (13.2 ± 0.1 units) (Table 4).

Colon length

There was no difference in colon length between MT + / + and MT − / − mice following any of the treatments. Overall, irrespective of genotype, control mice had longer colons compared to the DSS and DSS + Zn groups (Fig. 2). Shorter colons were found in MT + / + mice administered DSS

Table 3 Disease activity index (DAI) assessment of day 6 in metallothionein wild-type (MT + / +) and knockout (MT − / −) mice after consuming 2% dextran sulfate sodium (DSS) for 6 days with concurrent treatment

	DAI (U)			
	MT + / +	MT − / −	Mean[a]	
Treatment	2.2 ± 0.3	1.5 ± 0.2*		2-way ANOVA *P* value
Control	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.001, genotype
DSS	4.9 ± 0.1[y]	2.0 ± 0.2**,[y]	3.0 ± 0.3[y]	0.001, treatment
DSS + Zn	2.1 ± 0.2[z]	1.9 ± 0.2[y]	2.0 ± 0.2[z]	0.001, genotype × treatment

Note. The DAI of each variable was scored from 0 to 3 and values are the sum of scores of four independent variables as detailed under Materials and Methods. *Significantly ($P < 0.05$) different compared to MT + / + mice.**Significantly ($P < 0.05$) different compared to MT + / + mice treated with DSS. means within a column not sharing a common superscript letter (*y*, *z*) are significantly different, at $P < 0.05$.

[a]Geometric mean ± SD of row.

alone (61 ± 3 mm; mean ± SE) or DSS + Zn (62 ± 2 mm) compared to controls (77 ± 1 mm), and this was not observed in MT − / − mice (Fig. 2).

Crypt depth

MT + / + mice had shortened crypts compared to their MT − / − in both the proximal (Fig. 3A) and the distal (Fig. 3B) colon irrespective of treatment. In the proximal colon, MT − / − mice receiving DSS + Zn (165 ± 9 μm; mean ± SE) had longer crypts compared to their control MT − / − (134 ± 3 μm) counterparts (Fig. 3A). However, in the distal colon, MT − / − mice receiving DSS (148 ± 10 μm) resulted in a shorter crypt depth compared to control MT − / − (181 ± 5 μm) counterparts (Fig. 3B).

Myeloperoxidase activity

MT + / + mice had a marked ($P < 0.05$) neutrophil infiltration compared to their MT − / − counterparts irrespective of treatment (Fig. 4). MPO activity in MT + / + mice receiving DSS (148 ± 39 U/mg tissue; mean ± SE) was higher compared to that in control mice (26 ± 9 U/mg tissue) (Fig. 4). MT − / − mice receiving DSS alone (61 ± 17 U/mg tissue) or DSS + Zn (26 ± 5 U/mg tissue) had higher MPO activity compared to control MT − / − (8 ± 3 U/mg tissue) mice and there was a trend toward lower MPO activity in MT − / − mice receiving DSS + Zn (Fig. 4).

Metallothionein levels

Colonic mucosal MT levels did not change following treatment with DSS + Zn (1.6 ± 0.2 nmol of Cd bound/g wet weight; mean ± SE) compared to MT + / + mice receiving DSS alone (2.0 ± 0.2 nmol of Cd bound/g wet weight) and the control group (1.9 ± 0.2 nmol of Cd bound/g wet weight). MT − / − mice had MT levels < 0.5 nmol of Cd bound/g wet weight.

Discussion

ROS have been implicated in the pathogenesis of IBD [10], and animal models have been utilized to study the

Table 4 Histological severity score assessment of the proximal and distal colon of metallothionein wild-type (MT + / +) and null (MT − / −) mice after consuming 2% dextran sulfate sodium (DSS) for 6 days with concurrent treatment

	Histological severity score (U)						
	Control		DSS		DSS + Zn		
	2.1 ± 0.2		12.3 ± 0.1		13.2 ± 0.2		
	Proximal	Distal	Proximal	Distal	Proximal	Distal	3-way ANOVA *P* value
MT + / +	2.1 ± 0.2	2.1 ± 0.2	15.0 ± 0.1	12.9 ± 0.1	15.0 ± 0.1	7.0 ± 0.3*	0.001, treatment
MT − / −	2.0 ± 0.2	1.9 ± 0.2	10.7 ± 0.1	11.2 ± 0.1	11.3 ± 0.1	13.2 ± 0.1	0.50, genotype
							0.09, tissue
							0.06, treatment × genotype
							0.017, genotype × tissue
							0.19, treatment × tissue
							0.014, treatment × genotype × tissue

Note. Data are expressed as geometric mean ± SD. Values are the sum of scores of seven independent histologic criteria on colonic segments as detailed under Materials and Methods. The severity of each histologic parameter was scored from 0 to 3.

*Significantly ($P < 0.05$) different from other groups given the same treatment.

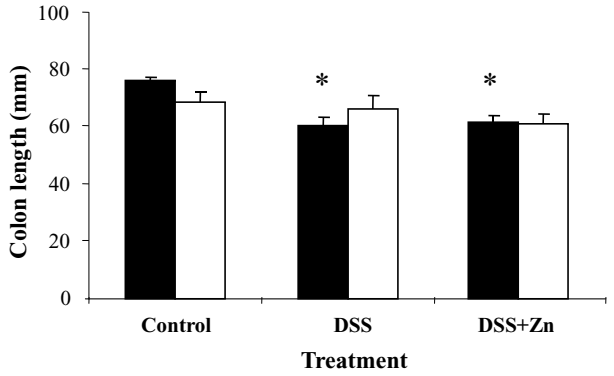

Fig. 2 Colon length (mm) in metallothionein wild-type (MT + / + ; ■) and null (MT − / − ; □) mice after consuming 2% dextran sulfate sodium (DSS) for 6 days with concurrent treatment. Data are expressed as mean ± SE. *Significantly ($P < 0.05$) different compared to control MT + / + mice

aetiology of this disease. The DSS-induced colitis model is a reproducible experimental model which produces acute and chronic inflammation and ulceration in the colon, with pathology resembling human UC. In the current study, we examined whether Zn administration and/or the presence of MT had a beneficial role in the clinical and histological features of DSS-induced colitis in mice.

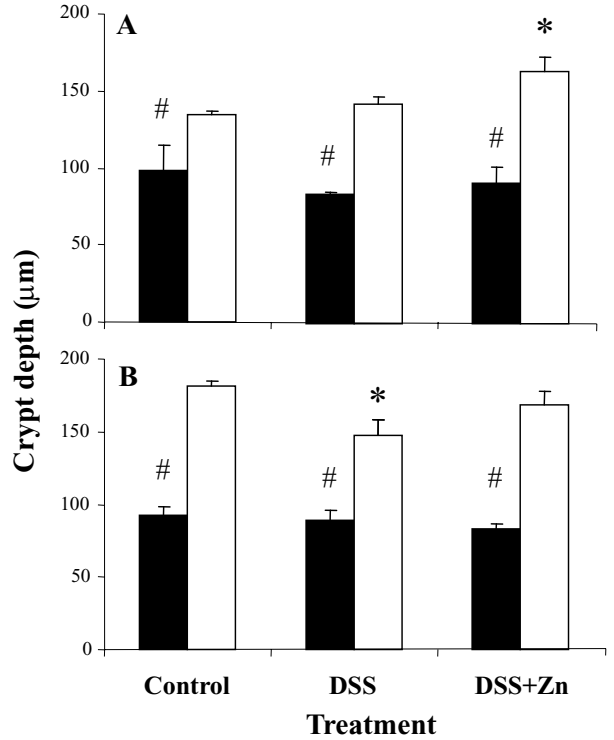

Fig. 3 Crypt depth (μm) in the proximal colon (A) and distal colon (B) of metallothionein wild-type (MT + / + ; ■) and null (MT − / − ; □) mice after consuming 2% dextran sulfate sodium (DSS) for 6 days with concurrent treatment. Data are expressed as mean ± SE. *Significantly ($P < 0.05$) different compared to control MT − / − mice.#Significantly ($P < 0.05$) different compared to MT − / − mice

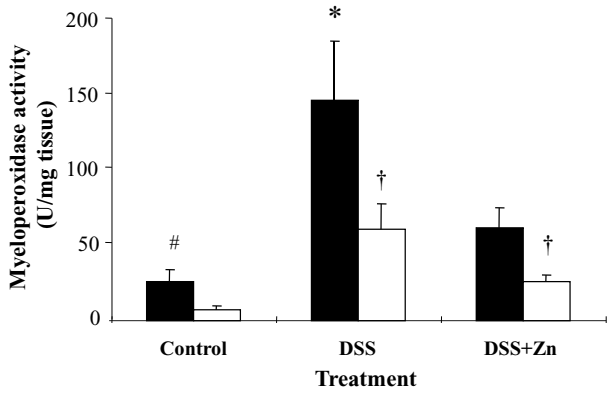

Fig. 4 Myeloperoxidase activity (U/mg tissue) in the colon of metallothionein wild-type (MT + / + ; ■) and null (MT − / − ; □) mice after consuming 2% dextran sulfate sodium (DSS) for 6 days with concurrent treatment. Data are expressed as mean ± SE. *Significantly ($P < 0.05$) different compared to control MT + / + mice. †Significantly ($P < 0.05$) different compared to control MT − / − mice. #Significantly ($P < 0.05$) different compared to control MT − / − mice

The results from the DSS dose-response study suggested that there was no significant protection from colitis offered by MT, since no difference was noted in DAI between MT + / + and MT − / − mice for 2%, 3%, or 4% DSS. This was consistent with a study by Oz et al. [26] in which there was no significant difference in pathology scores in MT transgenic, MT-null, or in wild-type mice administered the 4% dose of DSS only. Our results showed that, regardless of MT expression, all mice administered DSS had developed colitis from day 4 onward and, similarly, as reported by Oz et al. [26], when the onset of colitis occurred on day 6. However, it would appear that increasing the concentration up to 4% DSS was not a contributing factor to increasing pathology. To the best of our knowledge, the current study represents the first description of the severity scores in two regions of the colon after consumption of increasing concentration of DSS, with the distal colon being more affected compared to the proximal colon, especially at concentrations of 3% and 4% DSS. The crypts in the proximal colon of MT + / + mice receiving 3% and 4% DSS were shorter compared to the colons of rats consuming 2% DSS, representing a differential effect of DSS on the different regions of the colon.

In the present study, we demonstrated that Zn administration suppressed the development of DSS-induced colitis in mice as indicated by decreased clinical DAI and histological severity scores, respectively, in the distal colon. Clinically, as indicated by DAI, the absence of MT was beneficial in the suppression of colitis in MT − / − mice receiving DSS, suggesting that the presence of MT may have promoted the induction of colitis. Similarly, as indicated by the histological severity scores, MT + / + mice appeared to be more susceptible to DSS-induced colitis compared to MT − / − animals. However, Zn treatment suppressed DSS-induced

colitis, particularly in MT +/+ mice. This is consistent with other studies [10, 11] which have demonstrated that a high Zn dose administered rectally decreased the severity of experimentally induced colitis. The results of the present study and published studies [10–13] suggest that the inhibitory effect of Zn on the severity of colitis appeared to be via an anti-inflammatory effect of Zn, suggesting that Zn, a known mast cell stabilizer, may have had a therapeutic effect by inhibiting histamine release via the microtubule-stabilizing effect of Zn [10]. Furthermore, Zn has been reported to reduce prostaglandin E_2 and leukotriene B_4 levels in TNBS-induced colitis in rats [11]. By inhibiting leukotriene B_4 Zn may have down-regulated the recruitment of neutrophils and reduced the severity of inflammation. This is consistent with our findings that inflammatory indicators such as MPO activity were decreased by treatment with Zn.

It has been proposed that oral Zn administration induces MT in the colonic mucosa and MT then exerts its protective effects by sequestering and eliminating ROS produced during the disease process [11, 15]. In the present study, Zn administration to DSS-treated mice did not increase MT concentrations in the colonic mucosa, consistent with the study by Di Leo et al. [15] and in agreement with our previous studies where dietary Zn induction of MT in the colon was much lower that in the small intestine or the liver [16, 27]. There have been several studies determining MT expression in the colon of IBD patients. Kruidenier et al. [6] and Mulder et al. [28] reported that these patients had decreased levels of MT in the colonic mucosa. Sturniolo et al. [29] showed altered plasma and colonic concentrations of trace elements and reduced MT levels in UC. In contrast, Lih-Broody et al. [30] and Bruwer et al. [31] reported that MT was significantly increased in patients with active Crohn's disease. Taken together, these studies suggest that MT expression in the colon can be variable depending on the nature of the disease.

The inflammatory process of experimental colitis is characterized by an increase in mucosal permeability, increasing the recruitment and activation of polymorphonuclear cells [14, 32]. Sturniolo et al. [14] have shown that Zn administration reduced or prevented the loosening of tight junction complexes, however, the severity of colitis remained unaffected. The authors also proposed that Zn modulated the inflammatory cascade, which in turn regulates tight-junction physiology [14]. However, a direct effect of Zn on tight junctions remains to be demonstrated and merits further investigations.

In the present study, DSS resulted in shortening of the colon, consistent with previous studies [10, 11, 15, 22], however, Zn treatment did not reverse this effect. This shortening of the colon may have resulted from of crypt abnormalities and goblet cell loss [33]. This hypothesis was supported by the results of the current study, which showed significantly increased colonic disease severity when examined histologically. It is known that DSS is able to inhibit crypt cell proliferation, leading to a lowering of the number of crypt cells and promotion of apoptosis [33, 34]. Indeed, this disruption of apoptosis and proliferation may be causative in UC progression [34].

In conclusion, administration of Zn suppressed clinical features, histological pathology scores, and inflammatory indicators such as MPO activity in DSS-induced colitis. Further studies are warranted to determine the action by which Zn is able to protect against UC.

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