Effect of zinc supplementation on intestinal permeability in experimental colitis

GIACOMO CARLO STURNIOLO, WALTER FRIES, EMANUELA MAZZON, VINCENZA DI LEO, MICHELA BAROLLO, and RENATA D'INCA

PADUA and MESSINA, ITALY

Increased small-intestine permeability has been documented in experimental colitis in the rat. Zinc supplementation improves mucosal repair in patients with diarrhea, as well as paracellular permeability in malnourished guinea pigs. In this study, we sought to evaluate the effect of zinc supplementation on small-and large-intestine tight junctions in rats with acute colitis. Rats were given zinc at a dosage of 2 or 30 mg/kg body wt or glucose by gavage starting 3 days before colitis was induced through the intrarectal administration of dinitro-benzene-sulfonic acid and for 7 days thereafter. We evaluated small-intestine permeability by the number of tight junctions showing extravasation of lanthanum under electron microscopy. Low-dose zinc affected none of the examined parameters of colitis severity. Rats given high-dose zinc showed colitis of similar macroscopic and biochemical severity. However, zinc-treated rats weighed more than unsupplemented ones. The number of perfused tight-junction complexes was significantly higher in animals with colitis than in controls and in the rats with colitis given high-dose zinc. Zinc may regulate tight-junction permeability, with possible implications for healing processes in inflammatory bowel diseases. (J Lab Clin Med 2002;139:311-5)

Abbreviations: DNBS = 2,4,6-dinitrobenzenesulfonic acid

Intestinal permeability is increased in patients with inflammatory bowel disease, and this fact has been regarded as important in the evaluation of disease activity, response to therapy, and risk of relapse.[1-3] Furthermore, abnormal permeability may represent a primary defect in Crohn's disease; it has been found to be altered in some healthy first-degree relatives, leading to the hypothesis of increased absorption of antigens and macromolecules that are able to trigger and perpetuate inflammation.[4,5] Morphological studies have also shown that patients with Crohn's disease have abnormal intestinal tight junctions, with a reduced number of strands in normal-appearing ileal mucosa, supporting the theory of a primary permeability defect.[6]

Small-intestine permeability was recently shown to be altered in rats with experimentally induced colitis.[7] The structure of the tight junction-associated protein occludin showed a disrupted immunofluorescence signal and an irregular distribution pattern in small-intestine segments, and the degree of disruption correlated with macroscopic colonic damage.

Zinc supplementation in patients with chronic diarrhea promoted mucosal integrity and reduced the number of attacks, especially in malnourished patients.[8] A resolution of small-intestine mucosal damage with reduced permeability and increased coefficient of nitrogen absorption has also been demonstrated in children treated with zinc during acute shigellosis.[9] The positive effect of a high level of dietary zinc on intestinal

From the Department of Surgical and Gastroenterological Sciences, University of Padua; and the Departments of Internal Medicine and Biomorphology, University of Messina.

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Reprint requests: Professor Giacomo Carlo Sturniolo, Divisione di Gastroenterologia, Monoblocco Ospedaliero, Via Giustiniani, 2, 35127 Padova, Italia; e-mail: gc.turniolo@unipd.it.

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paracellular permeability is supported by evidence from a guinea pig model of malnutrition.[10] We recently found that zinc supplementation can resolve permeability alterations in patients with Crohn's disease in remission, probably contributing to a reduced risk of relapse in these patients.[11]

Oral zinc supplementation, on the other hand, leads to increased availability of antioxidant proteins, such as metallothionein, while having little effect on the course and severity of experimental colitis.[12]

In this study, we sought to determine whether small- and large-bowel permeability are affected by zinc supplementation in a model of acute experimental colitis in the rat. Segments of intestine were perfused with lanthanum nitrate, after which tight junctions showing extravasation of the marker were counted under electron microscopy.

MATERIALS

Animals. Male Sprague-Dawley rats weighing 250 to 275 g were purchased from Charles River (Calco, Italy). The animals were kept in plastic platform cages in a temperature-controlled room (22°C) adjusted for a 12-hour light-dark cycle, with free access to deionized water and standard chow containing 125 mg/kg zinc oxide. The experimental protocol was approved by the Veterinary and Health Committee of the University of Padua.

Materials. Lanthanum nitrate (molecular weight 433.029), glutaraldehyde, osmium tetroxide, and cacodylate buffer were obtained from Società Italiana Chimici (Rome, Italy). Agar 100 was purchased from Agar Scientific (Rome, Italy). DMBS, *O*-dianisidine dihydrochloride and common laboratory reagents were obtained from Sigma Chemical (Milan, Italy). Chloral hydrate was purchased from Carlo Erba (Milan, Italy).

Experimental protocol. Two groups of rats were randomized to receive oral zinc acetate at two different doses. A solution of zinc acetate (0.5 mL; 2 mg/kg or 30 mg/kg) was administered, dissolved in glucose 10%, twice a day by gavage starting 3 days before induction of colitis and for 7 days thereafter. Two control groups—one with and one without colitis—were given 0.5 mL glucose 10% by gavage starting 3 days before induction of colitis and for 7 days thereafter.

Colitis was induced by the intrarectal instillation of 25 mg DNBS dissolved in 50% ethanol (total vol 0.3 mL). The rats were anesthetized with ether, and a silicone catheter was introduced intrarectally up to 8 cm. Animals were kept in the Trendelenburg position for 15 minutes to avoid rapid evacuation of the enema. On day 8, one week after colitis induction, animals were weighed and anesthetized with intraperitoneal chloral hydrate (400 mg/kg), after which the abdomen was opened with a midline incision and exsanguination was performed.

Lanthanum perfusion was performed as described previously.[7] In brief, the abdominal aorta was cannulated and ligated proximal to the diaphragm, the portal vein was transected to avoid damage resulting from pressure overload, and the small intestine was first flushed with warm saline solution and then fixed by means of perfusion with a solution containing 2.5% glutaraldhyde and 4% lanthanum nitrate in 0.1 mol/L cacodylate buffer at the rate of 2 mL/hr.

The colon was removed, opened along the antimesenteric border, rinsed, weighed, and processed for histologic study. We assessed damage by scoring alterations observed macroscopically.[13]

The colonic mucosa was scraped 4 cm proximal to the anus, and neutrophil infiltration was assessed by means of myeloperoxidase assay in accordance with a method previously described.[14] In brief, myeloperoxidase activity was measured with the dianisidine-H_2O_2 assay: We put 0.1 mL of the sample in a 3-mL cuvette, after which 2.9 mL of *O*-dianisidine solution (16.7 mg *O*-dianisidine dihydrochloride dissolved in 90 mL of distilled water plus 10 mL potassium phosphate buffer and 50 μL of 1% H_2O_2) was added. Results are expressed as myeloperoxidase units per milligram of tissue weight.

Two-millimeter specimens of the duodenum (3 cm proximal to Treitz's ligament), terminal ileum, and intact ascending colon were removed and left in 2.5% glutaraldehyde for 2 hours. The specimens were then rinsed for 30 minutes in 0.1 mol/L cacodylate buffer and postfixed in osmium tetroxide (1%). Specimens were then dehydrated with graded ethanols and embedded in Agar 100. Sections (10 nm) were cut with an ultramicrotome (Ultratome, Bromme, Germany) and examined under a Hitachi H-600 electron microscope (Hitachi, Tokyo, Japan) at 50 kV and a magnification of $\times$35,000. We observed 150 to 250 consecutive tight junctions from each animal, avoiding those next to goblet cells, which are known to have less resistance. A "leaky" junction was regarded as one showing penetration of the electron-dense marker into the junction complex with extravasation identified on the luminal surface. Results are expressed as the percentage of leaky junctions.

Statistics. Results are expressed as mean $\pm$ standard error. Data were analyzed with one-way analysis of variance followed by a pairwise comparison with post hoc Bonferroni analysis. We considered P values less than .05 significant.

RESULTS

Colitis assessment. The clinical and biochemical results of colitis induction are summarized in Table I.

At the time of death, the animals treated with DNBS weighed significantly less than controls, whereas animals given zinc at the dosage of 30 mg/kg were similar in weight to controls. Colon wet weight was significantly increased in colitic animals compared with controls, whether or not they received zinc supplementation.

Myeloperoxidase activity in the colonic mucosa was significantly higher in all colitic rats compared with control animals. No difference in myeloperoxidase activity was observed between zinc-treated and untreated rats.

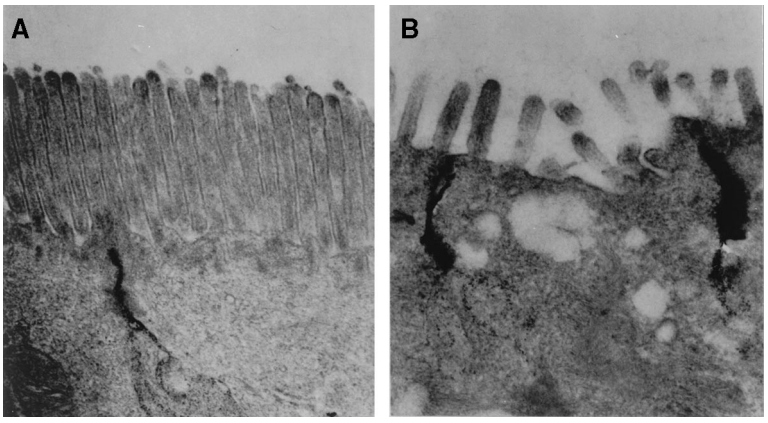

Fig 1. Electron microscopy of ileal tight junctions perfused with lanthanum nitrate. **A**, The marker approaches the junctional complex without reaching the epithelium in control animals (original magnification ×35,000). **B**, Penetration of the marker into the junctional complex with extravasation on the luminal surface in animals 7 days after induction of colitis induction with DNBS (original magnification ×53,500).

Table I. Clinical and biochemical parameters assessed in zinc-supplemented and unsupplemented rats 7 days after induction of colitis with DNBS

Parameters	Controls (n = 18)	Colitis (n = 9)	Colitis + 2 mg zinc (n = 9)	Colitis + 30 mg zinc (n = 6)	*P* value
Final body weight (g)	260 ± 10	189 ± 8*	210 ± 10	270 ± 11†‡	.001
Colon wet weight (g)	2.3 ± 0.1	4.9 ± 1.4*	5.1 ± 1.8*	3.5 ± 1.1	.174
Macroscopic-damage score	0 ± 0	7.2 ± 1.9*	3.2 ± 0.5*†	4.6 ± 0.9*	.001
Myeloperoxidase (U/mg)	0.5 ± 0.2	12.0 ± 3.0*	13.8 ± 4.8*	14.5 ± 5.0*	.001

*$P < .05$ vs controls.
†$P < .05$ vs colitis.
‡$P < .05$ vs colitis + 2 mg/kg zinc.

Table II. Percentages of opened tight junctions in the duodenum, terminal ileum, and colon in controls and in zinc-supplemented and unsupplemented rats with DNBS-induced colitis

Location	Controls (n = 5)	Colitis (n = 8)	Colitis + 2 mg zinc (n = 5)	Colitis + 30 mg zinc (n = 5)	*P* value
Duodenum	8 ± 3	58 ± 3*	35 ± 5*†	27 ± 4*†	.001
Terminal ileum	10 ± 4	60 ± 8*	39 ± 2*†	33 ± 4*†	.001
Ascending colon	10 ± 3	78 ± 4*	63 ± 9*	23 ± 4*†‡	.001

*$P < .05$ vs controls (corresponding gut segment).
†$P < .05$ vs colitis.
‡$P < .05$ vs colitis + 2 mg/kg zinc.

The macroscopic-damage score was significantly higher in colitic animals than in controls, and we observed reduced damage in the group of rats given low-dose zinc supplementation.

Tight-junction evaluation. Fig 1 and Table II summarize the results of lanthanum-perfusion studies. The percentage of open tight junctions in the intestines of control animals varied from 8 to 10 along the small and large intestine, whereas analysis of variance revealed a significantly higher number of lanthanum-perfused junctional complexes in colitic animals in each of the segments studied (Table II). Zinc supplementation reduced the number of opened junctions, which were significantly diminished in the group given high-dose zinc. Fig 1 shows normal ileal architecture with sealed tight-junction complexes (*A*) and the disrupted microvilli with lanthanum extravasation into the luminal surfaces of the colitic rats. No ultrastructural alterations

of cytoplasmic organelles were demonstrated in any group.

DISCUSSION

Experimental distal colitis is associated with increased tight-junction permeability in the small and large intestines, probably representing changes in response to the systemic effects of inflammation. In this study, zinc supplementation reduced or prevented the opening of junctional complexes, but the severity of colitis remained unaffected. The role of zinc in modifying intestinal permeability is still unknown.

Zinc, an essential trace element, is strongly implicated in cell turnover and repair systems, and the risk of zinc deficiency increases in the presence of inflammatory conditions. This holds true not only for chronic inflammatory states of the gastrointestinal tract such as inflammatory bowel diseases—in which increased intestinal losses, anorexia, and reduced absorption can explain deficiency—but for acute conditions such as infectious diarrhea.[8-9,15]

Al-Awadi et al[16] recently found that tissue levels of trace elements, including zinc, were reduced in rats with acetic acid– or DNBS-induced colitis. We recently examined trace elements and metallothionein concentrations in the colons of rats with DNBS-induced colitis and found reduced concentrations of metallothionein, a zinc-dependent enzymem, which may support the hypothesis of acute deficiency.[12]

The hypothesis of nutritional deficiency is not entirely supported by our findings; zinc supplementation at the dose of 2 mg/kg was ineffective despite being approximately 10 times the daily requirement for human beings. In this study, we did not measure plasma zinc levels because they are not a reliable indicator of zinc status, and at present the evaluation of zinc deficiency remains rather imprecise. The findings of earlier studies in children with acute shigellosis have suggested a nutritional role for zinc supplementation, which resulted in increased urinary mannitol excretion, considered a marker of epithelial-damage resolution.[9] Studies in guinea pigs showed that experimental malnutrition is associated with functional changes in the intestine such as hypersecretion, increased permeability, and a reduced number of tight-junction strands and that these alterations were reversed with pharmacologic doses of zinc.[10] In this study, rats given high-dose zinc supplementation did not lose weight as their colitic controls did; this can be regarded as the only element in favor of a protective nutritional effect.

Epithelial-barrier dysfunction in inflammatory bowel disease has important implications because it may allow the passage of noxious agents, bacterial particles, and antigens. This may represent the primary event responsible for disease onset, chronicization, and/or relapse.

Several in vitro studies have shown that zinc deficiency disrupts the barrier function of endothelial cells,[17] and the metal is known to protect essential sulfhydryl groups from oxidation in plasma membranes of rat erythrocytes[18] and against toxicity from *Staphylococcus aureus*, α toxin, and heat-inactivated Sendai virus.[19] On the other hand, tumor necrosis factor–α rapidly produces redistribution of zinc to the liver with low serum levels, probably mediated by the induction of metallothionein,[20] and graded increases in media zinc concentration can prevent the tumor necrosis factor–induced increase in albumin transfer, a measure of endothelial permeability.[21]

In addition to zinc's being essential to cell-membrane integrity, another possible mechanism of zinc protection involves its ability to retard oxidative processes by inducing the synthesis of metallothioneins.[22]

Tight junctions form the most important paracellular barrier in epithelia. The molecular identification of the structural components is still in process, but the available information allows us to conclude that their structure and function vary among cell types and species, possibly accounting for their different behaviors in terms of electrical resistance, ion and solute permeability, and charge selectivity. Cytokines, hormones, and a host of mediators may contribute to the regulation of opening and tightening of these structures, which have proved much more dynamic than previously thought.

Our recent data support the role of zinc supplementation in improving paracellular permeability in patients with Crohn's disease who are at high risk of relapse.[11] However, a direct effect of zinc on tight junctions remains to be demonstrated and merits further study. The most likely hypothesis is that zinc modulates the inflammatory cascade, which in turn regulates tight-junction physiology.

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