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Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs

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Abstract

Dietary supplementation with a high level of zinc oxide (ZnO) has been shown to reduce the incidence of diarrhea in weanling pigs, but the underlying mechanisms remain largely unknown. Intestinal–mucosal mast cells, whose maturation and proliferation is under the control of the stem cell factor (SCF), play an important role in the etiology of diarrhea by releasing histamine. The present study was conducted to test the novel hypothesis that supplementing ZnO to the diet for weanling piglets may inhibit SCF expression in the small intestine, thereby reducing the number of mast cells, histamine release, and diarrhea. In Experiment 1, 32 piglets (28 days of age) were weaned and fed diets containing 100 or 3000 mg zinc/kg (as ZnO) for 10 days (16 piglets per group). In Experiment 2, two groups of 28-day-old piglets (8 piglets per group) were fed the 100- or 3000-mg zinc/kg diet as in Experiment 1, except that they were pair-fed the same amounts of feed. Supplementation with a high level of ZnO reduced the incidence of diarrhea in weanling piglets. Dietary Zn supplementation reduced expression of the SCF gene at both mRNA and protein levels, the number of mast cells in the mucosa and submucosa of the small intestine and histamine release from mucosal mast cells. Collectively, our results indicate that dietary supplementation with ZnO inhibits SCF expression in the small intestine, leading to reductions in the number of mast cells and histamine release. These findings may have important implications for the prevention of weaning-associated diarrhea in piglets.
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Keywords: Zinc oxide; Mast cells; Histamine; Stem cell factor; Diarrhea in piglets

1. Introduction

Diarrhea is an important problem in young piglets and in children [1]. Recent studies have shown that dietary supplementation with a high level of zinc oxide (ZnO) (i.e., 1500 to 2000 ppm) is highly effective in preventing or alleviating diarrhea in infants and weanling piglets [2,3]. Although there is evidence suggesting that ZnO exerts antimicrobial and immunoregulatory actions in the small intestine [2–5], the underlying mechanisms remain largely unknown. Because piglets are widely used as an animal model to study human intestinal physiology and pathophysiology [6,7], understanding how supplementation with ZnO alleviates diarrhea in weanling piglets could have important implications for both animal nutrition and human medicine.

Mast cells contribute to the pathogenesis of diarrhea through the production and release of histamine, prostaglandins, leukotrienes, 5-hydroxytryptamine, and tumor necrosis factor-α [8]. These molecules play an important role in mediating the immunoglobulin E (IgE)-dependent hypersensitivity reactions and the increased vascular permeability of the gastrointestinal mucosa [9]. Indeed, an increase in the number of mucosal mast cells has been observed in the crypt and villus lamina propria of adult and pediatric cholera patients compared with healthy subjects [10]. Furthermore, available evidence suggests a critical role for mast-cell-derived histamine in the pathogenesis of diarrhea [8–10].

Precursor mast cells originate from the bone marrow [11] and mature into mast cells when they migrate to local tissues where they acquire appropriate stimulation from multiple factors, including stem cell factor (SCF) and interleukin-3 [12]. The SCF also stimulates mast cells to proliferate in the gut [12]. Interestingly, the expression of SCF is induced

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin E; RT-PCR, real-time polymerase chain reaction; SCF, stem cell factor; ZnO, zinc oxide.

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during the acute and convalescence phases of diarrhea in patients [10], suggesting a role for SCF in intestinal dysfunction. At present, evidence linking SCF expression with histamine release by the small intestine is lacking.

We hypothesized that feeding high levels of ZnO to weanling piglets may decrease SCF expression and the number of mast cells in the small intestine, thereby attenuating histamine release and preventing diarrhea. This hypothesis was tested using the weanling pig that naturally exhibits intestinal dysfunction and diarrhea [6].

2. Materials and methods

2.1. Experimental animals and diets

The experimental protocol used for the following experiment was approved by the China Agricultural University Animal Care and Use Committee.

2.2. Experiment 1

A total of 32 crossbred (Large White×Landrace×Pietran) barrows, weaned at 28 days of age (8.1±0.6 kg body weight), were assigned to one of two dietary groups (16 piglets per group) in a randomized complete-block design on the basis of body weight and litter of origin. Piglets were fed a corn- and soybean-meal-based diet containing 100 or 3000 ppm zinc (in the form of ZnO). The diets met or exceeded the requirements recommended by the National Research Council for all other nutrients by young pigs [13]. Weanling piglets were housed individually in 1.25×1.2-m pens with totally slotted stainless steel floors and had free access to feed and drinking water throughout the experiment. The temperature of the nursery room was set at 26–28°C and its light cycles were 16 h light and 8 h dark. The number of piglets scouring was recorded daily.

2.3. Experiment 2

Sixteen 28-day-old crossbred barrows (8.0±0.7 kg) were assigned randomly to one of the two dietary groups as in Experiment 1, with 8 pigs per group. The Zn-supplemented piglets were individually pair-fed to the control group, as described by Swamy et al. [14]. Pigs in the control group had free access to the diet, while Zn-supplemented pigs were provided with the same amount of feed consumed by the control group of pigs during the previous day. The pigs were fed twice daily with equal-sized meals and the number of piglets scouring was recorded daily.

2.4. Tissue collection and processing

In Experiments 1 and 2, after a 10-d period of dietary Zn supplementation, six piglets were chosen randomly from each treatment group for slaughter. Samples of the duodenum (5 cm from the pylorus), the proximal (150 cm from the pylorus), middle (middle of the whole jejunum) and distal jejunum (150 cm anterior to the ileocecal valve) and the ileum were acquired. The intestinal segments were thoroughly rinsed with saline to remove the intestinal contents and were then cut into smaller pieces (2 cm in length). Some of the samples were fixed in Carnoy's solution (60% absolute alcohol, 30% chloroform and 10% glacial acetic acid), whereas other samples were immediately frozen in liquid nitrogen for biochemical and molecular analyses.

2.5. Histochemical staining

The toluidine blue staining method was used to quantify mast cells, as described by Enerback [15]. Fixed tissues were dehydrated and embedded according to the standard general histochemical protocols. Sections were cut into 6-μm-thick sections, dewaxed and hydrated. The sections were then stained for 30 min with 0.5% (wt/vol) of the toluidine blue solution (Gurr, Poole, UK) in 0.5 mol/L HCl, washed for 30 s with 0.5 mol/L HCl, and counterstained for 30 s with 0.25% (wt/vol) of the Safranin O solution (Gurr, Poole, UK) in 0.125 mol/L HCl. Finally, all the sections were washed, dehydrated and mounted. Microcheck Grid (Wuhan Optical, China) was used to count the number of mast cells as previously described [16]. Mast cells in the small intestine may exhibit heterogeneity and their numbers may vary within the segment of the intestine. For this reason, we felt that it would be important to determine the numbers of mast cells in many sections of the gut.

2.6. Determination of histamine in the small intestine

The histamine content in tissue samples from the small intestine was measured using HPLC [17]. Briefly, a 1.5-g sample (cell pellet or supernatant) was immersed in 5 ml (or 1 ml) of 0.6 mol/L $HClO_4$ and immediately homogenized in ice at 15,000 rpm. The homogenates were centrifuged for 10 min at 800×g and at 10°C. The supernatant fluid was filtered through a 0.45-μm filter, and the solution obtained was analyzed for histamine by using postcolumn derivatization with *o*-phthaldialdehyde (Sigma, St. Louis, MO, USA) and fluorometric detection (Waters 2475 MultiλFluorescence Detector; Waters, Milford, MA, USA) at 340 nm excitation and 450 nm emission. The amounts of histamine in samples were quantified using known amounts of the standard (Sigma).

2.7. Determination of SCF expression in the small intestine

Concentrations of the SCF protein in the small intestine were determined using enzyme-linked immunosorbent assay (ELISA) as described by Gaca et al. [18]. Briefly, approximately 0.5 g of the middle jejunum was homogenized in phosphate-buffered saline. The homogenates were centrifuged for 10 min at 10,000 rpm and 10°C, and the supernatant fluids were obtained immediately for assays. Stem cell factor protein was quantified using a commercial ELISA kit specific for the porcine species (Fangbang., Dalian, China).

For determination of SCF mRNA levels in the same section of the small intestine, real-time polymerase chain reaction (RT-PCR) was carried out using the DNA Engine Opticon 2 Fluorescence Detection System (MJ Research). The total RNA was extracted using the RNAprep Tissue Extraction Kit DP401 (Tiangen, Beijing, China). The

Table 1
Feed intakes of piglets in Experiments 1 and 2

Item	Control	Treatment	S.E.M.	*P*
Experiment 1				
Feed intake (g/day)	466	588	27	.033
Experiment 2				
Feed intake (g/day)	489	496	24	.730

The treatment diet contained 3000 mg Zn per kilogram. Data are the means with pooled S.E.M. values, n=16 per treatment group for Experiment 1 and n=8 per treatment group for Experiment 2.

primers for the SCF and β-actin were designed according to GenBank (SCF, NM-214269; β-actin, AY550069). Three negative controls were performed to check the specificity of these primers for the porcine SCF and β-actin mRNAs. One microgram of total RNA was reverse-transcribed and the resultant cDNA was employed in the PCR using primers within the coding region of SCF mRNA. The PCR reaction system (10 μL) contained 5 μL DyNAmo SYBR Green qPCR mix, 0.48 μL primer (0.24 μmol/L forward and 0.24 μmol/L reverse), 3.2 μL ddH_2O and 1 μL cDNA template (<10 μg/l). The PCR was performed for 35 cycles at the following sequential temperatures: 94°C for 30 s; 57°C for 30 s; 72°C for 1 min; and 72°C for 5 min. Primers for β-actin, a housekeeping gene, ran for 35 cycles according to the following sequential temperatures: 94°C for 30 s; 64°C for 30 s; 72°C for 1 min; and 72°C for 5 min. To amplify SCF and β-actin cDNA fragments, the following sequences of PCR primer pairs were used: forward, 5′-GGATTTGGAGATGGTGGCAC-3′, and reverse 5′-AGAGAAGAATGCTGGCAATGC-3′ for SCF (223 bp); forward 5′-TGC GGG ACA TCA AGG AGA AG-3′, reverse 5′-AGT TGA AGG TGG TCT CGT GG-3′ for β-actin (216 bp). All samples were measured in triplicate.

2.8. Effect of ZnO on histamine release from intestinal–mucosal mast cells

Intestinal–mucosal mast cells were prepared from the jejunum of 30-day-old pigs, as previously described [19]. Briefly, 5 cm of the middle jejunum was removed and flushed with saline solution and then immersed in cold Tyrode's buffer B (NaCl, glucose, $NaHCO_3$, KCl, NaH_2PO_4) containing penicillium (100 UI/ml) and streptomycin (100 mg/ml). The mucus was scratched and sheared off. The tissue was incubated in 100 ml of Tyrode's buffer B containing 25 U/ml collagenase (Sigma) and 25 U/ml hyaluronidase (Sigma) for 120 min at 37°C with constant stirring.

Mast cells were separated from other cell types by using Percoll Discontinuous Gradient Centrifugation (Sigma). The mast cells were collected at the 60%/100% Percoll interface and passed through a complement-coated nylon wood column to remove contaminating eosinophils. Mast cell preparations were 90% pure, as assessed by toluidine blue staining. More than 95% of the cells were viable, as judged by trypan blue exclusion.

Purified mast cells were resuspended in Tyrode's buffer A (containing calcium) and divided into four groups supplemented with ZnO. Mast cell suspensions (1×10^6 cell/ml) were preincubated at 37°C for 2 h in Tyrode's buffer containing 0, 25, 50 or 100 μmol/L ZnO in a 5% CO_2 incubator and then incubated for 30 min in fresh medium containing 15 μg/ml concanavalin A (Sigma). At the end of the 30-min final incubation period, the reaction was stopped by cooling the plate on ice, followed by centrifugation at $400\times g$ and 4°C for 5 min to acquire the cell pellet and the supernatant fluid for histamine analysis, as previously described [17]. The measured values for the cell pellet plus the incubation medium were taken to indicate total histamine content in mast cells before the 30-min period of final incubation. Histamine release was expressed as the percentage of the histamine content in the incubation medium relative to the total cellular histamine content.

2.9. Statistical analysis

Results are expressed as mean±S.D. Data on gene expression as well as histamine concentrations and mast cell numbers were analyzed statistically by unpaired *t* test, using the SPSS statistical software package for Windows (version 13.0, SPSS, Chicago, IL, USA). Data on the incidence of diarrhea were analyzed statistically by using the SPSS Crosstabs of Descriptives method. Data on histamine release were analyzed statistically using one-way ANOVA. Probability values ≤.05 were taken to indicate statistical significance.

3. Results

3.1. Feed intakes of piglets

In Experiment 1 where all piglets had free access to diets, the high-zinc group of pigs consumed more feed than the control group (Table 1). To eliminate a potentially con-

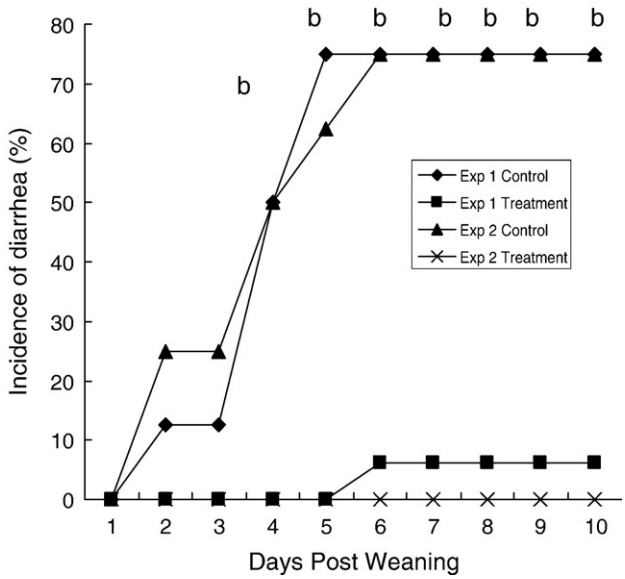

Fig. 1. The incidence of diarrhea in postweaning piglets fed diets containing normal and high levels of ZnO. The superscript b indicates a significant difference from the control group in each experiment ($P<.01$).

Table 2
Protein and mRNA levels for the SCF gene in the mid-jejunum of weanling piglets fed diets containing normal or high levels of ZnO

Item	SCF protein (μmol/g)		SCF mRNA	
	Control	Treatment	Control	Treatment
Experiment 1	16.20±1.40	11.14±0.88[a]	0.58±0.01	0.30±0.01[a]
Experiment 2	21.43±5.6	13.94±1.78[a]	0.64±0.24	0.21±0.06[a]

The treatment diet contained 3000 mg Zn per kilogram. SCF mRNA values are expressed as the ratios of SCF mRNA levels to β-actin mRNA levels. Data are means±S.D. n=6.

[a] $P<.05$ vs. the control group (normal Zn supplementation).

founding effect of a higher food intake by the Zn-supplemented pigs, all pigs were provided with the same amounts of feed in Experiment 2. Thus, feed intake did not differ ($P>.05$) between the control and Zn-supplemented piglets in Experiment 2 (Table 1).

3.2. Clinical and pathological symptoms

In Experiment 1, pigs fed the 100-ppm zinc diet started to scour on Day 2 postweaning (2/16), and most of the piglets (12/16) scoured between Days 6 and 10 postweaning (Fig. 1). Only one of the 16 pigs fed the 3000-ppm zinc diet scoured during the postweaning period. In Experiment 2, pigs fed the 100-ppm zinc diet started to scour on Day 2 postweaning (1/8), and most of the piglets (7/8) scoured between Days 5 and 10 postweaning. Dietary supplementation with a high level of Zn completely prevented the onset of diarrhea in weanling piglets (Fig. 1). In piglets fed the 100-ppm zinc diet, congestion and hemorrhages occurred in the small intestine and large intestine, and the chymes in the intestinal lumen appeared waterlike. However, in piglets fed the high-ZnO diet, congestion and hemorrhage were observed only sporadically, and the intestinal chymes were well shaped.

3.3. Stem cell factor protein and mRNA levels in jejunum

In Experiment 1, using the ELISA technique, we analyzed concentrations of the SCF protein in the mid-jejunum of piglets. Dietary Zn supplementation reduced ($P<.05$) intestinal SCF protein levels by 31% (Table 2). Similarly, SCF mRNA levels were 49% lower ($P<.05$) in piglets fed the high-ZnO diet in comparison with piglets fed the normal ZnO diet (Table 2). Similar results were obtained in Experiment 2 when feed intake was similar between the pair-fed control and Zn-supplemented piglets (Table 2).

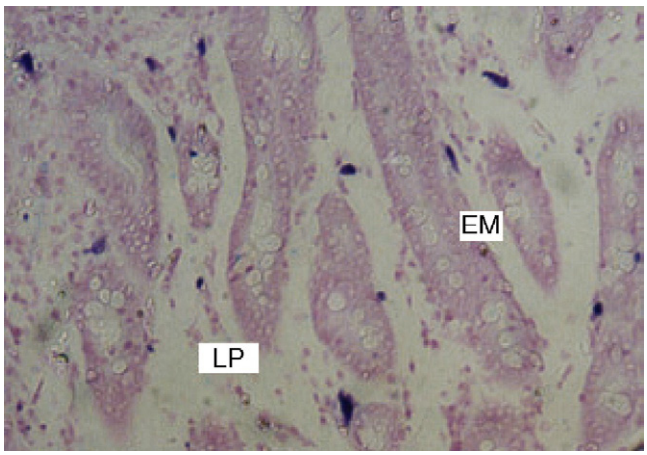

Fig. 2. Histological identification of mast cells in the piglet jejunum. EM, epithelial mucosa; LP, lamina propria. Mast cells are indicated in purple.

Table 3
The numbers of mast cells (cells/cm^2) in the small intestine of weanling piglets fed diets containing normal or high levels of ZnO in Experiment 1

Location	Control	Treatment	P value
Duodenum mucosa	64.9±10.2	23.4±4.8	<.01
Duodenum submucosa	100.8±9.7	38.9±4.8	<.01
Duodenum chorion	34.5±10.9	19.6±3.2	.22
Proximal jejunum mucosa	85.3±7.1	42.2±4.5	<.01
Proximal jejunum submucosa	119.0±7.1	70.4±4.3	<.01
Proximal jejunum chorion	28.6±2.9	14.1±2.0	<.01
Middle jejunum mucosa	88.5±5.9	40.5±4.9	<.01
Middle jejunum submucosa	143.3±7.4	79.9±10.4	<.01
Middle jejunum chorion	23.5±3.2	13.6±1.5	<.02
Distal jejunum mucosa	107.4±3.6	48.2±6.7	<.01
Distal jejunum submucosa	138.1±6.6	83.6±6.4	<.01
Distal jejunum chorion	16.6±3.1	19.6±4.9	.61
Ileum mucosa	79.2±5.7	51.2±3.7	<.01
Ileum submucosa	124.6±7.2	86.5±4.8	<.01
Ileum mucosa chorion	29.1±7.9	14.3±3.6	.12

The treatment diet contained 3000 mg Zn per kilogram. Data are means±S.D. n=6.

3.4. The number of mast cells

Histological identification of mast cells in the piglet jejunum is indicated in Fig. 2. Mast cells were estimated to account for approximately 2% to 4% of the total mucosal cells in the jejunum. In Experiment 1, mast cells were abundant in the mucosa, submucosa, and chorion of the small intestine (Table 3). The numbers of mast cells in the mucosa and submucosa of the duodenum, jejunum, and ileum were lower ($P<.01$) in the high ZnO group, compared with the normal ZnO group (Table 3). Similar findings were obtained for the

Table 4
The numbers of mast cells (cells/cm^2) in the small intestine of weanling piglets fed diets containing normal or high levels of ZnO in Experiment 2

Location	Control	Treatment	P value
Duodenum mucosa	74.9±21.9	36.7±8.9	<.01
Duodenum submucosa	127.9±19.1	68.8±13.2	<.01
Duodenum chorion	50.5±15.9	34.0±11.5	<.05
Proximal jejunum mucosa	91.7±13.1	59.7±19.8	<.01
Proximal jejunum submucosa	146.7±15.3	88.4±26.5	<.01
Proximal jejunum chorion	44.3±13.3	20.9±9.3	<.01
Middle jejunum mucosa	99.7.5±13.1	48.8±13.2	<.01
Middle jejunum submucosa	168.1±45.1	101.4±19.2	<.01
Middle jejunum chorion	34.7±9.3	20.9±2.4	<.05
Distal jejunum mucosa	86.1±8.5	39.8±14.1	<.01
Distal jejunum submucosa	137.3±19.5	91.6±12.6	<.01
Distal jejunum chorion	30.6±8.9	22.8±5.1	.11
Ileum mucosa	81.4±19.5	55.1±3.4	<.01
Ileum submucosa	121.3±17.3	84.5±8.1	<.01
Ileum mucosa chorion	34.1±14.0	22.5±4.3	.08

The treatment diet contained 3000 mg Zn per kilogram. Data are the means±S.D. n=6.

Table 5
Concentrations of histamine (ng/g) in the small intestine of weanling piglets fed diets containing normal or high levels of ZnO

Item	Duodenum	Proximal jejunum	Middle jejunum	Distal jejunum	Ileum
Experiment 1					
Control	0.87±0.11	0.92±0.11	0.75±0.15	0.70±0.14	1.00±0.15
Treatment	1.54±0.11[b]	1.48±0.20[b]	1.5±0.32[b]	1.12±0.10[a]	1.31±0.20
Experiment 2					
Control	0.84±0.05	0.87±0.11	0.89±0.04	0.85±0.13	0.87±0.22
Treatment	1.12±0.20[a]	1.17±0.18[a]	1.17±0.16[a]	1.05±0.12[a]	0.88±0.12

The treatment diet contained 3000 mg Zn per kilogram. Total histamine (extracellular and intracellular) in the segments of the small intestine was measured. Data are the means±S.D. n = 6.
[a] $P<.05$ vs. the control group.
[b] $P<.01$ vs. the control group.

numbers of mast cells in the chorion of the proximal and middle jejunum (Table 3). In Experiment 2 where feed intake did not differ between the control and Zn-supplemented piglets, dietary supplementation with Zn reduced ($P<.05$) the number of mast cells in the small intestine (Table 4).

3.5. Histamine concentrations in the small intestine

In both Experiments 1 and 2, concentrations of histamine in the duodenum and jejunum of piglets fed the high-ZnO diet were higher ($P<.05$) in comparison with the normal ZnO group (Table 5). There were no differences ($P>.05$) in ileal concentrations of histamine between the two groups of piglets (Table 5).

3.6. Histamine release from mast cells in vitro

Mast cells of the intestinal mucosa released a large amount of histamine during a 30-min period of incubation in the presence of concanavalin A (Fig. 3). Addition of 25 and 50 μmol/L ZnO to the incubation medium decreased ($P<.05$) histamine release from the cells by 21% and 22%, respectively, compared with the control (0 μmol/L ZnO) (Fig. 3).

4. Discussion

A zinc deficiency is known to result in diarrhea in humans and other animals [20], and high doses of zinc have been shown to prevent this intestinal abnormality in guinea pigs and rats [5,21]. Zinc supplementation also reduces the incidence, severity and duration of diarrhea in children [22–24]. Results of this study demonstrate that dietary supplementation with a high level of ZnO (3000 ppm) reduced the onset of diarrhea in weanling piglets. Our findings are similar to those reported for piglets by other investigators [3] and, therefore, establish a good animal model to determine a role for mast cells in the etiology of weaning-associated intestinal dysfunction.

Stress conditions (e.g., weaning) result in intestinal dysfunction by altering epithelial and immune responses in the gut [25]. Mast cells play an important role in this process [26]. These cells are activated by both IgE-dependent and independent mechanisms [27]. Mediators (i.e., histamine, prostaglandin, leukotriene, 5-hydroxytryptamine and tumor necrosis factor-α), released by the activated mast cells, trigger chloride and water secretion [28], leading to allergy and diarrhea in patients with cholera [10,29]. In the small intestine, mast cells are the major source of histamine, which has important pathological and physiological functions [27]. Particularly, histamine exerts secretory, contractile and immune effects on the intestine, and stimulates a short circuit current across the intestinal mucosa [30,31]. This amine also induces a net secretion of fluid from the intestinal epithelium, which may involve both H1 and H2 receptors. The H1 receptors directly produce cyclical chloride, which is secreted across the epithelium, whereas the H2 receptors act on the submucosal neurons [30–32]. Interestingly, zinc has previously been shown to block histamine release from cells in vitro [33,34]. Consistent with this report, we found that increasing extracellular concentration of ZnO inhibited histamine release from mast cells (Fig. 3), thereby increasing histamine concentration in the small intestine of weanling piglets (Table 3) and preventing diarrhea in these animals (Fig. 1).

A novel and important finding from the present study is that the number of mast cells in the small-intestinal muscosa and submucosa of the piglets fed the high ZnO diet were remarkably lower compared with the piglets fed the normal zinc diet (Table 3). This is expected to reduce extracellular histamine concentrations in the small intestine of weanling piglets, thereby minimizing an interaction of this amine with the H1 and H2 receptors and attenuating its potent effect on

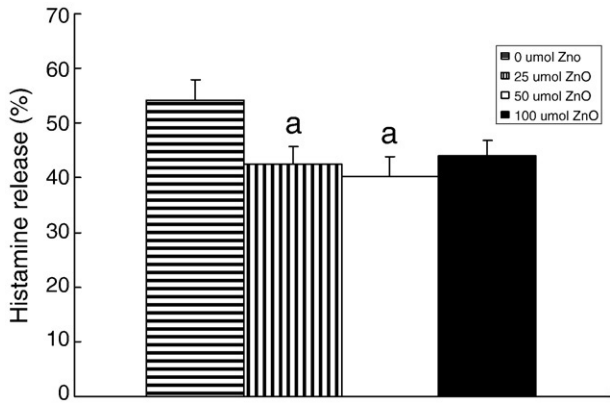

Fig. 3. Effect of different levels of ZnO on concanavalin A-induced histamine release from piglet intestinal–mucosal mast cells. [a]$P<.05$ vs. the 0 μmol ZnO treatment.

inducing the secretion of water and chloride by the intestinal epithelium. The close association between the prevention of diarrhea and the mast cell number suggests an important role for zinc in intestinal cell permeability. In support of this notion are the previous observations that net water and sodium transport across the small and large intestines were decreased in zinc-deficient rats when compared with Zn-sufficient rats [35] and that increasing extracellular concentrations of Zn reduced chloride secretion by preventing the rise in a short-circuit-current response to mast cell mediators (histamine and 5-hydroxytryptamine) in anaphylaxis [36]. Additionally, results of a recent study suggest that Zn supplementation reduces the incidence of diarrhea in rats through inhibition of cAMP-induced Cl secretion by the intestinal mucosa via the blocking of basolateral membrane K channels [37].

Stem cell factor is necessary for the development of mast cells from circulating and tissue-specific mast cell precursors, and SCF has been shown to promote mast cell growth in the bone marrow and peripheral blood [18]. Mature mast cells require an interaction with SCF and c-kit receptor to function [12]. In addition to its role in mast cell development, SCF is a chemotactic molecule for mast cells and induces their degranulation [38]. Furthermore, SCF stimulates human intestinal mast cells to release mediators as well as IgE and interleukin-3 [38,39]. Of note, mast cells express abundantly the receptor for SCF, which binds SCF upon its release from the cells. Through this autocrine stimulation of mast cells, SCF enhances their secretory function (i.e., histamine release) [40]. Thus, SCF treatment increases histamine synthesis and release from mast cells in vitro [41]. In keeping with this finding, our results demonstrate for the first time that dietary Zn supplementation decreased SCF expression in the small intestine of weanling piglets on the basis of reductions in both protein and mRNA levels. A reduced availability of SCF protein in the small intestine would restrain mast cells from proliferation and activation, thereby reducing the release of histamine.

Consistent with the above notion, intestinal concentrations of the SCF protein increase during acute and convalescence phases of gut dysfunction in cholera patients with diarrhea, while the numbers of mucosal mast cells also increase in the crypt and villus lamina propria of adult and pediatric cholera patients [10]. Furthermore, SCF induces the activation of mast cells and the overproduction of the c-kit ligand that could otherwise lead to mast cell proliferation in interstitial cystitis [42]. Collectively, down-regulation of SCF expression is likely beneficial for reducing the activation of mast cells and their release of histamine, thereby improving intestinal function in response to dietary supplementation with a high level of ZnO. Histamine is a product of histidine decarboxylation, further supporting an important role for amino acid catabolism in intestinal integrity and function [43]. Because diarrhea remains a significant problem for young mammals, including piglets and children [1,44], new knowledge about the role of zinc in regulating intestinal SCF expression and the activity of mast cells has important implications for reducing neonatal morbidity and mortality in both animal agriculture and human medicine.

Although the results of the present study indicate that dietary supplementation with ZnO inhibits SCF expression in the small intestine of weanling pigs, the underlying molecular mechanisms remain to be elucidated. Further studies are warranted to identify an effect of zinc supplementation on expression of SCF and related proteins in intestinal mucosal mast cells in vivo using proteomics technology [45]. Additionally, a role for c-kit in mediating an effect of Zn on histamine release can be defined if a specific inhibition of the SCF/c-kit pathway could be performed in vivo to prevent diarrhea in postweaning pigs and if mast cells with a c-kit receptor knockdown would be available to determine whether high levels of extracellular Zn can suppress their SCF expression and histamine release.

In summary, dietary supplementation with a high level of zinc (3000 ppm) reduced SCF expression, the number of mast cells and histamine production in the duodenum and jejunum of weanling piglets and effectively prevented diarrhea in these animals. This novel finding provides insight into the molecular mechanism for the efficacy of ZnO in treating and preventing diarrhea in weanling piglets.

Acknowledgments

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