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Zinc sulfate chronic exposure effects on large intestine of adult Wistar rats: a histological evaluation

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Abstract: The present study aimed to investigate possible morphological changes in the large intestine of adult Wistar rats submitted to chronic exposure to zinc sulfate at different doses.

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The animals were divided into three groups: control, treated with 5mg and treated with 20mg of zinc sulfate. The experiment had 56 days of exposure period and after this period the animals were anesthetized and euthanized. After dissection, a fragment of the cranial portion of the ascending colon of each animal was removed, fixed in Karnovsky solution for 24 hours, dehydrated in a growing ethanolic series and included in glycolmethacrylate resin. In a manual microtome, slices of 3µm thickness were obtained and then, stained with blue toluidine, for tissue description and morphometric analysis. The samples were also also submitted to histochemical techniques: Periodic acid of Schiff (PAS) and Alcian Blue (pH 2.5), for marking neutral and acidic mucus producing cells, respectively. The morphological parameters analyzed were: crypt depth, intestinal epithelium height, and number of PAS-positive and Alcian Blue-positive goblet cells. A decrease of neutral mucin-producing cells number and an increase in the height of the intestinal epithelium were observed in the animals treated with 20 mg of zinc sulfate. These results indicate morphological changes in the large intestine due to chronic zinc exposure, indicating a stress condition.

Keywords: Toxicity, Zinc, Intestine, Mammal, Morphology.

1. Introduction

Heavy metals are those chemical elements that have an atomic density higher than 4000kg/m^3. The elements included to the heavy metal group are: copper, cadmium, zinc, chromium, arsenic, boron, cobalt, titanium, strontium, tin, vanadium, nickel, molybdenum, mercury, lead, and others. Some of these metals are present in the biological systems as requirement for plants or animals normal growth. On the other hand, elements such as lead, mercury, cadmium, and arsenic are not essential for animal or plant physiology and may cause environmental pollution leading to serious consequences for plants, animals and humans [1].

Zinc is one of the heavy metals with biological relevance, being necessary for microorganisms, plants and animals cells, and is also the second most abundant transition metal in living organisms [2]. In the human body, zinc supports large number of biological functions: helps the normal formation of many enzymes, being sometimes, the core ion of their reaction center; improves the immune system activity; prevents apoptosis; helps normal growth and development of gonads; improves testosterone production and spermatogenesis; has a dermal protection function and helps the treatment of bacterial infections [3]. In the human activities, zinc is usually used in metallurgical industry, pharmaceutical, agricultural, and medicinal products that lead to Zn discharging in the environmental. This can be a major health problem once zinc poisoning causes diarrhea, liver failure, bloody urine, icterus, kidney failure, stomach cramps, abdominal cramps, epigastric pain, nausea, and vomiting, pancreatic harm, anemia, and lower levels of high-density lipoprotein cholesterol [1]. The laboratory findings of zinc high

exposure may include a regenerative anemia, neutrophilic leukocytosis, hemoglobinemia, bilirubinemia, hemoglobinuria, proteinuria, and elevated amylase, lipase, BUN, creatinine and hepatic enzymes [4].

Aquatic animals have been a recurrent model for zinc toxic effects. A study showed that $ZnCl_2$ increases necrosis and apoptosis rates in epidermal cells from the rainbow trout [5]. Toxicity, accumulation and retention of zinc by the common carp were demonstrated under normoxic and hypoxic conditions [6]. On the other way, recently, another study about the rainbow trout showed that zinc was the least cytotoxic element to a gut cell line, in comparison with copper, silver and cadmium [7]. Similarly, in developing tadpoles of the Chiricahua leopard frog, zinc did not appear to have a negative impact during the acute or chronic exposures, unlike copper and cadmium [8].

In mammals, is proven that, in mice, excess free zinc may induce pancreatic acinar necrosis. Necrosis is also directly induced by zinc ions on renal tubular epithelial cells, and is reported the occurrence of dehydration and gastrointestinal blood loss [4]. It is also known that zinc might be the major contributor to the observed thyroid toxicity caused by metals presents in environmental contaminations [9]. In Bama mini pigs, chronic excessive zinc diet impacted testicular Zn concentration and made the testes more vulnerable to heat, leading to testicular toxicity [10]. Another study showed that after intravenous injection on rats, uptake of zinc was high in liver, spleen, pancreas, kidney, and intestine.

To the oral exposure in humans, recommended dietary allowance (RDA) for zinc is 11 mg/day for men and 8 mg/day for women. These values are 2-3mg/day for infants and 5-9mg/day for children.The oral ingestion of zinc affects directly the organs of the gastrointestinal tract, even before it is distributed to the whole body [11].

Despite of the primary contact of digestive organs to zinc after oral exposure, there are only a few studies about the zinc effects on organs such as esophagus, stomach, small and large intestines. A recent study previously published by our research group, showed that the large intestine of male Wistar rats are morphologically affected by chronic exposure to nickel [12].

Because of its role on water and salts absorption, and mucus production to the fecal bolus formation, the large intestine is a very important organ to indicate zinc poisoning.

Thus, this study aimed to elucidate some morphological aspects of the large intestine that can be associated with zinc chronic oral exposure. We evaluated and quantified histological and cellular components of the organ and established their relationship with the gastrointestinal tract functions.

2. Material and Methods

2.1 Animals, Zinc Oral Exposure and Material Collection

The whole experiment was conducted according to the "Ethical Principles for the Use of Laboratory Animals" (COBEA, 1991), having been the project previously accepted by the Animal Research Ethics Committee of the Federal University of Viçosa (Protocol 001/11).

Fifteen adult male Wistar rats (90 days old) obtained from the Central Biottery of the Center for Biological and Health Sciences at Universidade Federal de Viçosa (UFV, Viçosa, Minas Gerais, Brazil) were kept in the biotery of the Department of Nutrition and Health of the Universidade Federal de Viçosa. The animals were maintained in individual cages being fed daily with 30g of pelleted commercial feed (Labcil– SOCIL, São Paulo, Brazil), for laboratory animals. The ambient temperature was 22 ± 1 °C and the photoperiod 12:12h (light: dark). The animals were divided into experimental groups containing five animals each. The control group received free water, the group 2 received 5mg/day of zinc sulfate, and the group 3 received 20mg/day of zinc sulfate. Zinc sulfate was supplied in the drinking water. The experiment lasted 56 days and after the experimental period the animals were anesthetized with 30mg/Kg of the commercial combination of ketamine and xylazine (Xylazin and Cetamin, Syntec, Santana de Parnaíba, São Paulo, Brasil) and euthanized by prolongation of the anesthesia. After that, the animals were weighed, and a cranial portion fragment of the ascending colon was removed of each animal. The fragments were then fixed in Karnovsky solution for 24 h and then left in 70% alcohol.

2.2 Hystometric Evaluations

The colon fragments were dehydrated in a growing ethyl series (alcohol 80, 90, 95% and absolute I) and included in historesin. Semi serial sections of 3µm thickness were obtained in a rotating microtome (RM2245, Leica, Lincolnshire, Buffalo Grove, United States), using a glass knife. The sections were stained with toluidine blue (1% sodium borate) for tissue description and morphometric analysis, and also submitted to histochemical techniques: Periodic acid of Schiff (PAS) and Alcian Blue (pH 2.5), for the identification of neutral and acidic mucus producing cells, respectively.

Photographic documentation of the preparations was performed under a optical microscope (Olympus, Tokyo, Japan), with a digital camera (Olympus, Tokyo, Japan). Photomicrographs were obtained using the 10x objectives for the intestinal crypt's depth and width (at midpoint) and for intestinal epithelium eighth. Objectives of 20x were used for photomicrographs used at the quantification of other goblet cells and PAS- and AB-positive mucus-producing cells. Five fragments of each animal were randomly selected, and photomicrographs were obtained of five fragment fields per each fragment, totaling 25 photomicrographs per animal and 150 photomicrographs per animal group (control and zinc-contaminated animals). To obtain the histometric data, the photomicrographs were digitalized and analyzed using the Image J software (Media Cybernetcs, Inc, Madison, Wisconsin, United States). From the histological images, we conducted five measurements for each parameter (depth and width of intestinal crypts and height of the apical epithelium), obtaining 125 measurements/animal/parameter. The quantification of goblet cells and PAS-positive/AB-positive mucus-producing cells per μm^2 were obtained by counting all stained cells in the photographic field.

2.3 Statistical Analyses

The normality of Kolmogorov-Smirnov and homoscedasticity of Cochran tests were conducted using the Prisma (Graphpad software, San Diego, California, United States) software. Once these assumptions were achieved, a Tukey test ($P<0.05$) was conducted for comparing the values obtained for zinc-unexposed (control) and zinc-treated animals. When the normality and homoscedasticity were not achieved, it was conducted such comparison using the Mann-Whitney nonparametric test ($P< 0.05$).

3.Results

In the present study, the animals of control group did not show significance changes in crypt depth from animals tread with 5mg Zn/day and 20mg Zn/day (Figure 1A, 1B and 1C) (Table 1). Our findings show that zinc-exposed animals had no significance change in AB-positives goblet cell number, when compared to control group (Figure 1D, 1E and 1F). The PAS-positive goblet cells (Figure 1G, 1H and 1I) number was significantly lower in 20mg Zn/day-exposed animals (Table 1).

The 20mg Zn/day-exposed animals showed significantly bigger values of height of the intestinal epithelium (Figure 1C) than other groups (Table 1).

(Figure1)

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Table 1

Group	DC	HE	PAS^{+}	AB^{+}
20mgZn	232,15 ± 47,9^{A}	36,4 ± 4,7 ^{B}	277,28 ±153,5^{B}	199,28 ± 72,5 $^{A\,A}$
5mgZn	255,66 ± 53,8^{A}	31,9 ± 4,2 ^{A}	586,24 ±236,8^{A}	289,24 ±195,8^{A}
Control	258,65 ± 57,8^{A}	32,1 ± 3,2 ^{A}	469,44 ±173,5^{A}	299,28 ±173,3^{A}

Hystometric quantification (mean ± standard deviation) of intestinal parameters of adult male Wistar rats treated with different daily doses of zinc, for 56 days.

^{DC} = depth of crypt (µm); ^{HE} = height of the epithelium (µm); $^{PAS+}$ = number of PAS positive goblet cells; $^{AB+}$ = number of AB positive goblet cells. Means followed by equal letters within the same column do not differ from one another by the analysis of variances at the 5% level of significance.

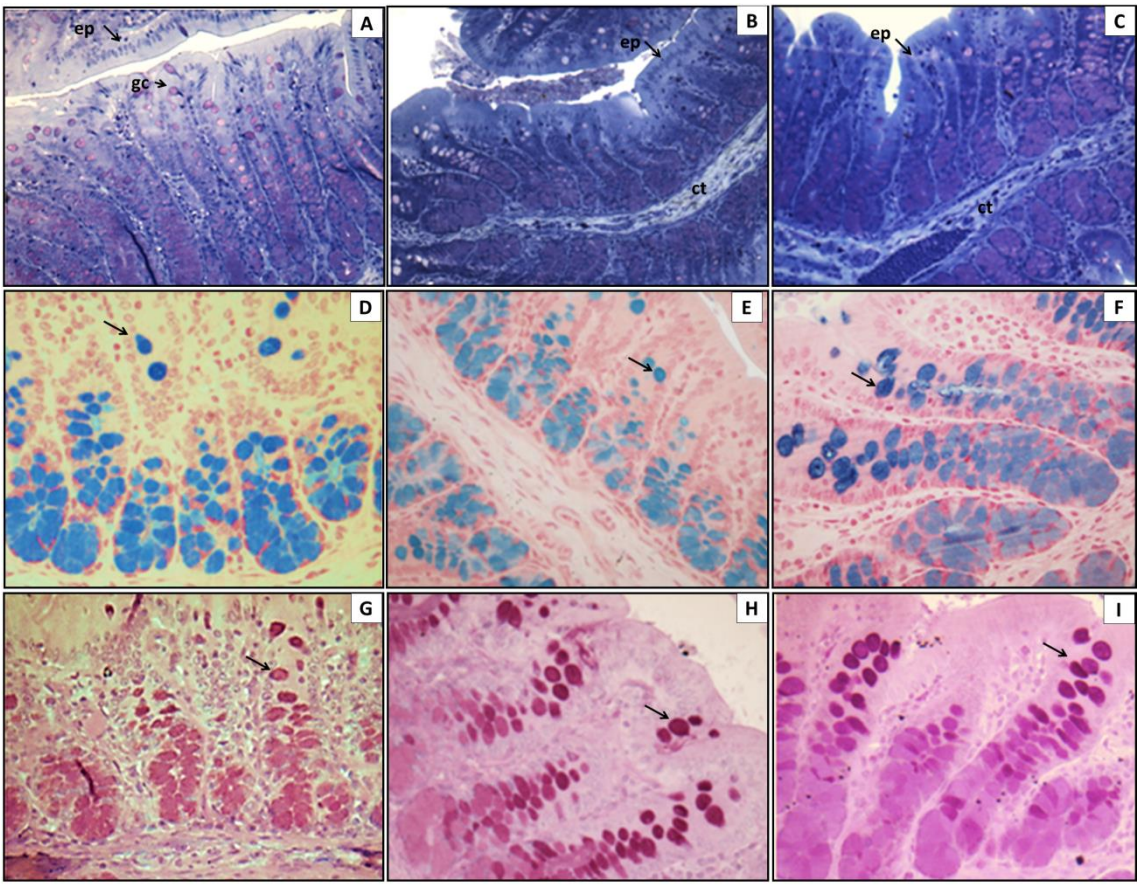

Fig.1. Large intestine of Wistar rats (A), (D), and (G) control group. (B), (F), and (H) 5mg Zn/day- exposed. (C), (F), (I) 20mg Zn/day-exposed. ep = epithelium; ct = connective tissue; gc = goblet cells; (D), (E), (F) Arrow =Alcian Blue-positive goblet cell. (G), (H), (I) Arrow = PAS-positive goblet cell. Magnification: (A), (B), (C) 100X; (D), (E), (F), (G), (H), (I) 200X; Toluidine Blue – Sodium Borate (A), (B).(C). Alcian Blue (pH 2.5) (C), (D), (E). Periodic-acid-Schiff (PAS) (G), (H),(I).

4.Discussion

The structural organization of mammalian intestinal epithelium is based on serial finger-like projections that reach the lumen, called villi, and invaginations into the mesenchyme called crypts. The villi is composed by specific cell types such as absorptive enterocytes, goblet cells, and enteroendocrine cells, whereas the crypt contains stem and transit amplifying cells [13]. Usually, increased villus height to crypt depth ratio is a useful criterion to estimate and suggestive of greater nutrient absorption [14]. The increase of crypt depth decreased the absorption in the small intestine [15].

Some studies evaluate the occurrence of changes in structural components of mammalian intestine under dietary variations. The crypt depth is one of the relevant parameters to discuss morphological alterations in the intestine [14, 16].

Our results suggest that zinc, at the used dosages by chronic exposure, possibly do not influence intestinal absorption.

A study reported a reduced crypt depth in the colon of rats after nickel chronic exposure [12]. These findings may suggest that zinc is less harmful than nickel, concerning crypt depth alterations.

Crypt depth in the colon of rats is associated with changes in the intestinal transit in a study that showed that crypt depth decreased after exclusion of intestinal transit [17]. The absence of significance changes in crypt depth after zinc chronic exposure indicates no intestinal transit alterations in the zinc-exposed animals.

Mucins are the main component of mucus, a selective and protective barrier for body mucosal tissues. Mucus is also responsible for many other physiological functions. In the gastrointestinal tract, major intestinal mucins are produced by goblet cells. Mucus deposition of large intestine and small intestine is not the same. The mucus layer of large intestine is divided into an inner layer and an outer layer [18]. In the large intestine, the inner mucus layer separates the commensal bacteria from the host epithelium and the outer colonic mucus layer is the natural habitat for the commensal bacteria [19].

Abnormalities in the secretion, composition and distribution pattern of mucins in the intestinal crypts have been a relevant aspect that affects the colon under different stress conditions [18].

Sharma and Schumacher showed that feeding a commercial diet reduced the volume density of cells containing neutral mucins in the jejunum of rats. These results demonstrate that dietary changes are influential in modifying the amount and proportion of mucins in the small

intestine [20]. The chronic ingestion of nickel induced a decrease in AB-positive and PAS-positive goblet cells in colon of rats [12]. Another study using rats, reported a decrease in goblet cells number in animals without fecal transit [17].

Anyway, it is possible to presume that zinc chronic exposure affects the mucins production, especially the acid mucins. As the PAS-positive goblet cells, after zinc chronic ingestion, follows the same decreasing pattern observed in other stressing events, we can assume that zinc prolonged ingestion at high doses can induce some degree of intestine dysfunction.

The intestinal epithelium is a cytoprotection to the intestinal mucosa, being a barrier against infectious and disruptors agents. The enterocytes are the epithelial cell line related to the barrier function of the intestine and morphological and cytological alterations in the organ are associated with stress conditions [21].

Since it is known that stress conditions may induce the barrier function failure in the intestine, it is possible to link the increase in the height of the intestinal epithelium with a compensation mechanism, in response to zinc chronic ingestion. Similar results were obtained after nickel prolonged administration in rats [12].

5. Conclusion

The zinc chronic exposure has a functional impact in the large intestine of male rats which reflects on its histological aspects. Since triggers the decrease of mucin production and the increase in the epithelium height, the zinc exposure can be considered a stressing condition and can be associated with intestinal dysfunctions. However, zinc appears being less harmful than other heavy metals such as nickel.

More investigations would be a perspective to evaluate the effects of chronic zinc ingestion in intestinal transit, occurrence of exogenous infections, intestinal immune response, stem cell proliferation and cell renewal in the organ.

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