Pharmacology Biochemistry & Behavior, Vol. 26, pp. 293–297. Copyright © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Zinc Deficiency: Its Role in Gastric Secretion and Stress-Induced Gastric Ulceration in Rats

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Received 11 September 1986

CHO, C. H., L. Y. Y. FONG, P. C. C. MA AND C. W. OGLE. *Zinc deficiency: Its role in gastric secretion and stress-induced gastric ulceration in rats.* PHARMACOL BIOCHEM BEHAV **26**(2) 293–297, 1987.—The effects of zinc deficiency on gastric secretion and on cold-restraint stress-induced ulceration in rat stomachs have been studied. Administration of graded zinc deficient diets for 5 weeks significantly depressed the serum zinc concentration and decreased body weight gain in the rats. These diets significantly increased the gastric secretory volume, acid and pepsin. Zinc deficiency produced or aggravated the formation of glandular ulceration in the absence or presence of stress, respectively; it also decreased the mast cell count in the gastric glandular mucosa. It is concluded that zinc deficiency adversely affects the rats by reducing the body weight gain and producing ulceration which is probably mast cell-mediated. On the other hand, it increases gastric secretory functions.

Zinc Gastric secretion Stress Mast cell Gastric ulceration

ZINC ions, by their known biological membrane stabilizing action [9], have been shown to have a significant antiulcer effect on several experimental gastric ulcer models in rats [4, 5, 12, 14, 15, 18]. Support for this is found in the stabilizing activities of zinc on lysosomal and mast cell biomembranes [4, 5, 9, 13, 18]. Degranulation of the latter in the gastric mucosa has been further shown to be one of the ulcerogenic factors accounting for stress ulceration in animals [4,5]. Dietary supplementation of zinc has also been shown to depress or enhance gastric secretion [7,22]. The aim of our work is to determine the effects of nutritional zinc deficiency on gastric secretion and on stress-induced ulceration in rats.

METHOD

Animals

Weanling male Sprague-Dawley rats (45–55 g) were obtained from the Laboratory Animal Unit, University of Hong Kong. The animals were distributed randomly in plastic cages as described previously [11]. They were housed in an air-conditioned room in which the temperature (22±1°C) and relative humdidity (65–70%) were kept constant. The animals were fed different dietary regimens and had free access to deionized water. They were fasted 24 hr before experimentation.

Experimental Groups

The animals were randomly divided into 4 groups and given the following dietary regimens for 5 weeks before experimentation:

A. Zinc sufficiency (pair-fed with group B, diet contained zinc 80–100 ppm).

B. Mild zinc deficiency (diet contained zinc 20–25 ppm).

C. Zinc sufficiency (pair-fed with group D, diet contained zinc 80–100 ppm).

D. Severe zinc deficiency (diet contained zinc 7–10 ppm).

The food was prepared by mixing the essential nutrients, vitamins and minerals in appropriate proportions. Soybean protein (International Corporation of Nutrition, USA) was washed thrice with the disodium salt of EDTA at pH 4.3 to reduce the zinc content [20]. The protein was then washed with distilled water and finally allowed to dry overnight at room temperature. The different proportions of zinc content in the 3 types of diet were achieved by adding appropriate amounts of zinc sulphate (Merck) when mixing the other ingredients.

Determination of Zinc Status in Animals and Zinc Content in the Diet

The zinc status in the rats was determined by measuring

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the serum zinc levels after the animals had been given experimental diets for 5 weeks. Rats were lightly anaesthetized with ether and blood samples obtained from the retro-orbital plexus through a heparinized capillary tube. The blood, collected in a centrifuge tube, was allowed to clot and then spun (Beckman TJ-6) for 15 min at 3000 rpm. The serum was diluted 10-fold with deionized water and the zinc level determined by an atomic absorption spectrophotometer (Instrumentation Laboratory, Model 651).

The zinc content in the diet was measured by mixing 50 mg food with 4 ml of a concentrated $HNO_3/HClO_3$ solution (4:1 v/v). This mixture was digested in an acid-washed teflon beaker at 160–170°C on an electric hot plate. The residue was then dissolved in 10 ml of deionized water and its zinc level determined by atomic absorption spectrophotometry.

Pylorus Occlusion and Gastric Secretion Measurement

The pylorus was occluded by the method of Shay *et al.* [19]. The rats were lightly anesthetized with ether, an abdominal mid-line incision was made and the duodenum exposed. The pylorus was ligated, leaving the circulation intact. The abdominal wound was sutured and the animals were returned to their starvation cages to recover from anaesthesia. They were killed 2 hr later by a sharp blow on the head. Stomachs were isolated and the gastric secretion collected through an incision in the forestomach. The total volume of gastric secretion was measured and the acid output was determined by titration with 0.01 N NaOH to pH 7.4 using an autotitration system (Radiometer, model TTT80). The stomachs were cut open along the greater curvation and examined for lesions.

Induction of Cold-Restraint Stress Gastric Ulcers and Determination of Mast Cell Numbers

Rats were starved for 24 hr before experimentation. They were placed in individual close-fitting cylindrical wire mesh cages and exposed to 4°C for 1 hr. Controls remained in their starvation cages, in the air-conditioned room (22°C), for a similar period of time. The animals were killed by a sharp blow on the head and their stomachs cut open for measurement of the mucosal ulcer severity. The mast cell count was carried out according to the method of Cho and Ogle [4]. The glandular portion of the stomach was fixed in freshly prepared 4% lead acetate (Merck) solution for 48 hr. Tissue blocks were cut and stained with toluidine blue. The number of mast cells seen under a light microscope was counted in 40 adjacent oil immersion fields (magnification 1000×) in the glandular mucosa.

Measurement of Gastric Mucosal Lesions and Statistical Analysis

The severity of mucosal lesions was determined with an illuminated magnifier (3×). Lesion size (mm) was measured along its greatest length; in the case of petechiae, five such lesions were considered the equivalent of a 1 mm ulcer. The sum of the lesion lengths in each group of animals was divided by its number and expressed as the ulcer index [5].

The data were analyzed for statistical significance by means of the two-tailed Student *t*-test.

RESULTS

Rats fed zinc-deficient diets had a significantly lower serum zinc level when compared with their pair-fed counterparts (Table 1). In both the mild and severe zinc-deficient rats, the body weights were comparatively less than those of their pair-fed controls (Tables 2 and 3). In the pylorus-occluded rats, the severity of haemorrhagic ulcers was higher in the zinc-deficient rats than in their corresponding pair-fed groups. The degree of ulceration also depended on the severity of zinc deficiency (Table 2). Severe zinc deficiency significantly increased the volume and the amount of titratable acid and pepsin in the gastric secretion. Mild zinc deficiency also markedly elevated the quantity of gastric acid and pepsin secretion (Table 2).

TABLE 1

SERUM ZINC CONCENTRATIONS IN RATS FED THE VARIOUS DIETARY REGIMENS

Groups	Serum Zinc Concentration (μg/100 ml)
A. Zinc sufficiency (zinc 80–100 ppm; pair-fed with group B)	123.2 ± 6.3
B. Mild zinc deficiency (zinc 20–25 ppm)	36.4 ± 4.0*
C. Zinc sufficiency (zinc 80–100 ppm; pair-fed with group D)	116.2 ± 8.3
D. Severe zinc deficiency (zinc 7–10 ppm)	16.7 ± 3.2*

The values are means ± S.D. of 8 rats.
*$p<0.001$ when compared with their corresponding pair-fed controls.

The glandular mucosal wall mast cell count in zinc-deficient rats was less than that in their corresponding pair-fed controls under nonstress conditions. The ulcer index was also found to be significantly higher than that of the controls (Table 3). Cold-restraint stress for 1 hr produced severe haemorrhagic glandular ulceration which was much greater in zinc-deficient rats (Table 3). Stress also markedly depleted the mucosal mast cell counts in both the zinc-sufficient and -deficient rats. However, the number of mast cells depleted by stress was higher in the zinc-sufficient groups (Table 3), in spite of the percentage of cells degranulating in the zinc-deficient and normal groups being roughly similar.

DISCUSSION

The present study demonstrates the effects of nutritional zinc deficiency on gastric secretion and on stress ulceration in the rat. It also shows that zinc deficiency influences the gastric glandular mucosal wall mast cell population under both normal and stress conditions. Rats fed zinc-deficient diets for 5 weeks had a markedly depleted store of the metal in the body as shown by a low serum zinc level. The growth of animals was also adversely affected [20]. However, the motor activity of the animals was not decreased (unpublished findings).

Zinc deficiency has been shown to decrease gastric acid

TABLE 2

EFFECTS OF DIETARY-ZINC DEFICIENCY ON GASTRIC GLANDULAR ULCERATION AND SECRETION IN PYLORUS-OCCLUDED RATS (ANIMALS KILLED 2 HR AFTER OCCLUSION)

Groups	No. of Rats	Body Weight (g)	Ulcer Index (mm)	Gastric secretion		
				Volume (ml/hr/100 g)	Titratable acid (μEq HCl hr/100 g)	Pepsin (μg/hr/100 g)
A. Zinc sufficiency (zinc 80–100 ppm; pair-fed with group B)	8	166.3 ± 11.5	0.31 ± 0.16	0.35 ± 0.13	32.2 ± 20.0	105.5 ± 34.4
B. Mild zinc deficiency (zinc 20–25 ppm)	8	148.3 ± 12.6†	1.13 ± 0.78†	0.63 ± 0.14	67.0 ± 25.0†	181.2 ± 76.8*
C. Zinc sufficiency (zinc 80–100 ppm; pair-fed with group D)	9	166.2 ± 9.7	0.23 ± 0.23	0.37 ± 0.13	34.5 ± 18.6	142.7 ± 44.6
D. Severe zinc deficiency (zinc 7–10 ppm)	11	83.7 ± 5.7‡	2.25 ± 0.82‡	1.28 ± 0.37‡	102.8 ± 41.1‡	223.2 ± 73.6*

The values are means ± S.D.
*$p<0.05$, †$p<0.02$, ‡$p<0.001$ when compared with their corresponding pair-fed controls.

TABLE 3

EFFECTS OF DIETARY-ZINC DEFICIENCY ON STRESS-INDUCED ULCERATION AND CHANGES OF MAST CELL COUNT IN THE GASTRIC GLANDULAR MUCOSA

Groups	No. of Rats	Body Weight	Ulcer Index (mm)	Mast Cell Count/40 o.i.f.
No stress (unrestrained at 22°C for 1 hr)				
A. Zinc sufficiency (zinc 80–100 ppm; pair-fed with group B)	5	174.3 ± 23.3	0.1 ± 0.1	86.2 ± 12.7
B. Mild zinc deficiency (zinc 20–25 ppm)	7	115.4 ± 26.7†	1.2 ± 0.6†	34.4 ± 9.9‡
C. Zinc sufficiency (zinc 80–100 ppm; pair-fed with Group D)	5	144.3 ± 26.8	0.9 ± 0.7	73.0 ± 5.8
D. Severe zinc deficiency (zinc 7–10 ppm)	6	78.7 ± 9.8‡	1.4 ± 0.7	23.4 ± 6.5‡
Stress (restrained at 4°C for 1 hr)				
A. Zinc sufficiency (zinc 80–100 ppm; pair-fed with group B)	5	170.3 ± 29.3	4.5 ± 2.5§	24.5 ± 4.0¶
B. Mild zinc deficiency (zinc 20–25 ppm)	6	117.4 ± 18.3†	15.4 ± 4.7‡§	18.0 ± 2.6§
C. Zinc sufficiency (zinc 80–100 ppm; pair-fed with group D)	5	152.2 ± 31.3	4.2 ± 3.0	27.3 ± 6.6¶
D. Severe zinc deficiency (zinc 7–10 ppm)	7	99.4 ± 13.4†	15.3 ± 8.1*§	6.5 ± 3.0‡¶

The values are means ± S.D. o.i.f.=oil immersion fields (1000×).
*$p<0.05$, †$p<0.01$, ‡$p<0.001$ when compared with their corresponding pair-fed controls.
§$p<0.01$, ¶$p<0.001$ when compared with its corresponding nonstressed controls.

secretion in rats [17]; however, the acid output per unit time was calculated without taking into account the body weight of the animal. The present study demonstrates that zinc deficiency significantly increased the acid and pepsin output when the rate of secretion was expressed as per unit time and body weight. This increase in all three gastric secretory parameters was also seen when the data were expressed only in relation to unit time. Pylorus occlusion for 2 hr at 22°C (Table 2) did not significantly increase the ulcer index when compared with the corresponding nonstressed control (Table 3), indicating that accummulation of gastric secretion for a relatively short period of time did not produce a significant damaging effect on the glandular mucosa.

Zinc deficiency has been shown not to affect the mast cell population in the skin, thyroid and the metaphysis of the tibia [1]. The present study shows that zinc deficiency greatly reduced the number of granulated mast cells in the gastric glandular mucosa. Zinc has been shown to stabilize the biological membrane, especially those of mast cells found in the stomach [3,4] and peritoneal fluid [10]. Deficiency of this metal in the body could labilize the cells and reduce the number of mast cells in the stomach. Degranulation of the gastric mast cell has been shown to be associated with the release of histamine [15] which is thought to be responsible for gastric secretion [3] and stress ulcer formation [5,16]. This phenomenon has been confirmed in experiments where pretreatment with zinc reduces gastric acid secretion [7] and stress ulceration [5,14]. However, oral administration of zinc compounds in high doses has been reported to increase gastric acid secretion in young rats. This discrepancy may be due to the difference in age and species of the animals used in these studies.

The present study also confirms that zinc deficiency produces gastric ulceration in rats [17]. The high ulcer index in nonstressed animals could be due to chronic depletion of mast cells in the stomach, which have been shown to produce ulcers [4,5]. Other ulcerogenic factors, including breakdown of the gastric mucosal barrier, cannot be excluded [17].

It has been found that mast cell counts in the gastric glandular mucosa are inversely proportional to the severity of gastric ulceration [4,5]. The acutal number of mast cells which had degranulated in the zinc-deficient rats under stress conditions was less than that in the zinc-sufficient animals; this observation cannot explain the higher ulcer index in the former group (Table 3). These findings, therefore, imply that aggravation of stress ulceration by zinc deficiency is probably not wholly due to increased numbers of mast cells being degranulated. Zinc compounds have been found to protect against stress ulceration [1, 2, 5] and ethanol lesion formation [8, 10, 21]. The antiulcer effect appears to be due also the activation of the gastric mucosal defensive systems, e.g., preservation of gastric mucus from depletion [6, 10, 21]. Thus, it is conceivable that dietary zinc deficiency weakens the mucosal barrier [17] to contribute to the observed worsening of stress ulceration (Table 3) in rats. These findings also imply that the zinc ion could be an important natural defensive element in stomachs. The possibility that supplementations with this metal could increase the healing rate of gastric ulcers in man does indeed merit serious consideration.

ACKNOWLEDGEMENTS

The work was partially supported by research grants from the University of Hong Kong and the Wing Lung Bank Medical Research Fund.

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