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Original Article

Inflammatory response to zinc deficiency may be related to the number of white blood cells and platelets

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Abstract

Nutritional zinc deficiency leads to immune dysfunction and aggravates inflammation. The number of white blood cells (WBCs) and platelets increases as part of the inflammatory response and may aggravate inflammation due to viral or bacterial infections. We elucidated whether the effects of zinc deficiency on blood indices were related to the inflammatory response. Five-week-old male Sprague-Dawley rats were fed a zinc-deficient diet (without zinc additives) or a standard diet (containing 0.01% zinc) for 6 weeks. The mean body weight in the zinc-deficient group decreased compared to the standard group. The mean value of the serum malondialdehyde concentration in the zinc-deficient group was higher than that in the standard group. Furthermore, serum total superoxide dismutase (SOD) and Cu/Zn-SOD activities in the zinc-deficient group were lower than those in the standard group. The numbers of WBCs and platelets in the zinc-deficient group were higher than those in the standard group. These findings indicate that the inflammatory response, and concurrently WBC and platelet numbers, was increased after 6 weeks of zinc deficiency. Augmentation of the inflammatory response may have involved infiltration of bacteria and viruses from dermatitis, as well as changes in the balance between zinc and copper in the blood, which inhibited the antioxidant enzymes Cu/Zn-SOD, ultimately resulting in the accumulation of reactive oxygen species.

Introduction

Zinc is an essential trace element for humans that is required for more than 300 zinc-finger transcription factors and enzymes, and for gene expression[1-3)]. The mean daily dietary zinc intake from several countries ranges from 4.7 to 18.6 mg. The main human dietary sources of zinc include oysters, fish, red meat, nuts, and dairy products. The recommended daily zinc intakes of men and women over 20 years of age are 13.5 and 9.5 mg, respectively[4)]. However, it was reported recently that zinc intake was deficient in the United States[5-7)], Mexico, Colombia, and Japan[8)].

Zinc deficiency leads to growth retardation, taste abnormalities, dermatitis, depilation, immune dysfunction[9)], and a higher risk of inflammatory diseases[10)]. Overexpression of the pro-inflammatory proteins, inducible nitric oxide synthase and interleukin (IL)-1β, aggravated lung inflammation in rats with zinc deficiency[11)]. In vitro studies showed that the levels of inflammatory cytokines (IL-1β, IL-6, and tumor necrosis factor-α) increased due to reactive oxygen species (ROS) in immune cells under a zinc-deficient condition[12, 13)]. Based on these findings, and because blood cell indices such as white blood cell (WBC) and platelet numbers increase in response to inflammation, including viral and bacterial infections[14)], WBC and platelet numbers may also be affected by zinc deficiency. However, previous results focused on effects on rat lungs, or immune cells in vitro. Therefore, it is important to evaluate whether WBCs and platelets are affected by zinc deficiency-induced inflammation.

In this study, we analyzed blood from rats fed a standard or a zinc-deficient diet to assess the number of WBCs, red blood cells (RBCs), and platelets, as well as hemoglobin (HGB) levels, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Additionally, we determined zinc and copper concentrations, and superoxide dismutase (SOD) activities in serum. The results indicated that the effects of zinc deficiency on blood indices were related to the inflammatory response.

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Materials and methods

Animal model

Five-week-old male Sprague-Dawley rats (n = 5/group), weighing 180–200 g, were obtained from Charles River (Tokyo, Japan). Rats were divided into two feeding groups: 17 g/day of either a zinc-deficient (additive-free of zinc) or standard (containing 0.01% of zinc) diet for 6 weeks. Standard and zinc-deficient diets were specially made by Oriental Yeast (Tokyo, Japan) (Table 1). Body weights were determined twice a week. Animals were housed in separate cages at 22℃ with a 12-h light/dark cycle, in accordance with protocols and guidelines approved by the Animal Experimentation and Ethics Committee of The Jikei University School of Medicine. After dietary manipulation, blood samples were collected from the abdominal aorta.

Serum zinc, copper, and malondialdehyde (MDA) levels, and superoxide dismutase (SOD) activities

For obtaining serum, blood was transferred to a 15 mL centrifuge tube and centrifuged at 3000 × g for 10 min. Serum zinc and copper levels were quantified with the ACCURASAUTO zinc and QUICKAUTO NEO copper kits (Shino-test, Kanagawa, Japan) as described previously[15, 16]. MDA was quantified using a thiobarbituric acid assay kit (Japan Institute for the Control of Aging, Shizuoka, Japan), following the manufacturer's protocol[17]. Total SOD activity (Mn, Cu/Zn, and extracellular) was measured using the SOD Assay Kit-WST (Dojindo, Rockville, MD, USA) according to the manufacturer's instructions. Mn-SOD activity was determined by adding 1.5 mM diethyldithiocarbamate to serum to inhibit Mn-SOD and incubating the mixture for 30 min at 37℃. Subsequently, Cu/Zn-SOD activity were calculated as total SOD activity – Mn SOD activity.

Determination of blood indices

Blood was transferred to a blood collection tube containing EDTA. Determinations of WBCs, RBCs, HGB, HCT, MCV, MCH, MCHC, and platelets were done with a cytometer (Nihon Kohden, Tokyo, Japan).

Statistical analysis

Groups fed the standard and zinc-deficient diets were compared by Student's t test[18]. Data were analyzed using JMP 11.2 software (SAS Institute, Cary, NC, USA). Differences were considered significant at $P < 0.05$.

Results

Body weights

Figure 1 shows the daily changes in body weights in the standard and zinc-deficient diet groups after 6 weeks. The mean body weight in the zinc-deficient group was decreased compared to the standard group at 10–42 days.

Serum zinc and copper concentrations, and copper/zinc ratio analyses

Figure 2 shows the serum zinc and copper concentrations, and copper/zinc ratio in rats fed the standard and zinc-deficient diets. The mean serum zinc concentration in the zinc-deficient group was lower than that in the standard group. The mean serum copper concentration and copper/zinc ratio in the zinc-deficient group were higher than those in the standard group.

Serum MDA concentrations, and superoxide dismutase activity analyses

Figure 3 shows the serum MDA concentration and SOD activities (total, Mn, and Cu/Zn) in the standard and zinc-deficient diet groups. The mean serum MDA concentration in the zinc-deficient group was higher than that in the standard group. Serum total SOD, and Cu/Zn-SOD activities in the zinc-deficient group were lower than those in the standard group. However, Mn-SOD activity was comparable between the two groups.

Blood indices analyses

Table 1 shows values for WBCs, RBCs, HGB, HCT, MCV, MCH, MCHC, and platelets in the standard and zinc-deficient diet groups. The numbers of WBCs and platelets in the zinc-deficient group were higher than

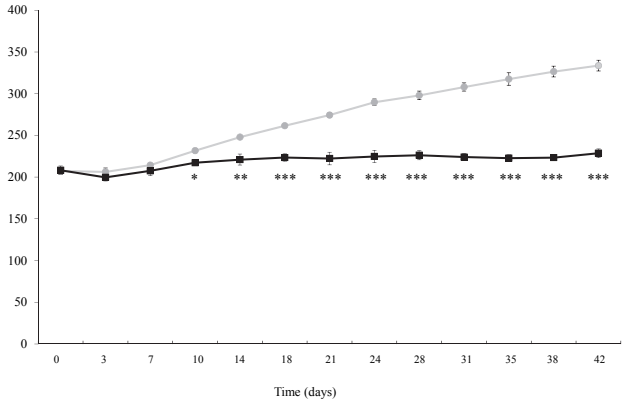

Fig. 1 Daily changes in body weights in rats on standard and zinc-deficient diets.
Data are expressed as means ± SEM (n = 5/group). *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the standard diet by Student's t test.

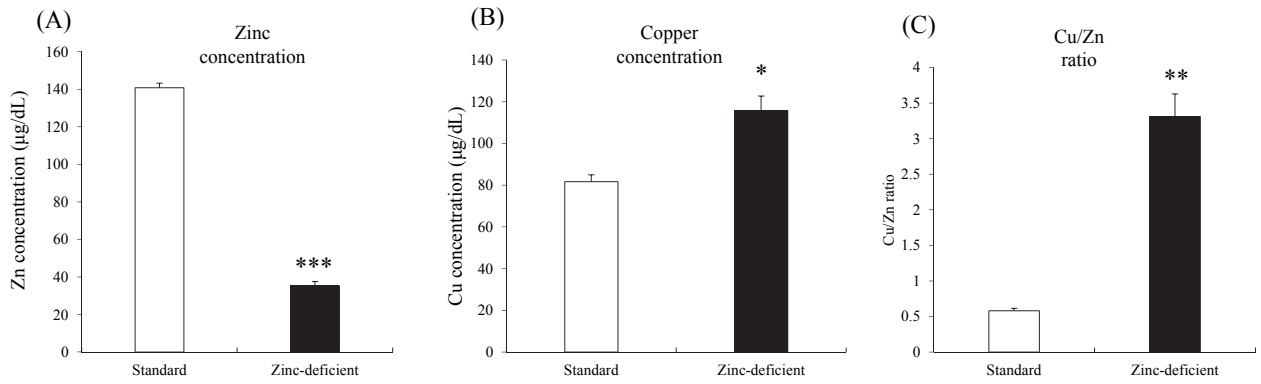

Fig. 2 Serum zinc and copper concentrations in rats on standard and zinc-deficient diets.
Zinc (A) and copper (B) concentrations, and the Cu/Zn ratio (C) in serum. Data are expressed as means ± SEM (n = 5/group). *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the standard diet by Student's t test.

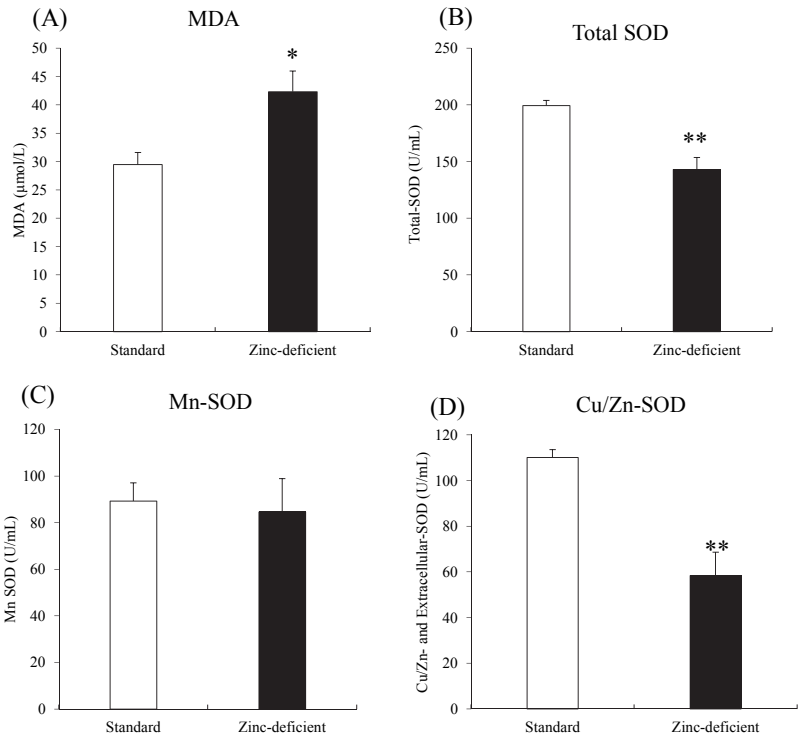

Fig. 3 Serum malondialdehyde (MDA) levels, and superoxide dismutase (SOD) activities in rats on standard and zinc-deficient diets.
MDA concentrations (A). total (Cu/Zn, Mn, and extracellular) SOD activities (B). Mn-SOD activities (C). Cu/Zn-SOD activities (D). Cu/Zn-SOD activity was calculated as total SOD activity - Mn-SOD activity. All SOD activities were measured in serum. Data are expressed as means±SEM (n = 5/group). *P < 0.05 and **P < 0.01 vs. the standard diet by Student's t test.

those in the standard group. However, the RBC, HGB, HCT, MCV, MCH, and MCHC indices were comparable between the two groups.

Discussion

Zinc deficiency leads to immune dysfunction[9] and a higher risk of inflammatory diseases[10]. We found that the inflammatory response due to zinc deficiency was caused by changes in blood cells, similar to the increase in WBCs and platelets caused by the inflammatory response from acute coronary syndrome, and viral and bacterial infec-

Table 1 Components of special diet used this study

Component	(g)
Protein	20
Glucose	10
Corn oil	10
Powdery cellulose	2
Cornstarch	48.7
α - Cornstarch	5
Minerals	3.13
Vitamins	1.17
Total	100

Standard and zinc-deficient diets were specially made by Oriental Yeast Co. Ltd., Tokyo, Japan.

tions[14]. We also found that zinc-deficient rats gained significantly less body weight at 10–42 days of feeding than rats on a standard diet (Fig. 1). This confirmed that zinc deficiency delayed growth, probably because of inefficient DNA/RNA synthesis and cell division[19].

The low zinc concentration of serum resulted from decreased absorption of zinc from the zinc-deficient food. In contrast, the high serum copper concentration resulted from an imbalance of zinc-copper antagonism leading to increased copper absorption in the small intestine[1–3, 20, 21]. Therefore, zinc deficiency also altered the balance between the absorption of zinc and copper and thus, their ratio, in blood. These results suggest that the alterations of serum zinc and copper resulting from the zinc-deficient diet caused the low Cu/Zn-SOD activity. Also, total SOD activity was decreased in zinc-deficient rats. Therefore, the imbalance between zinc and copper in the blood inhibited the antioxidant enzymes, Cu/Zn-SOD[20]. The diminished effectiveness of these important antioxidant enzymes ultimately resulted in the accumulation of ROS, as indicated by an increase in MDA. Therefore, zinc deficiency may aggravate inflammatory reactions via enhancement of ROS[3].

Changes in WBCs and platelets have been related to the inflammatory response. In the current study, zinc deficiency increased the number of WBCs and platelets in blood. In addition, feeding a zinc-deficient diet for six weeks observed dermatitis and depilation (Supplemental fig. 1). Therefore, local dermatitis caused by zinc deficiency can aggravate the inflammatory response caused by the infiltration of bacteria and viruses[22], and increase the number of WBCs and platelets.

In summary, the inflammatory response, and concurrently WBC and platelet numbers, were increased by six weeks of zinc deficiency. Zinc deficiency altered the balance between serum concentrations of Zn and Cu, and thus their ratio in blood. The imbalance between zinc and copper in the blood inhibited the antioxidant enzymes Cu/Zn-SOD, ultimately resulting in the accumulation of ROS, as indicated by the increase in MDA, that can induce inflammation. In conclusion, inflammatory factors that increase in response to zinc deficiency may be related to an increase in the number of WBCs and platelets derived from dermatitis.

Acknowledgments

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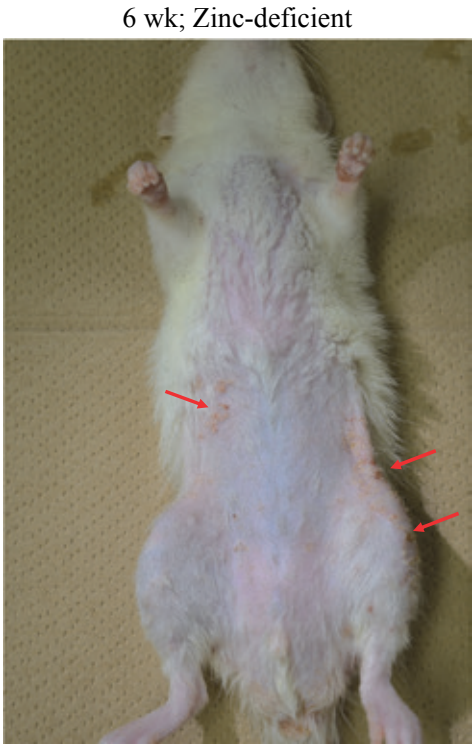

Supplemental fig. 1 Representative dermatitis and depilation in a rat on the zinc-deficient diet.
Dermatitis and depilation are evident at 6 weeks. The arrows indicate dermatitis.

Table 2 Blood indices in rats on standard and zinc-deficient diets

	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
standard	74.20 ± 5.03	759.0 ± 18.2	15.60 ± 0.23	41.28 ± 0.76	54.42 ± 0.42	20.54 ± 0.19	37.80 ± 0.23	93.24 ± 4.13
zinc-deficient	217.2 ± 28.5**	643.0 ± 70.8	13.42 ± 1.22	36.18 ± 2.66	57.36 ± 2.57	21.06 ± 0.52	36.88 ± 0.75	135.7 ± 9.97*

Data are expressed as means ± SEM (n = 5 per group). *P < 0.05 and **P < 0.01 vs. the standard diet by Student's t test.
WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets.

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