韓國營養學會誌 27(9) : 949~956, 1994
Korean J Nutrition 27(9) : 949~956, 1994

Lymphopenia by Pure Zinc Deficiency : Role of Corticosterone

Jung Han Yoon Park · Sang Sun Lee*

Department of Food and Nutrition, Hallym University, Chunchon, Korea
Department of Food and Nutrition, Hanyang University, Seoul, Korea*

ABSTRACT

The effect of zinc deficiency on certain immunological parameters was investigated using intragastric tube feeding to obviate decreased food intake and altered eating pattern. Male, Fischer 344 rats were bilaterally adrenalectomized(ADX) or sham operated(SHAM). ADX rats received 0.9% NaCl in their drinking water and corticosterone injections at the dose of 1mg/kg of body weight three times per day. After recovery, one half of ADX and SHAM animals were tube-fed a purified, liquid diet containing either two ppm of zinc(zinc-deficient, force-fed ; ZDF) or 50ppm(zinc-replete, force-fed ; ZRF) for 19 days. They received identical amounts of diet based on the intake of ad libitum-fed, zinc-replete rats. Although they received identical amounts of food, ZDF rats grew at a slower rate compared to ZRF rats in both SHAM and ADX rats. Regardless of surgery, force-feeding rats the zinc-deficient diet resulted in a substantial decrease in serum zinc levels. The weights of the thymus, lymph node, and spleen were lower in SHAM-ZDF rats compared to SHAM-ZRF rats. Marginal zinc deficiency caused lymphopenia in SHAM animals. However, these differences in lymphoid tissues and cells between SHAM-ZDF and SHAM-ZRF rats disappeared in ADX rats. These results indicate that the impaired growth of lymphoid tissues observed in zinc-deficient, sham-operated animals can be attributed to elevated serum corticosterone levels under the conditions of our experiments.

KEY WORDS : pure zinc deficiency · adrenal cortex · corticosterone · lymphocyte.

Introduction

In 1970, two hereditary zinc-deficient states, the lethal trait A-46 in Friesian cattle[1] and acrodermatitis enteropathica in human[2-4], drew the attention of immunologists because of the associated immunodeficiency with these diseases. Severe immunodeficiency symptoms were also observed in patient suffering from zinc deficiency due to inadequate zinc supplementation as part of total parenteral nutrition[5-7]. Involution of the thymus and other lymphoid tissues has been found in many species of zinc-deficient animals when zinc deficiency is produced in the laboratory[8-11].

Most investigators have evaluated the effect of zinc on immune response by studying animals fed a zinc-deficient diet ad libitum for several weeks. However, reduced food intake, which is the earliest and most striking effect of zinc defi-

Accepted : November 8, 1994

ciency[12)13)], is also known to impair the immune system[14-17)]. Thus, many of the immune dysfunctions observed in the previous studies may have been due to decreased dietary intake or the combined effects of protein-calorie malnutrition and zinc deficiency. Therefore, it is important to determine if pure zinc deficiency without reduced food intake produces altered immune function.

Zinc deficiency may produce stress resulting in activation of the hypothalamus-pituitary-adrenal cortical axis[18)]. This ultimately would result in stimulation of the adrenal cortex and a rise in serum glucocorticoid. The injurious effects of the glucocorticoid on thymocyte are well known [19)20)] and might account in part for the rapid atrophy of the thymus observed in zinc deficiency. Mice fed a zinc-deficient diet ad libitum were reported to have increased levels of plasma corticosterone[21)22)]. Children with protein-calorie malnutrition are also known to have elevated levels of plasma cortisol[23)]. Thus, relationships between zinc deficiency and the adrenal cortex and their effects on the immune system need to be investigated.

The present study was performed to determine 1) if pure zinc deficiency in the absence of reduced food intake causes alterations in lymphohematopoietic tissues and 2) the role of corticosterone in lymphohematopoietic tissues during pure zinc deficiency.

Methods

1. Animals and Diets

Weanling, male, Fischer 344 rats were housed individually in hanging stainless steel cages under a fixed light-dark cycle(light being on from 0800 to 2300 hours). They were given an unrefined diet (Rodent Laboratory Chow, Ralston Purina Co., St. Louis, MO, USA) and tap water ad libitum for three days to allow acclimation to laboratory conditions. After acclimation, the rats were randomly divided into two groups. One group was bilaterally adrenalectomized(ADX) via a dorsal incision, and the other group was sham operated (SHAM). The sham-operated rats had free access to deionized water and the adrenalectomized rats were provided with 0.9% NaCl in deionized water for drinking. Corticosterone was administered by subcutaneous injection to the adrenalectomized animals three times per day(1mg/kg of body weight for each injection). Corticosterone was dissolved in ethanol and then physiological saline was added to make a concentration of 1mg/ml(final ethanol concentration : 10%). The sham operated animals received vehicle only. Four days after surgery, all animals became accustomed to an intra gastric tube-feeding procedure by receiving increasing amounts of liquid diet three times a day for three days. The liquid diet was a purified diet(Tecklad, Harland Sprague-Dawley, Inc., Madison, WI, USA) containing 50 ppm of zinc blended with deionized water[24)]. Then each group was further divided into two groups. One half of the adrenalectomized and sham-operated rats(10 rats/group) were force fed the liquid diet containing 50ppm of zinc(zinc-replete, force-fed(ZRF)) and the other half(10 rats/group) received a similar diet containing 2ppm of zinc(zinc-deficient, force-fed(ZDF)). The zinc-deficient diet containing 2ppm of zinc was also purchased from Teklad. An additional 8 animals were included to determine the food intake of force-fed rats.

They were given a powdered control diet containing 50ppm of zinc ad libitum and their food intake was monitored. This group is included only to determine food intake of the ZRF and ZDF groups. The feeding procedures and food intakes of animals were essentially the same as those published previously[24)]. After 19 days of feeding the

assigned diets, the following studies were performed.

2. The Concentration of Serum Corticosterone

A number of precautions were taken to minimize elevation of corticosterone before and during the blood sampling. Blood was collected at 0900 hours from the orbital plexus of each rat under ether anesthesia within one minute of initial cage disturbance. Entrance to the animal room was not allowed for a three-hour period prior to the bleeding in order to maintain basal levels of corticosterone. The concentration of serum corticosterone was determined by using a radioimmunoassay kit(Radioassay system Laboratories, Inc., Carson, CA, USA).

3. Zinc Concentration in the Thymus, Spleen, Lymph Nodes, and Serum

Serum was diluted with deionized water and the concentration of zinc was determined by atomic absorption spectrophotometry[25]. After the blood samples were collected, the rats were killed by cervical dislocation. The thymus, spleen and cervical and mesenteric lymph nodes were removed, weighed, wet ashed with concentrated nitric acid and vacuum evaporated to dryness.

The resulting mixture was reconstituted with 5% nitric acid and zinc concentration was determined by atomic absorption spectrometry.

4. Hematologic Studies

Hematocrit values were determined by using a microprocedure. Total leucocyte counts were made using a Coulter counter after treating heparinized blood samples with Zap-O globin. Differential white cell counts were estimated from blood smears prepared with Wright-Giemsa stain.

Results

Effects of dietary zinc deficiency and adrenalectomy on body weights and tissue weights are shown in Table 1. Adrenalectomy did not influence the growth of animals. Zinc-deficient, force-fed animals were smaller than zinc-replete, force-fed animals whether the animals were adrenalectomized or sham operated. Adrenal weights were higher in SHAM-ZDF rats than those of SHAM-ZRF rats. Corticosterone levels in the serum were also significantly higher in SHAM-ZDF rats(329 ± 44ng/ml) compared to SHAM-ZRF rats(175± 29ng/ml). Blood levels of the hormone were very low in adrenalectomized rats(ADX-ZDF, 8± 1 ng/ml and ADX-ZRF, 10± 2ng/ml) because the blood samples for the hormone assay were taken

Table 1. Effects of pure zinc deficiency on body weights and organ weights in rats

Groups (n)	SHAM-ZDF (10)	SHAM-ZRF (10)	ADX-ZDF (10)	ADX-ZRF (10)
Body wt(g)	150 ± 2[a]	165 ± 1[b]	146 ± 4[a]	168 ± 2[b]
Liver wt(g/100g body wt)	3.93± 0.06[a]	3.75± 0.07[a]	3.25± 0.07[b]	3.02± 0.04[b]
Thymus wt(mg/100g body wt)	177 ± 5[a]	222 ± 8[b]	242 ± 5[c]	254 ± 8[c]
Lymph node wt(mg/100g body wt)	29 ± 1.5[a]	33 ± 3.1[ab]	38 ± 2.6[b]	38 ± 3.1[b]
Spleen wt(mg/100g Body wt)	180 ± 5[a]	226 ± 1[b]	230 ± 5[b]	233 ± 5[b]
Adrenal wt(mg/100g body wt)	37 ± 1.6[a]	24 ± 0.3[b]		

Values are means± SEM ; n, number of rats. SHAM-ZDF ; sham-operated, zinc-deficient, force-fed : SHAM-ZRF ; sham-operated, zinc-replete, force-fed : ADX-ZDF ; adrenalectomized, zinc-deficient, force-fed : ADX-ZRF ; adrenalectomized, zinc-replete, force-fed.

A corticosterone replacement therapy was given to the ADX rats as described in Method.

Values with the same letter within rows are not statistically different($p>0.05$) among different groups.

8 hours after the last injection of the hormone. Relative liver weights, expressed as g per 100g of body weight, were lower in the adrenalectomized groups compared to sham-operated groups. There were no statistically significant differences in relative liver weights between zinc-replete and zinc-deficient groups. Force-feeding rats the zinc-deficient diet for 19 days resulted in lower thymus, lymph node, and spleen weights in sham-operated animals. However, the difference due to dietary zinc disappeared in adrenalectomized animals.

Zinc concentrations in the serum and tissues are shown in Table 2. Regardless of surgery, feeding rats the zinc-deficient diet for 19 days resulted in a substantial decrease in serum zinc levels. Relative to SHAM-ZRF, animals in the SHAM-ZDF group had lower zinc contents in the liver. There were no differences in zinc contents in lymph nodes, thymus, and spleen among four dietary groups.

Effects of pure zinc deficiency on hematological values are shown in Table 3. There was a significant elevation in hematocrit values in zinc-deficient groups compared to zinc-replete groups, regardless of surgery. Hematocrit values were higher in adrenalectomized rats compared to sham-operated rats. A similar trend was seen in hemoglobin values, but the differences were not statistically

Table 2. Zinc concentration in serum and tissues from rats fed different amounts of zinc

Groups	SHAM-ZDF	SHAM-ZRF	ADX-ZDF	ADX-ZRF
(n)	(10)	(10)	(10)	(10)
Serum(μg/100ml)	38± 3[a]	130± 5[b]	40± 2[a]	131± 3[b]
Liver(μg/g)	31± 1[a]	42± 1[b]	ND	ND
Lymph node(μg/g)	23± 2[a]	20± 1[a]	24± 2[a]	20± 2[a]
Thymus(μg/g)	20± 0.8[a]	20± 0.5[a]	19± 0.5[a]	19± 0.5[a]
Spleen(μg/g)	22± 0.4[a]	21± 0.4[a]	19± 0.7[a]	23± 1.8[a]

Values are means± SEM ; n, number of rats. SHAM-ZDF ; sham-operated, zinc-deficient, force-fed : SHAM-ZRF ; sham-operated, zinc-replete, force-fed : ADX-ZDF ; adrenalectomized, zinc-deficient, force-fed : ADX-ZRF ; adrenalectomized, zinc-replete, force-fed.

A corticosterone replacement therapy was given to the ADX rats.

Values with the same letter within rows are not statistically different($p>0.05$) among different groups.

ND, not determined.

Table 3. Effects of pure zinc deficiency on hematological parameters in rats

Groups	SHAM-ZDF	SHAM-ZRF	ADX-ZDF	ADX-ZRF
(n)	(10)	(10)	(10)	(10)
Hematocrit(%)	37 ± 0.8[b]	34 ± 0.6[a]	49 ± 0.9[d]	46 ± 0.8[c]
Hemoglobin(g/100ml)	12.5 ± 0.37[a]	11.8 ± 0.21[a]	13.0 ± 0.30[a]	12.7 ± 0.19[a]
RBC($\times 10^9$/ml)	3.88± 0.08[a]	4.13± 0.09[a]	5.27± 0.15[b]	5.32± 0.06[b]
WBC($\times 10^6$/ml)	6.73± 0.58[a]	7.20± 0.36[a]	9.27± 0.32[b]	8.24± 0.36[b]
Lymphocytes(%)	65 ± 3[a]	74 ± 3[b]	76 ± 3[b]	78 ± 5[b]
Granulocyte(%)	30 ± 2[a]	22 ± 3[b]	20 ± 2[b]	19 ± 2[b]
Monocytes(%)	5 ± 0.6[a]	54 ± 1.0[a]	4 ± 0.5[a]	3 ± 0.3[a]

Values are means± SEM ; n, number of rats. SHAM-ZDF ; sham-operated, zinc-deficient, force-fed : SHAM-ZRF ; sham-operated, zinc-replete, force-fed : ADX-ZDF ; adrenalectomized, zinc-deficient, force-fed : ADX-ZRF ; adrenalectomized, zinc-replete, force-fed.

A corticosterone replacement therapy was given to the ADX rats.

Values with the same letter within rows are not statistically different($p>0.05$) among different groups.

significant. RBC and WBC concentrations were also increased in the adrenalectomized group compared to sham-operated groups, but dietary zinc did not influence these parameters. Marginal zinc deficiency resulted in lymphopenia in the sham-operated groups, but not in the adrenalectomized groups.

Discussion

The immune response to zinc-deficiency has been evaluated in a variety of animal models[8-11]. However, most of these zinc-deficient animals were also protein-calorie malnourished because the decrease in food intake is the most prominent feature of zinc deficiency[12][13].

Among the many adverse effects of protein-energy malnutrition, the most prominent one is the frequent occurrence of infection and a significant atrophy in lymphoid tissues[14]. The experiment described herein is the first one that shows that zinc-deficiency per ce without protein-calorie malnutrition causes an atrophy in the thymus, spleen and lymph node and lymphopenia.

The mechanisms by which zinc deficiency causes immunomodulation are not clear. We hypothesized that zinc deficiency constitutes a stress that results in the activation of the hypothalamus-pituitary-adrenal cortex axis. This would result in an increase in serum levels of glucocorticoid. Indeed, corticosterone concentrations are elevated in our SHAM-ZDF rats. To assess the role of glucocorticoids in zinc deficiency, we utilized adrenalectomized rats injected with corticosterone. Similar to SHAM-ZDF rats, ADX-ZDF rats did not grow as well as ZRF rats. Serum zinc levels in ADX-ZDF rats were markedly lower than ADX-ZRF rats indicating that they are zinc-deficient as SHAM-ZDF rats. However, by contrast to SHAM animals, there was no decrease in the weight of thymus, lymph node, and spleen of ADX-ZDF rats. These results indicate that the atrophy of the lymphoid tissue and lymphopenia observed during zinc deficiency are due to an increased concentration of adrenal hormones. These results do not agree with results by DePasquale-Jardieu and Fraker[21] who showed that their adrenalectomized, zinc-deficient mice lost T cell function. However, their zinc-deficient mice were fed ad libitum and thereby undernourished, which may explain the observed decreased immune response. Of course we cannot prove if our ADX-ZDF rats did not lost any T cell functions. However, it is unlikely that these animals lost T cell functions without the atrophy of the lymphoid tissue or lymphopenia.

ADX rats had high values of hematocrit, RBC, WBC compared to SHAM animals. Their thymus, lymph nodes, and spleen were heavier than those of SHAM animals. These results suggest that ADX rats did not receive enough corticosterone. The ADX rats in the present experiments received corticosteron at the dose of 1mg/kg body weight every eight hours. Blood levels of the hormone in ADX were very low at 8 hours after the last injection of the hormone. The selection of an appropriate dose of corticosterone was difficult because corticosterone has a very short half life[26] and we were afraid that corticosterone levels in the blood might reach high after each injection. In the present experiment we tried to maintain the minimum levels of corticosterone to withstand the stress of force-feeding. ADX rats grew at a similar rate to SHAM animals. Serum zinc concentrations that are sensitive to glucocorticoid levels [27] did not differ between ADX and SHAM rats indicating that the adrenalectomy and corticosterone replacement therapy did not alter the zinc metabolism in our adrenalectomized rats.

There was no difference in zinc concentration

in the thymus, spleen, and lymph nodes between SHAM-ZRF and SHAM-ZDF rats. It appears that the growth of lymphoid tissues stops when serum zinc concentrations are too low to maintain normal tissue zinc levels. Zinc supports functions of numerous metalloenzymes including those synthesize DNA, RNA, and protein. In contrast the lymphoid tissues there was a decrease in the zinc concentration of the liver in SHAM-ZDF rats. In the liver, there may have been an increase in the concentration of lipid and glycogen which are not normally associated with zinc in the tissue. Our previous results[25] have shown that glycogen and lipid levels were significantly elevated in zinc-deficient, force-fed rats.

In summary, these results indicate that zinc-deficiency in the absence of protein calorie malnutrition results in impaired growth of lymphoid tissues and cells. Under the conditions of our experiments, the impaired growth of lymphoid tissues observed in zinc-deficient animals can be attributed to elevated serum corticosterone levels.

■ Acknowledgement

Special thanks are given to Dr. D.A. Crouse for his valuable guidance throughout the study.

Literature cited

1) Brummerstedt E, Basse A, Flagstad T, Andresen E. Animal model of human disease. *Am J Pathol* 87 : 725-728, 1977
2) Julius R, Schulkind M, Sprinkle T, Rennert O. Acrodermatitis enteropathica with immune deficiency. *J Pediatr* 83 : 1007-1011, 1973
3) Oleske JM, Westphal ML, Shore S, Gorden D, Bogden JD, Nahmias A. Zinc therapy of depressed cellular immunity in acrodermatitis enteropathica. *Am J Dis Child* 133 : 915-918, 1979
4) Rodin AE, Goldman AS : Autopsy findings in acrodermatitis enteropathica. *Am J Clin Pathol* 51 : 315-321, 1969
5) Allen JI, Kay NE, McClain CJ. Severe zinc deficiency in humans : Association with a reversible T-lymphocyte dysfunction. *Ann Intern Med* 95 : 154-157, 1981
6) Srouji MN, Balistrerei WF, Caleb MH, South MA, Starr S. Zinc deficiency during parenteral nutrition : Skin manifestations and immune incompetence in a premature infant. *J Pediatr Surg* 13 : 570-575, 1978
7) Pakarek RS, Sandstead HH, Jacob RA, Barcome DF. Abnormal cellular immune responses during acquired zinc deficiency. *Am J Clin Nutr* 32 : 1466-1471, 1979
8) Fraker PJ, DePasquale-Jardieu P, Zwickl CM, Luecke RW. Regeneration of T-cell helper function in zinc-deficient adult mice. *Proc Natl Acad Sci USA* 75 : 5660- 5664, 1978
9) Golub MS, Gershwin ME, Hurley LS, Baly DL, Hendrickx AG. Studies of marginal zinc deprivation in rhesus monkeys. I. Influence on pregnant dams. *Am J Clin Nutr* 39 : 265-280, 1984
10) Macapinlac MP, Pearson WN, Darby WJ. Some characteristics of zinc deficiency in the albino rats. In : Zinc Metabolism, edited by AS Prasad. Springfield, C.C. Thomas, 1966, pp142-166
11) Keen CL, Gershwin ME. Zinc deficiency and immune function. *Annu Rev Nutr* 10 : 415-431, 1990
12) Chester JK, Quarterman J. Effects of zinc deficiency on appetite and plasma amino acid concentrations in the rat. *Br J Nutr* 24 : 1061-1069, 1970
13) Wallwork JC, Fosmire GJ, Sanstead HH. Effects of zinc deficiency on appetite and plasma amino acid concentrations in the rat. *Br J Nutr* 45 : 127-136, 1981
14) Chandra RK. Protein-energy malnutrition and immunological responses. *J Nutr* 122 : 597-600, 1992
15) Corman LC. The relationship between nutrition, infection, and immunity. *Med Clin N Am* 69 : 519-529, 1985
16) Beisel WR. History of nutritional immunology : Introduction and overview. *J Nutr* 122 : 591-596, 1992
17) Chandra RK. Nutrition and immunoregulation. Significance for host resistance to tumors and infec-

tious diseases in human and rodents. *J Nutr* 122 : 754-757, 1992

18) Quarterman J, Humphries WR. Effect of zinc deficiency and zinc supplementation on adrenals, plasma steroids and thymus in rats. *Life Sci* 24 : 177-184, 1979

19) Bach JF, Duval D, Dardenne M, Salomon JC, Tursz T, Fournier C. The effects of steroids on T cells. *Transplant Proc* 7 : 25-30, 1975

20) Claman HN. Corticosteroids and lymphoid cells. *N Engl J Med* 287 : 388-397, 1972

21) DePasquale-Jardieu P, Fraker PJ. Further characterization of the role of corticosterone in the loss of humoral immunity in zinc-deficient A/J mice as determined by adrenalectomy. *J Immunol* 124 : 2650-2655, 1980

22) DePasquale-Jardieu P, Fraker PJ. The role of corticosterone in the loss in immune function in the zinc-deficient A/J mice. *J Nutr* 109 : 1847-1855, 1979

23) Schonland MM, Shandlely BC, Loening WEK, Parent MA, Coovadia HM. Plasma cortisol and immunosuppression in protein-calorie malnutrition. *Lancet* 2 : 435-436, 1972

24) Park JHY, Grandjean CJ, Hart MH, Erdman SH, Pour P, Vanderhoof JA. Effect of pure zinc deficiency on glucose tolerance and insulin and glucagon levels. *Am J Physiol* 251(Endocrinol Metab 14) : E 273-E278, 1986

25) Park JHY, Grandjean CJ, Antonson DL, Vanderhoof JA. Effects of isolated zinc deficiency on the composition of skeletal muscle, liver, and bone during growth in rats. *J Nutr* 116 : 610-617, 1986

26) Schapiro S, Percin CJ, Kotichas FJ. Half life of plasma corticosterone during development. *Endocrinology* 89 : 284-286, 1971

27) Flynn A, Pories WJ. Strain WH, Hill OA Jr, Fratianne RB. Rapid serum-zinc depletion associated with corticosteroid therapy. *Lancet ii* 1169-1171, 1971

Pure zinc deficiency and lymphopenia

= 국문초록 =

순수 아연 결핍증에 의한 림프구감소증 : 코르티코스테론의 역할

윤 정 한 · 이 상 선*
한림대학교 자연과학대학 식품영양학과
한양대학교 가정대학 식품영양학과*

아연결핍에서 나타나는 식이섭취량 감소에 따른 영향을 배제하기 위해 쥐에게 위장경관을 통해 강제급식을 하면서 순수한 아연 결핍이 면역기능에 미치는 영향을 조사하였다. Fischer 344종의 수컷 쥐를 부신절제 수술을 한 군(ADX)과 부신은 절제하지 않고 수술과정을 똑같이 겪은 대조군(SHAM)으로 나누어 ADX군에게 0.9% 생리식염수를 물 대신 공급하고 코르티코스테론을 체중 kg당 1mg이 되도록 하루에 3번 주사하였다. 수술에서 회복된후 ADX군과 SHAM군을 각각 둘로 나누어 아연 함량이 2ppm인 유동식과 (아연 결핍, 강제급식 ; ZDF), 50ppm인 유동식을 (아연 보충, 강제급식 ; ZRF) 각각 위장관 튜브를 통해 19일간 강제급식하였다. 동물의 식이공급량은 아연이 보충된 식이를 자유급식시킨 대조군의 섭취량을 측정하여 조절하였다. 같은 량의 식이를 공급 받았지만 아연 결핍군이(ZDF) 아연 보충군(ZRF)에 비해 성장이 저하되었고 혈청 아연수준이 현저하게 낮아졌다. SHAM－ZDF 동물에서 혈청 코르티코스테론 수준이 상승되었다. 흉선, 림프절, 비장의 무게는 SHAM－ZDF군이 SHAM－ZRF군에 비해 낮게 나타났다. 아연 결핍이 SHAM군에서는 림프구감소증(*lymphopenia*)을 유발했으나 ADX군에서는 이러한 현상을 볼 수 없었다. 그러므로 본 연구에서 나타난 SHAM－ZDF군의 림프조직의 손상은 혈청 코르티코스테론의 상승에 기인한 것으로 생각된다.