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Inhibition of bone marrow immature B lymphocytes from zinc deficient mice by methionine enkephalin[1]

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ABSTRACT Bone marrow cells (BMC) from Zn deficient mice were used to measure the anti-TNP plaque forming cell (PFC) response in the presence of methionine enkephalin (Met-Enk). The results demonstrated that the PFC response of BMC was suppressed by Met-Enk. In the case of Zn deficient group, the inhibitions were up to 51% compared to 27% for the control and became higher as the increasing concentration of Met-Enk. It seems that the sensitivity to Met-Enk was related to degree of Zn deficiency. These findings suggest that Zn may interfere with the role of Met-Enk on BMC.

KEY WORDS zinc; methionine enkephalin; bone marrow; B-lymphocytes; plaque assay; lipopolysaccharides

Opioid peptides as the neuroimmunomodulators play a role on the regulation of the immune system[1-3]. Evidences accumulated recently also indicate that the enkephalin system is activated and released with other hormones from central and peripheral sites in exposure to stress. Stress can alter the susceptibility of animals to a wide variety of diseases by different mechanisms including the alteration of immune functions. Since Zn deficiency may influence the function of the immune system in human and animals, it is reasonable to think that there may exist some relationship between methionine enkephalin (Met-Enk) and Zn in immune system[1].

Although some works have been done to evaluate the effect of Met-Enk on antibody reaction of spleen cells, little attention has been paid to that in bone marrow cells (BMC). Bone marrow contain immature B lymphocytes that can respond to antigen. It is interesting to investigate the susceptibility of BMC to Met-Enk when they are exposed to the challenge of antigen in the status of Zn deficiency.

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MATERIALS AND METHODS

Mice and Diets A/J mice ♀, 3-wk-old, were randomly divided into two dietary groups and fed with a synthetic, biotin-fortified egg white diet containing adequate (26 $\mu g \cdot g^{-1}$) or deficient (0.1 $\mu g \cdot g^{-1}$) levels of zinc carbonate. Diet consumption was measured daily and mice were weighed at least once a week[1].

Agents Met-Enk purchased from Sigma was dissolved in 1 $mg \cdot ml^{-1}$ phosphate buffer saline (PBS) solution (pH 7.2) and diluted to corresponding concentrations in RPMI 1640 medium before use. Trinitrophenylated lipopolysaccharide (TNP-LPS) was prepared by conjugating trichloroacetic acid extracted LPS from *E coli* 055 : B5 (Difco, USA) to *2,4,6*-trinitrobenzene sulfonate (Sigma, USA). After extensive dialysis against sterile PBS, TNP-LPS samples were sonicated, filtered, sterilized and stored away from the light at 4℃[4].

Collection of BMC BMC were obtained with Medina's method[4]. BMC were resuspended at a concentration of 5×10^5 cells / ml in RPMI 1640 medium supplemented with 0.5% of normal mouse serum and 0.5% of delipidated bovine serum albumin (BSA).

Culture of BMC The cells were cultured in 24 well-plate at a density of 5×10^5 cells / ml and TNP-LPS was added to triplicate wells at concentrations of 0.01 or 0.1 $\mu g \cdot ml^{-1}$ and Met-Enk of 0.2, 0.5, or 1

μmol · L^{-1} separately. The cells were incubated for 5 d at 37℃ in a humidified atmosphere of CO_2 10% + O_2 7% + N_2 83%.

Plaque forming cell assay for BMC After 5 d of incubation, the total number of direct anti-TNP plaque forming cell (PFC) per well was determined using a modification of the Jerne plaque assay and the data were presented as PFC per 10^7 nucleated BMC[5].

Statistical analysis All the date ware expressed as $\bar{x} \pm s$. P values were determined by t test.

RESULTS

Food consumption and body weights of the two dietary groups After a 26-d feeding period, the mice fed Zn adequate food averaged 20.9 g, whereas those fed Zn deficient diet, as a result of Zn deficiency, averaged 14.4 g and weighed 69% as much as the control group. The average food consumption by Zn deficient mice was 81% of that of Zn adequate mice (Tab 1). The values of the body weights from the two groups were different significantly ($P<0.01$).

Tab 1. Effect of dietary Zn on body weight and food consumption of mice. $n=10$. $\bar{x} \pm s$. $^{***}P<0.01$ *vs* **Zn adequate group.**

Dietary groups	Body weight, g d 0	d 26	Food Consumed, g / mouse
−zinc	17.4 ± 0.06	14.4 ± 0.2***	81.2 ± 4.5***
+zinc	17.3 ± 0.06	20.9 ± 0.2	99.8 ± 4.8

To avoid the influence of reduced diet intake of mice by Zn deficiency, an analysis of covariance for determining the effect of Zn deficient food on losing body weight was undertaken. The result showed that losing body weights of mice were related to Zn deficiency rather than reduced diet intake ($F=68.2$, $P<0.01$).

Effects of Zn deficiency on response of BMC to Met-Enk A higher number of PFC was obtained at TNP-LPS 0.01 μg · ml^{-1} than that at TNP-LPS 0.1 μg · ml^{-1} (Fig 1). BMC from Zn adequate mice gave more PFC than those from Zn deficient mice. On the other hand, the number of anti-TNP PFC of either Zn deficient or Zn adequate groups was reduced as the concentration of Met-Enk was increased in a range of 0.25-1 μmol · L^{-1} (Fig 1). In the case of Zn adequate mice, the reductions were from 8.8% to 28% and 27.2% to 51.8% in the presence of different concentrations of TNP-LPS, whereas in the case of Zn deficient mice, the reductions were from 31.3% to 50.0% and 51.1% to 68.1%. The results suggest that Met-Enk inhibited the PFC responses of both groups and that more suppressed effects of Met-Enk was observed in the Zn deficient group.

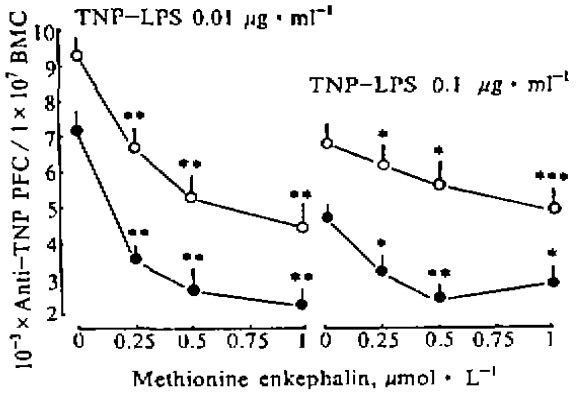

Fig 1. Effects of Met-Enk on anti-TNP PFC responses obtained by incubation of BMC from Zn-deficient (●) and control (○) A / J mice with TNP-LPS and Met-Enk. $n=3$-4. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.**

In order to determine the inhibitory effect of Met-Enk on different degrees of Zn deficiency, the Zn deficient mice were divided into 2 groups, ie, moderate deficiency and severe deficiency which weighed 73% and 63% as much as the body weight of the control group respectively. Met-Enk inhibited the PFC response of all groups, but more

inhibitory effects were observed with the increasing degree of Zn deficiency (Fig 2). The percentages of inhibition in the moderate deficient and severe deficient groups were 60.7% and 71.1% *vs* 41.7% of the control when Met-Enk 0.2 μmol · L^{-1} was used and 69.4% and 84.6% *vs* 56.7% of the control when Met-Enk 1 μmol · L^{-1} was used.

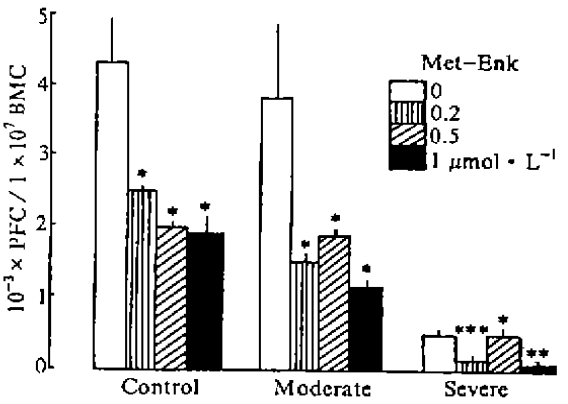

Fig 2. Effects of Met-Enk on anti-TNP PFC responses obtained by incubation of BMC from severe and moderate Zn deficient A / J mice with TNP-LPS and Met-Enk. $n=3$–4. $\bar{x} \pm s$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

DISCUSSION

In this experiment, all mice fed with Zn-deficient food lost weight from the second week. Previous studies have shown that the body weight lost was due to Zn deficiency that could led to stress in the body. Zn malnutrition is simultaneously accompanied with the impairment of immune capacity in the body[6-7].

It has been proved that Met-Enk can be released from adrenal medulla into peripheral blood and influences the function of immune system in exposure to stress[8]. From our data, the effects of Met-Enk on BMC are readily discerned and the results are consistent with that obtained from spleen cells[9]. It is especially interesting in light of the fact that BMC from the Zn deficient group was more sensitive to Met-Enk than that from Zn adequate group. Although the number of PFC was different in the two concentrations of TNP-LPS, BMC from Zn deficient mice always got a higher percentage of inhibition under every concentration of Met-Enk.

Previous studies *in vitro* have shown that some cells possess receptors for opioid peptides[10]. A high-affinity binding site for Met-Enk has been reported to be present on mouse spleen cell membranes that was similar to those found in the brain[9]. Our data suggest that Met-Enk receptors are also present on mouse BMC and seem to be related to Zn status in body.

Some interactions between Zn and Met-Enk have been found in the studies of brain[11]. Zn and enkephalin-containing opioid peptides are coexistent in the brain. Zn ions can block met-enkephalinamide binding to rat brain membranes[12]. Zn has a high affinity for thiol groups and alteration of the SH-groups in opioid receptors suffice to block opioid binding[13]. In our experiment, Zn deficiency might influence the behaviors of opioid receptors.

In addition, a major enkephalin-degrading enzyme, aminopeptidase, is reported to be Zn metalloenzyme[14]. Zn also has been considered to complex *in vitro* with Met-Enk. The interaction may interfere with the binding of Met-Enk to receptors. It is speculative that the binding might be increased when Zn was deprived from the body.

REFERENCES

1 Li G, Fraker PJ. Methionine-enkephalin alteration of mitogenic and mixed lymphocyte culture responses in zinc-deficient mice. *Acta Pharmacol Sin* 1989; **10** : 216

2 Blalock JE, Smith EM. A complete regulatory loop between the immune and neuroendocrine systems. *Fed Proc* 1985; **44** : 108

3 Wybran J. Enkephalins and endorphins as

modifiers of the immune system: present and future. *Fed Proc* 1985; **44** : 92
4 Medina CA, Li G, Fraker PJ. Improved plaque production for shortterm bone marrow and fetal liver cultures. *J Immunol Methods* 1988; **111** : 233
5 Li G, Fraker PJ. Effect of dexamethasone of bone marrow cells from zinc deficient mice. *J Xi'an med Univ* 1989; **1** : 112
6 Prasad AS. *Clinical biochemical and nutritional aspects of trace elements.* NY: : Alan R. Liss, 1982 : 64
7 DePasquale-Jardieu P, Fraker PJ. Interference in the development of a secondary immune response in mice by zinc deprivation: persistence of effects *J Nutr* 1984; **114** : 1762
8 Hanbauer I, Kelly GO, Saiani L, Yang HYT. [Met^5]-enkephalin-like peptides of the adrenal medulla: release by nerve stimulation and functional implication. *Peptides* 1982; **3** : 469
9 Johnson HM, Smith EM, Torres BA, Blalock JE. Regulation of the *in vitro* antibody response by neuroendocrine hormones. *Proc Natl Acad Sci USA* 1982; **79** : 4171
10 Brown SL, Tokuda S, Saland LC, Van Epps DE. Opioid peptide effects on leukocytes migration. In: Plotnikoff NP, Faith RE, Murgo AJ, Good RA, eds. *Enkephalins and endorphins: stress and the immune system.* New York: Plenum Press, 1986 : 367-86
11 McGinty JF, Henriksen SJ, Chavkin C. Is there an interaction between zinc and opioid peptides in hippocampal neurons? In: Frederickson LJ, Howell GA, Kassarskis EJ, eds. *The neurobiology of zinc.* NY: Alan R. Liss, 1984 : 73-89
12 Stengaard-Pedersen K. Inhibition of enkephalin binding to opiate receptors by zinc ions: possible physiological importance in the brain. *Acta Pharmacol Toxical* 1982; **50** : 213
13 Simon EJ, Groth J. Kinetics of opiate receptor inactivation by sulfhydryl reagents: evidence for conformational change in presence of sodium ions. *Proc Natl Acad Sci USA* 1975; **72** : 2404
14 Barclay RK, Philips MA. Inhibition of enkephalin- degrading aminopeptidase activity by certain peptides. *Biochem Biophys Res Commun* 1980; **96** : 1732

甲硫氨酸脑啡肽对缺锌小鼠骨髓未成熟 B 淋巴细胞的抑制作用

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提要 本试验测定缺锌小鼠骨髓细胞(BMC)在甲硫氨酸脑啡肽(Met-Enk)存在下的抗 TNP 空斑形成细胞(PFC)反应. 结果显示 Met-Enk 能抑制 BMC 的 PFC 反应. 对缺锌组的抑制可达 51%，而对照组为 27%，抑制程度随 Met-Enk 浓度增高而加大. 这种对 Met-Enk 的敏感性似乎与锌缺乏程度有关. 上述发现提示锌可以干扰 Met-Enk 在 BMC 上的作用.

关键词 锌; 甲硫氨酸脑啡肽; 骨髓; B-淋巴细胞; 空斑测定; 脂多糖