ELSEVIER

Nutrition 23 (2007) 157–163

NUTRITION

www.elsevier.com/locate/nut

Basic nutritional investigation

T-helper type 1 cytokine release is enhanced by in vitro zinc supplementation due to increased natural killer cells

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Manuscript received February 3, 2006; accepted October 24, 2006.

Abstract

Objective: We examined the influence of zinc on T-helper type 1 (Th1)/T-helper type 2 (Th2) balance in human lymphocytes.

Methods: Human peripheral blood mononuclear cells or diluted whole blood were cultured for 8 d in the presence of zinc (30 or 60 μM) or 1 μM of N, N, N', N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) (a zinc-specific chelator). Phytohemagglutinin-induced cytokine release was measured by enzyme-linked immunosorbent assay, and expression of CD56/CD69, CCR4/CD3, and CCR5/CD3 and intracellular labile zinc were detected by flow cytometry.

Results: We found that our in vitro supplementation resulted in an increase of intracellular labile zinc comparable to that of a 7-wk administration of 10 mg of zinc per day in vivo. Zinc triggered interferon-γ release and impaired interleukin-10 release. Phenotypically, a Th2/Th1 shift could not be confirmed after detecting the Th1-specific chemokine receptor CCR5 or CCR4 for Th2 cells. Surprisingly, we detected a larger amount of $CD56^+$ cells after zinc stimulation, leading us to the conclusion that the amount of interferon-γ release after zinc supplementation might be attributed to the upregulation of natural killer cells after in vitro zinc supplementation rather than to a Th2/Th1 shift.

Conclusion: We suggest that a nutritional intake of 10 mg of zinc increases the quantity of interferon-γ–producing natural killer cells and strengthens the immune system against neoplasms and viral infections.

Keywords: Zinc; T-helper 1; T-helper 2; Cytokines; Natural killer cells; Trace elements

Introduction

Zinc (Zn) is an important trace element and plays a significant role in initiating and maintaining the immune response [1–6]. It is essential for proper functioning of >300 enzymes [7] and is involved in apoptosis [8], signal transduction [9], cell growth and proliferation, and DNA synthesis [10–12]. In particular, Zn deficiency affects highly proliferating tissues such as hair, skin, and cells of the immune system.

Although the human body contains a total of 2–3 g of Zn, the physiologic serum Zn level ranges from 12 to 16 μM [13]. Most of the body Zn can be detected intracellular or bound to albumin, α2-macroglobulin, and transferrin [14]. The human body has no specific storage pool for Zn as does, e.g., iron; therefore a regular intake of Zn is crucial [15]. The severe effects of Zn shortage were first observed by Prasad et al. [3] in 1963 in a group of Egyptian adolescent boys with the malabsorption syndrome acrodermatitis enteropathica, a disease accompanied with thymic atrophy, impaired leukocyte function, growth retardation, loss of hair, skin problems, and recurrent infections. This disease can be cured with Zn supplementation. Zn homeostasis is known for its efficiency in the body. Large ingestion of Zn compounds normally do not cause long-term toxic side effects [16]. Inhalation of atomic Zn causes Zn fume fever, and the symptoms are completely reversible,

The work was supported in part by the EU Project ZINCAGE (Food-CT-2003-506850).

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0899-9007/07/$ – see front matter
doi:10.1016/j.nut.2006.10.007

but this should not be compared with effects mediated by Zn ions [17].

Zn deficiency can be attributed to inherited diseases, caused by malabsorption, increased need, or excretion [18]. A suboptimal diet is associated with impaired immune function, and lack of Zn has a negative effect on the host defense system [19]. Zn deficiency leads to thymic atrophy and lymphopenia, which weakens an adequate immune response [20]. It plays a critical role in lymphocyte proliferation, T-cell functions, antibody production and natural killer (NK) cell activity, and chemotactic functions of monocytes and neutrophils [21].

Studies from Prasad [22] clearly show that Zn deficiency reduces the T-helper type 1 (Th1) cell cytokines interferon-γ (IFN-γ) and interleukin (IL)-2, with no effect on T-helper type 2 (Th2) cytokines including IL-4, IL-6, and IL-10. Greater susceptibility to viral and bacterial infections might be due to this lack of Th1 cytokines. Decreased NK cell cytotoxic activity has been observed in Zn-deficient individuals [23,24].

In addition to the Thl system, NK cells produce IFN-γ and thus modulate the immune system. Lack of Zn due to malnutrition, e.g., in the elderly, may lead to dysregulation of the immune system and to a higher incidence of viral and bacterial infections [25] resulting from a Th1/Th2 imbalance [26–28]. We investigated whether Zn supplementation in vitro resulted in a Th1/Th2 shift and we studied the effects of Zn on NK cells.

Materials and methods

Preparation of cells and culture conditions

For phytohemagglutinin (PHA) mitogen stimulation, experiments were conducted with peripheral blood mononuclear cells (PBMCs) derived from the buffy coats or whole blood of healthy blood donors (anonymous, 18–60 y of age; whole blood, 23–44 y of age) to observe the possible changes in Zn homeostasis after a delayed isolation of PBMCs from buffy coats, which contain citrate. After density centrifugation over Ficoll Hypaque (Ficoll-separating solution, 1.077 g/mL; Biochrom, Berlin, Germany), PBMCs were collected from the interphase, washed twice with phosphate buffered saline (without Ca^{2+} or Mg^{2+}, single concentrated; BioWhittaker, Heidelberg, Germany) and resuspended in RPMI-1640 (BioWhittaker) supplemented with 10% heat-inactivated fetal calf serum (PAA, Coelbe, Germany), 1% penicillin (10 000 U/mL)/streptomycin (10 000 μg/mL; BioWhittaker), and 1% L-glutamine (2 mM; BioWhittaker) at a concentration of 1×10^6 cells/mL.

Isolated PBMCs were cultured in 24-well culture plates (Becton Dickinson, Heidelberg, Germany) at volumes of 1 mL. After isolation, Zn (30 or 60 μM) or N, N, N', N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) (1 μM) was added to the different wells. With no change of medium, the cells were incubated over 8 d at 37°C in a 5% humidified CO_2 atmosphere as previously described [28]. On day 7, the cultured cells were stimulated with PHA (Becton Dickinson) or an equal volume of supplemented RPMI-1640 for control. Cells were incubated with 10 μg of PHA/mL dissolved in RPMI-1640 to induce IL-10 and IFN-γ. The supernatants of cultures were harvested for cytokine detection and stored at −20°C before testing.

Detection of CD56/CD69, CCR4/CD3, and CCR5/CD3 cells

Whole blood was taken from healthy blood donors and diluted with RPMI-1640 containing 1% penicillin (10 000 U/mL)/streptomycin (10 000 μg/mL) and 1% L-glutamine (2 mM) at a ratio of 1:10. Cells were cultured in PPN tubes (polypropylene tubes with lid, 5 mL; Greiner, Nuertingen, Germany). Zn was added to produce concentrations of 30 and 60 μM (two to four times the physiologic serum concentration), whereas TPEN was used at a concentration of 1 μM. Cells were incubated over 8 d at 37°C in a humidified atmosphere with 5% CO_2. At the same time every day, the PPN tubes were shaken for 3 s to dissolve the pellets. The number of viable cells was ≥80% at the end of the experiments as detected by propidium iodide staining and analysis by flow cytometry (data not shown).

Zinc preparation

Zinc sulfate was dissolved in sterile water and sterile filtered to obtain a 100-mM stock solution. *Aqua ad injectabile* was used for further dilution at a ratio of 1:10 and then unsupplemented Ultra Doma serum free (BioWhittaker) was used to reach a working solution of 200 μM.

Determination of cytokines

Commercially available enzyme-linked immunosorbent assay (ELISA) kits (BD Biosciences Pharmingen, Heidelberg, Germany) were obtained and used for quantification of IFN-γ and IL-10. With each assay, controls and standards were included. For detection of cytokines, an ELISA reader was used.

Flow cytometry

Using two-color fluorescence gating including forward and side scatter, we were able to distinguish the lymphocyte population from other cells. Cells were labeled with CD56/fluorescein isothiocyanate and CD69/phycoerythrin (BD Biosciences Pharmingen) to identify NK cells. T-cell subpopulations were stained with CD3/fluorescein isothiocyanate and CCR5/phycoerythrin or CCR4/phycoerythrin (all from BD Biosciences Pharmingen) for identification of Th1 or Th2 cells.