Zinc in cancer prevention

Ananda S. Prasad1,2 and Omer Kucuk1
1Department of Medicine (Division of Hematology–Oncology), 2Department of Molecular Medicine and Genetics, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine Detroit, MI, USA

Key words: zinc, cancer prevention, immune function, nutrition, head and neck cancer

Abstract

Epidemiologic studies suggest that zinc deficiency may be associated with increased risk of cancer. However, few studies have been conducted with zinc supplementation in animals and humans. Most previous intervention studies have used zinc in combination with several other micronutrients, which make them difficult to interpret. Zinc supplementation is associated with decreased oxidative stress and improved immune function, which may be among the possible mechanisms for its cancer preventive activity. Preclinical and clinical studies need to investigate modulation of genetic and epigenetic pathways of carcinogenesis by zinc.

Introduction

Essentiality of zinc and its deficiency in humans was first recognized in the early sixties of the last century [1,2]. The current estimate is that a nutritional deficiency of zinc may affect over 2 billion subjects in the developing world. Growth retardation, immune dysfunction and cognitive impairment are among major consequences of zinc deficiency [3]. These effects are reversible with zinc supplementation. Conditioned deficiency of zinc is known to occur in many diseases, such as malabsorption syndrome, chronic liver and renal diseases, sickle cell disease, excessive intake of alcohol, malignancies and other chronic debilitating conditions [3,4].

Zinc is required for the activity of over 300 enzymes, as such it participates in many enzymatic and metabolic functions in the body. There are over two thousand transcription factors involved in gene expression which require zinc for the maintenance of their structural integrity and binding to DNA [3,4].

Nutritional deficiency of zinc is widespread throughout developing countries, and zinc deficient persons have increased susceptibility to a variety of pathogens. Zinc deficiency in an experimental human model caused an imbalance between Th1 and Th2 functions. Production of interferon-γ and interleukin (IL)-2 (products of Th1) were decreased, whereas production of IL-4, IL-6 and IL-10 (products of Th2) were not affected during zinc deficiency. Zinc deficiency decreased natural killer cell lytic activity and percentage of precursors of cytolytic T cells. In HUT-78, a Th0 cell line, zinc deficiency decreased gene expression of thymidine kinase, delayed cell cycle, and decreased cell growth. Gene expression of IL-2 and IL-2 receptors (both α and β) and binding of NF-κB to DNA were decreased by zinc deficiency in HUT-78. Decreased production of IL-2 in zinc deficiency may be due to decreased activation of NF-κB and subsequent decreased gene expression of IL-2 and IL-2 receptors [5].

We investigated subjects with newly diagnosed squamous cell carcinoma of the oral cavity, oropharynx, larynx, and hypopharynx. Patients with metastatic disease and with severe co-morbidity were excluded. Nutritional assessment included dietary history, body composition, and prognostic nutritional index (PNI) determination. Zinc status was determined by zinc assay in plasma, lymphocytes, and granulocytes. Pretreatment zinc status and nutritional status were correlated with clinical outcomes in 47 patients. Assessment of immune functions included production of Th1 and Th2 cytokines, T cell subpopulations and cutaneous delayed hypersensitivity reaction to common antigens.

At baseline approximately 50% of our subjects were zinc deficient based on cellular zinc criteria and had decreased production of Th1 cytokines but not Th2 cytokines, decreased NK cell lytic activity and decreased proportion of CD4+ CD45RA+ cells in the
peripheral blood. The tumor size and overall stage of the disease correlated with baseline zinc status but not with PNI, alcohol intake, or smoking. Zinc deficiency was associated with increased number of unplanned hospitalizations. The disease-free interval was highest for the group, which had both zinc sufficient and nutrition sufficient status.

Zinc deficiency and cell mediated immune dysfunctions were frequently present in patients with head and neck cancer when seen initially. Zinc deficiency resulted in an imbalance of Th1 and Th2 functions. Zinc deficiency was associated with increased tumor size and overall stage of the cancer. These observations have broad implications in the management of patients with head and neck cancer [6].

**Zinc as an antioxidant**

There is now increasing evidence that oxidative stress is an important contributing factor in several chronic human diseases, such as atherosclerosis and related vascular diseases, mutagenesis and cancer, neurodegeneration, immunologic disorders, and the aging process [7].

A free radical is defined as any species that has one or more unpaired electrons. Greater than 95% of the oxygen consumption in aerobic organisms is the result of enzymatic reduction to H₂O in mitochondria by the terminal oxidase of the respiratory chain. Superoxide radical is produced when molecular oxygen is reduced by one electron. The addition of a second electron to superoxide radical gives rise to H₂O₂, an oxidizing species that has no unpaired electrons and thus is not a free radical. The one electron reduction of H₂O₂ yields H₂O and hydroxyl radical which is a strong oxidant. Generation of hydroxyl radical from H₂O₂ is catalyzed by iron and copper. Finally, hydroxyl radical reduction produces a second molecule of H₂O. Together superoxide radical, H₂O₂ and hydroxyl radical are known as reactive oxygen species (ROS) and these are produced continuously by aerobic growing cells.

In eukaryotic cells, the mitochondrial respiratory chain, microsomal cytochrome P450 enzymes, flavoprotein oxidases, and peroxisomal fatty acid metabolism are the most important intracellular sources of ROS [7]. The NADPH oxidases are a group of plasma membrane associated enzymes, which catalyze the production of superoxide radical from oxygen by using NADPH as the electron donor. Zinc is an inhibitor of this enzyme. Cytotoxic cytokines such as TNF-α, IL-1β, and IL-8 generate increased amount of free radicals. In HL-60, a malignant human monocyte macrophage cell line, our studies have shown that zinc decreased the production of cytotoxic cytokines (unpublished data). The dismutation of superoxide radical to H₂O₂ is catalyzed by an enzyme superoxide dismutase (SOD) which contains both copper and zinc. Zinc is known to induce production of metallothionein, which is very rich in cysteine, and this is an excellent scavenger of hydroxyl radical. Iron and copper ions catalyze the production of hydroxyl radical from H₂O₂, and zinc is known to compete with both iron and copper from binding to cell membrane, thus decreasing the production of hydroxyl radical. Thus it is clear that zinc has multiple roles as antioxidant and is therefore, an excellent candidate for clinical chemoprevention trials in humans.

**Preliminary unpublished studies in HL-60 cells**

We have utilized HL-60 a malignant human monocyte-macrophage cell line to study the effect of zinc on cytotoxic cytokines, TNF-α, IL-1β and IL-8.

Zinc-deficient medium was prepared by removing zinc from the fetal bovine serum (FBS) used with RPMI-1640 medium (Blo-Whittaker, Walkersville, MD) for cell culture. A solid chelating resin (iminodiacetic acid, Sigma Chemicals, St Louis, MO), was used to remove all minerals from FBS, and then was reconstituted with sufficient salts, copper, calcium, manganese, magnesium, and iron-but not zinc to bring the electrolytes in the FBS back to the original concentration. Zinc deficient and sufficient media contain 1 and 15 μM zinc, respectively.

HL-60, human malignant macrophage-like cell line (NIAIDS, MD) were utilized in this study, and the cells were maintained in RPMI-1640 culture medium containing L-glutamine and supplemented with 10% regular FBS (without chelation), 100 U/ml penicillin, 100 μg/ml streptomycin, and 1.5 gm/l sodium bicarbonate at 37°C in a humidified atmosphere of 5% CO₂ in air. Without the addition of 10% FBS overnight, 5 x 10⁵ per ml cells were serum arrested or seeded in the culture medium described above. Then the cells were separated into two groups. One set of cells was further incubated in zinc-deficient medium, and the other set was incubated in zinc-sufficient medium for 10 days.

After incubation for 10 days in the media containing different levels of zinc concentration, the cells (2 x 10⁶/ml) were stimulated with 5 ng/ml phorbol-12 myristate 13 acetate (PMA) and 10 μg/ml phytohemagglutinin-p (PHA) for 1, 3 and 6 h (as early