Chapter 10

Zinc in Human Health

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Additional information is available at the end of the chapter

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Abstract

The essential role of zinc in human health was first suggested by our studies in growth-retarded Iranian villagers in 1961. Our later studies in 1963 established conclusively that zinc was essential for human and that zinc deficiency resulted in severe growth retardation, hypogonadism in males, immune dysfunctions, and cognitive function impairment. The suggestion that zinc was an essential element for humans remained very controversial, but in 1974, the USA National Academy of Sciences declared zinc as an essential element for humans and established the recommended dietary allowances. In 1978, the FDA and other regulatory agencies made it mandatory to include zinc in total parenteral nutrition fluids, which resulted in saving many lives. During the past five decades, tremendous progress has been made in the understanding of the biochemical role of zinc, and we now know that zinc therapy has impacted significantly on human health and diseases. In this review, I plan to present a brief historical review of the discovery of zinc as an essential element for humans, the clinical manifestations of zinc deficiency, its therapeutic impact on human health and diseases, biomarkers of human zinc deficiency, and its biochemical role.

Keywords: zinc, anti-inflammatory agent, antioxidant agent, oxidative stress, inflammatory cytokines

1. Historical review

In 1869, Raulin [1] reported for the first time that zinc was essential for the growth of *Aspergillus niger*. In 1926, it was reported that zinc was required for the growth of the plants, and in 1934, it was shown to be a growth factor for the rats [2].

I received my training in medicine as a clinical scientist under Professor Cecil James Watson at the University of Minnesota Medical School. The purpose of this training was to train

physicians not only in clinical medicine but also in basic sciences so that the clinical scientists could investigate the bedside clinical problems in research laboratories and to understand the basic mechanisms involved in clinical disorders [1–4].

Following the completion of my training under Dr. Watson in 1958, I was contacted by Professor Hobart Reimann, Chief of Medicine, Jefferson Medical College in Philadelphia. Professor Reimann who was a personal friend of the Shah of Iran had just accepted a position as Chief of Medicine at Shiraz University, Iran, and he offered me a position at the Shiraz Medical School to set up a medical curriculum for students and physicians in training on an American pattern. Although I was initially very reluctant, I did accept this offer and arrived in Shiraz, Iran, in July 1958.

The story of zinc began when an Iranian physician presented to me a severely anemic 21-year-old male, who looked like a 10-year-old boy, at the medical school grand round. His genitalia were infantile. He had rough and dry skin, was mentally lethargic, and had hepatosplenomegaly. He ate only bread made of whole wheat flour, he had no intake of animal protein, and in addition he ate 1 pound of clay every day. His severe anemia was due to iron deficiency but he had no blood loss. Iron deficiency in adult males without blood loss is a very unusual phenomenon.

Iron deficiency alone could not account for the severe growth retardation and infantile genitalia as these features are not seen in iron-deficient experimental animals. An examination of the periodic table suggested to me that deficiency of another transitional element, perhaps zinc, may also have been present, which could account for growth retardation and hypogonadism. We considered the possibility that a high phosphate content of the diet and clay may have decreased the availability of both iron and zinc which resulted in their deficiencies [3].

Our studies in Egypt later documented that zinc deficiency occurred in humans and that zinc supplementation resulted in 5–6 in. of longitudinal growth in 1 year and the genitalia became normal within 3–6 months of zinc supplementation [5].

The details of circumstances leading to the discovery of human zinc deficiency have been published recently [6].

2. Clinical manifestations of human zinc deficiency

The clinical manifestations of a moderate deficiency of zinc as described in the Middle East included growth retardation, hypogonadism in the males, rough skin, poor appetite, mental lethargy, delayed wound healing, cell-mediated immune dysfunctions, and abnormal neurosensory changes. These manifestations were reported in subjects with nutritional deficiency of zinc [5, 6] and in subjects with conditional deficiency of zinc [7, 8].

It is now apparent that a nutritional deficiency of zinc in humans is globally widespread, particularly in areas where cereal proteins are primarily consumed. Their diets are high in phytate, an organic phosphate compound which complexes both zinc and iron, and this deficiency may be affecting nearly 2 billion subjects in the world [7, 8].

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Zinc deficiency is also prevalent in females in the developing world. Cavdar et al. [9] observed decreased plasma zinc levels in almost 30% of the low-socioeconomic-status pregnant women in Turkey. Maternal zinc deficiency was associated with severe congenital malformation of the central nervous system in the fetuses and increased maternal morbidity [9].

In 1973, Barnes and Moynahan [10] reported a 2-year-old girl with severe acrodermatitis enteropathica (AE) who was being treated with diiodohydroxyquinoline and a lactose-deficient synthetic diet, but she was unresponsive to this management. The serum zinc level was low and this prompted the physicians to administer zinc sulfate. Surprisingly this resulted in complete recovery of this patient. This observation was quickly confirmed by others throughout the world, and many lives were saved by zinc therapy of AE patients.

AE used to be a lethal disease. This is caused by an autosomal recessive genetic disorder which usually occurs in infants of Italian, Armenian, or Iranian lineage [7, 8]. The disease manifests in the early months of life soon after weaning from breastfeeding. The dermatologic manifestations of severe zinc deficiency in AE patients include bullous pustular dermatitis of the extremities and the oral, anal, and genital areas around the orifices, paronychia, and alopecia. Ophthalmic signs include blepharitis, conjunctivitis, photophobia, and corneal opacities. Neuropsychiatric signs include irritability, emotional instability, tremors, and cerebellar ataxia. Other manifestations include growth retardation, weight loss, and male hypogonadism. Congenital malformation of fetuses and infants born of pregnant women with AE has been frequently observed [7, 8].

AE patients have increased susceptibility to infections. Immune dysfunction is due to abnormal Th1 functions. Clinical course is downhill with failure to thrive and complicated by intercurrent bacterial, viral, fungal, or parasitic infections. The disease if unrecognized is fatal. Zinc therapy is very effective and is curative.

AE gene has been identified as SCL39A4 and is localized to a ~3.5cm region on 8q24.3 chromosome. The gene encodes a histidine-rich protein which is now referred to as ZIP-4, which is a member of a large family of transmembrane proteins, known as zinc transporters. In patients with AE, mutations in this gene have been demonstrated [11]. So far 31 different mutations or variants of the SCL39A4 gene have been identified in AE patients throughout the world [12–14].

Covering about 4.5 kb of chromosomal region 8q24.3, the human SLC39A4 gene is composed of 12 exons ranging from 55 bP (exon 9) to 292 bP (exon 1) in size and 11 introns ranging from 76 bP (intron 7) to 506 bP (intron 1). The SLC39A4 gene encodes a 647-amino acid protein of about 68 kDa. This protein, which is designated as ZIP-4, belongs to the family of 14 members of specific ZIP zinc transporters (for zinc-/iron-regulated transporter-like protein) which facilitate zinc influx from outside the cell to cellular compartments into the cytoplasm [11–14]. We have previously reported a new mutation in exon 3 of the SCL39A4 gene in a Tunisian family with severe AE [14], and recently we have observed two new mutations (unpublished), one in a United Arab Emirate (UAE) family, which showed a mutation in exon 7, Gly 409→Arg, and the other in a patient from Turkey, which showed the mutation in exon 7 Leu 415→Pro.

3. Severe zinc deficiency in total parenteral nutrition (TPN) patients

Kay and Tasman-Jones reported the occurrence of severe zinc deficiency in subjects receiving TPN without zinc for prolonged periods [15]. Okada et al. [16] also reported similar observations in 1976. The clinical features were similar to those reported in AE patients. These complications of TPN fluids were completely prevented after 1978 when it was made mandatory to include zinc in TPN fluids. In 1976 we reported a severe deficiency of zinc in a patient with Wilson's disease who was treated with penicillamine and eventually became severely deficient of zinc due to chelation therapy. The manifestations were similar to AE patients [17].

We developed an experimental model of mild deficiency of zinc in human volunteers in 1978 [18]. The details of dietary preparation and experimental model studies have been published in detail before [18]. A semi-purified diet which supplied all essential nutrients in RDA amounts except zinc which was restricted to approximately 3.0–5.0 mg of zinc daily was used to induce zinc deficiency [18].

In this model as a result of specific mild deficiency of zinc, we observed decreased serum testosterone level, oligospermia, decreased natural killer (NK) cell activity, decreased IL-2 (interleukin-2) activity of T helper cells, decreased serum thymulin activity, hyperammonemia, hypogeusia, decreased dark adaptation, and decreased lean body mass [18–21]. Our study clearly established that even a mild deficiency of zinc in humans adversely affects clinical, biochemical, and immunological functions.

4. Biomarkers of zinc deficiency

In the Middle East, we assayed zinc in plasma, red blood cells, 24-h urine, and hair by dithizone technique [4]. This technique was very difficult and labor intensive but there was nothing easier available. Extreme precautions were taken to avoid contamination. We also utilized Zn^{65} to study zinc metabolism in our subjects [4].

Zinc levels were decreased in plasma, red blood cells, 24-h urine, and hair in the growth-retarded subjects in comparison to the controls. The plasma zinc turnover rate was greater, and the 24-h zinc exchangeable pool was decreased significantly in the dwarfs. The cumulative excretion of zinc in urine and stool in 13 days was decreased in the dwarfs, in comparison to the controls, indicating body conservation of zinc in the zinc-deficient subjects. We concluded from these results that the dwarfs were zinc deficient. This was the first demonstration that zinc deficiency in humans occurred [4].

On daily oral supplementation with 15 mg zinc as sulfate, the growth rate was 5–6 in. per year in the dwarfs, and the external genitalia became adult like within 6 months of supplementation [5].

5. Atomic absorption spectrophotometric assay for zinc (AAS)

In 1965 we published the first technique of zinc measurement in plasma and blood cells by the use of atomic absorption spectrophotometer (AAS), and this technique is currently being used all over the world [22].

At present plasma zinc by AAS is being widely used as a biomarker of zinc deficiency globally. The machine is expensive, needs careful maintenance, and is not easily available in developing countries. Furthermore, plasma zinc assay is not a specific biomarker of zinc deficiency in humans inasmuch as the plasma zinc pool changes as a result of infections, exercise, and stress. Also, even slight hemolysis increases the plasma zinc inasmuch as the red cells are very rich in zinc.

6. Biomarkers of zinc deficiency in experimental human zinc deficiency model

We used a semi-purified diet based on texturized soy protein which provided all nutrients in RDA amounts except for zinc which was 3–5 mg/d. The RDA for zinc is 12–15 mg/d. The details of experimental model studies have been published before [18].

In this model we studied the effect of zinc deficiency on zinc levels in plasma, blood cells, zinc-dependent enzymes, and immunological functions.

We observed that the assay of ecto 5′ nucleotidase (5′NT), a zinc-dependent enzyme, which is present in plasma membrane of lymphocytes and is a marker of cell maturity, was a very sensitive test for human zinc deficiency [23]. We observed that the activity of 5′NT decreased during early zinc depletion phase (4–8 weeks). Plasma zinc did not change until 24 weeks of zinc restriction.

We also assayed serum zinc thymulin activity in our experimental model of human zinc deficiency subjects [21]. Thymulin is a thymic hormone which requires zinc for its activity. Thymulin is required for the development and differentiation of T helper cells. In our subjects, we observed that serum thymulin activity decreased within 8–12 weeks following zinc-restricted diet, suggesting that this test was also a very sensitive biomarker of zinc deficiency. This correlated well with our observation that the generation of IL-2 and its mRNA were also decreased during the early zinc depletion phase, 8–12 weeks after initiation of zinc-restricted diet [20, 24, 25].

Thus, our studies revealed that the assay of immunological markers is perhaps the most sensitive biomarker of human zinc deficiency. We have published that the assay of IL-2 mRNA in peripheral blood mononuclear cells by RT-PCR was a very good indicator of zinc deficiency in humans [25].

7. Endogenous excretion of zinc as a biomarker of zinc deficiency

Humans maintain zinc homeostasis by increasing efficacy of zinc absorption and decreasing endogenous excretion of zinc when they are subjected to short-term dietary zinc restriction. However, a mild deficiency of zinc in humans is usually an outcome of chronic exposure to low dietary zinc for many months and years.

We, therefore, assessed the efficiency of zinc absorption as well as endogenous zinc excretion during a 6-month period of dietary zinc restriction by the use of Zn^{70}. Our studies showed that the efficiency of zinc absorption was not sustained and decreased in the volunteers when the zinc-restricted diet was continued for 6 months [26]. On the other hand, prolonged dietary zinc restriction did not impair the functional role of endogenous zinc excretion in zinc homeostasis. We observed a significant reduction of endogenous zinc excretion by restricting dietary zinc for 6 months. Our studies thus showed that the measurement of endogenous zinc excretion may also be a sensitive biomarker of human zinc deficiency.

In collaboration with Dr. Chris Fredrickson, we are currently developing a cost-effective, exportable instrument which uses laser-induced background spectroscopy (LIBS) for the measurement of zinc in tissue, plasma, and blood cells. Our preliminary studies have shown that measurement of zinc by LIBS technique in nails is an excellent technique for assessing chronic zinc deficiency in humans.

8. Therapeutic impact of zinc

8.1. Acute diarrhea in children

Zinc supplementation has been shown to prevent and treat diarrhea in children under 5 years of age decreasing both diarrhea and mortality [27, 28]. Zinc deficiency is also correlated with risk of respiratory tract infections, but the benefits of supplementation appears to be limited to more severe episodes and in populations with high incidence of zinc deficiency [28].

Diarrhea causes damage to absorptive mucosa of the intestines and decreases absorption of nutrients including zinc. Children with low plasma zinc were observed to be more susceptible to diarrhea, thus resulting in a vicious cycle of zinc deficiency and infection.

In 2004 the World Health Organization (WHO) issued a global recommendation for the daily supplementation of 20 mg zinc in children ≥6 months for 10–14 days upon diarrheal onset [28].

Meta-analysis of routine supplementation for up to 3 months in seven studies providing one to two times RDA elemental zinc five to seven times per week found an 18% reduction in diarrheal incidence, or 25% decrease in diarrhea prevalence, and a 33% reduction in persistent diarrhea episodes among supplemented children in comparison to those who received placebo [28]. A meta-analysis of three randomized controlled trials providing short course of zinc supplementation with two to four times the daily RDA for 2 weeks following the onset of acute or persistent diarrhea was also reported. The pooled analysis showed an 11%

decrease in diarrhea incidence and a 34% decrease in diarrhea prevalence during 3 months of observation.

8.2. Zinc for the treatment of common cold

Common cold is one of the most frequently occurring diseases in the world [29]. More than 20 viruses cause common cold, and these include rhinoviruses, corona viruses, adenoviruses, respiratory syncytial viruses, and parainfluenza viruses. In the USA, adults may suffer of common cold two to four times and children six to eight times per year. The morbidity and subsequent financial loss resulting from absenteeism from work are considerable. Previous treatments have not resulted in a consistent relief of symptoms.

We tested the efficacy of zinc acetate lozenges in common cold in 50 volunteers who were recruited within 24 h of the onset of common cold symptoms, and we carried out a double-blind placebo-controlled trial [29]. Participants took one lozenge containing 12-8 mg zinc (as acetate) or placebo every 2–3 h while awake as soon as they developed common cold symptoms. Subjective symptom scores for sore throat, nasal discharge, nasal congestion, sneezing, cough, scratchy throat, hoarseness, muscle ache, fever, and headache were recorded daily for 12 d. Plasma zinc and pro-inflammatory cytokines were assayed on day 1 and at the end when subjects were well.

Compared to the placebo group, the zinc group had shorter overall duration of cold symptoms (4.5 d vs 8.1 d, $p < 0.01$), nasal discharge (4.1 d vs 5.8 d, $p = 0.02$), and decreased total severity scores for all symptoms ($p = 0.02$).

In another study, we recruited 50 ambulatory volunteers within 24 h of the onset of common cold for randomized placebo-controlled trial of zinc lozenges [30]. Each lozenge contained 13.3 mg of zinc as acetate. Plasma zinc, soluble interleukin (IL-1) receptor antagonist (sIL-1ra), soluble tumor necrosis factor receptor-1, and soluble vascular endothelial cell adhesion molecule (SICAM)-1 were assayed on days 1 and 5 [30].

Compared with the placebo group, the zinc group had a shorter mean duration of common cold (4.0 d vs 7.1 d, $p = 0.001$), shorter duration of cough (2.1 d vs 5.0 d, $p < 0.001$), and nasal discharge (3.0 d vs 4.5 d, $p = 0.02$). The mean changes between zinc and the placebo groups (before vs after therapy) showed significant difference in sIL-1-ra (interleukin-1 receptor antagonist) ($p = 0.033$) and sICAM-1 ($p = 0.04$). Both decreased in the zinc group, and the mean changes between zinc and placebo group (before vs after therapy) showed significant differences ($p < 0.001$). Our results suggest that zinc decreased oxidative stress of monocytes and macrophages induced by the common cold viruses.

Human rhinovirus type 24 "docks" at ICAM-1 on the surface of the somatic cells [30]. Our results showed that zinc may have acted as an antiviral agent by reducing the ICAM-1 levels. We have also reported earlier that zinc downregulates NF-κB activity which is involved in gene regulation of ICAM-1 [30].

We conclude that zinc acetate lozenges are effective in decreasing the duration and severity of common cold symptoms. We also conclude that these beneficial effects of zinc on common

cold symptoms are due to the anti-inflammatory and antioxidant effect of zinc. A meta-analysis published by Cochrane has confirmed our results and conclusions [31].

8.3. Zinc deficiency in sickle cell disease (SCD)

Our extensive studies have documented the occurrence of zinc deficiency in SCD adult patients [31]. Growth retardation, male hypogonadism, hyperammonemia, abnormal dark adaptation, and cell-mediated immune dysfunctions in SCD patients have been related to a deficiency of zinc. Zinc deficiency was associated with decreased levels of zinc in plasma, erythrocytes, lymphocytes, and hair; hyperzincuria; decreased activity of zinc-dependent enzymes such as carbonic anhydrase activity in erythrocytes, alkaline phosphatase activity in granulocytes, and deoxythymidine kinase activity in newly synthesizing collagen connective tissue; and hyperammonemia [32]. Zinc supplementation to SCD patients resulted in significant improvement in growth; secondary sexual characteristics; normalization of plasma ammonia levels; correction of dark adaptation; increased zinc levels in plasma, erythrocytes, lymphocytes, and granulocytes; and expected response of zinc on zinc-dependent enzymes [32, 33]. Zinc supplementation also corrected impaired delayed-type hypersensitivity (DTH).

A recent Cochrane review has concluded that zinc is the only therapeutic modality which results in decreased incidence of infections and pain crises [34].

8.4. Zinc therapy for Wilson's disease (WD)

Wilson's disease is an inherited autosomal disorder due to copper accumulation. The excretion of liver copper in the bile is deceased which leads to the decreased loss of copper in the stool. This leads to accumulation of copper in the liver. Eventually copper accumulates in the brain, kidneys, and other organs. Patients present with liver disease, neurological disease (movement disorder), or psychiatric disturbance in the second to fourth decades of life. In many cases the diagnosis is missed by the physician [35].

The genetic mutation of Wilson's disease gene leads to a defective generation of a protein called ATP 7B which is responsible for a key step in biliary excretion of copper [35–37]. The disease is recessive, and thus both copies of the ATP 7B gene have to be mutated to cause the disease. A large number of mutations in this gene causing Wilson's disease have been reported.

Early diagnosis of Wilson's disease is important inasmuch as effective therapy may prevent toxic accumulation of copper and damage to organs.

Ninety percent of the WD patients have low levels of ceruloplasmin and ceruloplasmin-bound copper, but non-ceruloplasmin copper is elevated in the plasma. Twenty four hour urinary copper is markedly elevated and this is a helpful diagnostic tool. Urinary copper, however, may be elevated also in patients with obstructive liver disease.

A slit lamp examination for copper deposits in the cornea (Kayser-Fleischer rings) is a very useful noninvasive diagnostic test for WD. This is positive in nearly 50% of the cases.

Several years ago, we were using 150 mg elemental zinc in six divided doses as an effective anti-sickling agent for the treatment of patients with sickle cell disease (SCD) [38]. At this level of zinc therapy, we observed that we induced copper deficiency in SCD patients [38]. This led Brewer et al. [35–37] to develop zinc as an effective anti-copper drug for WD.

Zinc competes with copper for similar binding sites, and oral doses of zinc efficiently decrease the uptake of copper [39]. Zinc may act by inducing intestinal metallothionein (MT) which has a high affinity for binding copper. This prevents the serosal transfer of copper into the blood pool. The intestinal cells turn over rapidly and take the complexed copper in stool for excretion. Zinc not only blocks food copper but also the copper which is endogenously excreted via salivary, gastric, and other gastrointestinal juices. Thus zinc is effective in producing a negative balance of copper.

Fifty milligrams of zinc (as acetate) is given orally three times a day to WD patients. Zinc must be given in a fasting or post absorptive state. The only side effect of zinc is that nearly 10% of the subjects may experience mild gastric discomfort. This can be avoided if zinc is administered between breakfast and lunch or after dinner before going to bed.

Zinc is the drug of choice for maintenance therapy. Zinc has no toxicity and is non-teratogenic, and it can be prescribed to subjects of all ages and even to pregnant women. Zinc has been approved by the FDA for the treatment of WD patients.

8.5. Zinc and age-related macular degeneration (AMD)

Age-related macular degeneration (AMD) affects nearly 25% of the subjects over 65 years of age, and late stage of disease accounts for nearly 50% of legal blindness in Europe and North America [40]. Newsome et al. [40] reported for the first time that zinc concentration is reduced in the human eye in patients with AMD, and they suggested that zinc deficiency may have led to oxidative stress and retinal damage.

The Age-Related Eye Disease Study (AREDS) group, supported by the National Eye Institute, NIH, conducted a double-blind clinical trial in patients with dry-type AMD in 11 centers [41]. They enrolled 3640 patients for trial. Their ages ranged from 55 to 80 years, and the average follow-up period was 6.3 years. Participants were assigned randomly to receive orally one of the following: (1) antioxidants (vitamin C, 500 mg; vitamin E, 400 IU; and beta carotene, 15 mg); (2) zinc 80 mg as zinc oxide and copper 2 mg as copper oxide, to prevent copper deficiency induced by zinc; (3) antioxidants plus zinc; or (4) placebo.

The group taking the antioxidant plus zinc reduced the risk of developing advanced AMD by about 25% and the vision loss by 19%.

The group taking zinc alone reduced the risk of developing advanced AMD by about 21% and the vision loss by 11%. In the group taking the vitamins alone, the risk of developing advanced AMD was decreased by 17%, and the vision loss was decreased by 10%. Only the zinc-supplemented group showed increased longevity [42, 43]. The risk of mortality was reduced by 27% in subjects who received only zinc. In a later publication, the AREDS group reported that a decrease in mortality was due to a decrease in the adverse cardiovascular

events [43]. These results of zinc supplementation in the elderly are remarkable and suggest that the anti-inflammatory effect of zinc may be beneficial in patients with atherosclerosis.

8.6. Zinc deficiency in the elderly

The daily intake of zinc in the elderly in the Western world including the USA is only approximately 8–10 mg, whereas the RDA as reported in 1974 is 15 mg [44]. Frequently the elderly do not routinely eat three meals a day. Many live alone and do not cook a proper meal.

Our study in Detroit showed that 35% of the well-to-do ambulatory elderly subjects may have a deficiency of zinc [44–46]. Results of the third National Health and Nutrition Examination Survey (1988–1994) also reported that the elderly subjects >71 years were at great risk of inadequate zinc intake.

Oxidative stress and increased inflammatory cytokines have been recognized as important contributing factors for several chronic diseases attributed to aging, such as atherosclerosis and related cardiovascular disorders, mutagenesis and cancer, neurodegenerative disorders, type 2 diabetes, and Alzheimer's disease. Together, O^{2-}, H_2O_2, and OH·– radicals are known as reactive oxygen species (ROS), and excessive generation of ROS causes oxidative stress. Inflammatory cytokines such as TNF-α, IL-1β, and IL-6 generated by activated monocytes and macrophages are also known to generate excessive ROS.

We have shown that zinc supplementation to subjects ages 20–50 decreased oxidative stress markers such as malondialdehyde (MDA), 4-hydroxy-alkelans (HAE), and 8-hydroxydeoxyguanine in the plasma and downregulated the ex vivo induction of TNF-α and IL-1β mRNA in mononuclear cells (MNCs) by decreasing TNF-α induced NF-κB induction [46]. We have also reported that in the promyelocytic leukemia cell line HL60, which differentiates to the monocyte and macrophage phenotype in response to phorbol-12-myristate-13 acetate (PMA), zinc upregulated the expression of A20 and the binding of A20 to trans-activating factor to DNA which resulted in the inhibition of NF-κB activation [45, 46].

We carried out a randomized placebo-controlled trial of zinc supplementation in 50 healthy elderly subjects (55–87 y) of both sexes and all ethnic groups in Detroit, MI. Exclusion criteria were life expectancy of <8 mo, progressive neoplastic disease, severe cardiac dysfunction, significant renal disease, significant liver disease, and subjects who were mentally incompetent. Zinc supplementation consisted of 45 mg elemental zinc as gluconate daily for 12 mo.

A comparison of our baseline data in the elderly with the younger adults showed that in the elderly, plasma zinc was lower and the percentage of cells producing IL-1β and TNF-α and the generated cytokines were significantly higher [45, 46]. IL-10 generated by Th2 cells which is known to downregulate IL-2 generation from Th1 cells was significantly higher in the elderly. The oxidative stress markers were also significantly higher in the elderly than the younger adults [45].

The mean incidence of infections was lower ($p < 0.01$) in the zinc-supplemented group (1.4 ± 0.95) vs placebo group (20.29 ± 0.46). The plasma zinc increased, and ex vivo generation of TNF-α and IL-10 significantly decreased in the zinc group in comparison to the placebo group [45, 46]. Oxidative stress markers in the plasma also decreased in the zinc group in comparison to the placebo group [46].

In MNCs isolated from zinc-deficient elderly subjects, zinc supplementation increased the ex vivo PHA induced IL-2 mRNA expression and plasma zinc concentration when compared to the placebo group.

Thus our study showed that zinc supplementation to the elderly subjects decreased the incidence of infection by nearly 66%. Following zinc supplementation the oxidative stress markers and the generation of inflammatory cytokines which were increased prior to supplementation significantly declined. These are very significant effects of zinc supplementation and may imply that zinc may prove to be an excellent agent for prevention of some of the chronic diseases in the elderly.

8.7. Biochemical mechanisms of zinc

When I started my studies in zinc metabolism in the early sixties, I knew of only three enzymes which required zinc for their functions. These were carbonic anhydrase, carboxypeptidase, and alcohol dehydrogenase. Now we understand that there is hardly any cellular process that does not depend upon zinc in some way. Zinc is a constituent of at least 2800 human protein structures, enzymatic catalysis, and cellular regulation. The largest group of zinc metalloenzymes is proteinases [47]. Zinc has a major role in the structural organization of protein domains that interact with DNA/RNA, other proteins, and lipids. Several dozen proteins control cellular and subcellular zinc homeostasis and redistribution. This control is essential for regulatory functions of free zinc (II) ions.

Free zinc intracellularly regulates the activity of several kinases, phosphatases, phosphodiesterases, caspases, and transcription factors.

8.8. Zinc and growth

IGF-1 is a zinc-dependent growth factor. Zinc is required for generation of IGF-1, and its receptor tyrosine kinase requires zinc for phosphorylation. IGF-1 intracellularly upregulates thymidine uptake by the nucleus which is converted to dTMP and dTTP for DNA synthesis. The enzyme deoxy thymidine kinase (TK) is required for the synthesis of TTP. Our studies have shown that zinc is required for the gene expression of TK. Thus zinc is involved at various steps for DNA synthesis and cell proliferation. Other enzymes such as DNA polymerase and RNA polymerase are also zinc dependent.

8.9. Zinc and immune cells

Zinc is a second messenger for immune cells, and intracellular zinc participates in signaling events [21, 48–51]. Hirano et al. [48, 49] have shown that a decrease in intracellular free zinc in T cells is essential for LPS-mediated CD4+ activation by dendritic cells (DCs). LPS binds to TLR-4 on DCs and activates Myd88 and TRIF-mediated signaling [48]. TRIF-mediated signaling upregulates ZnT-5 mRNA and downregulates ZIP-6 mRNA, resulting in a decrease of intracellular free zinc in DCs. Reduction in intracellular free zinc increases the expression of MHC class II molecules which is required for the activation of CD4+ T cells [48].

Zinc activates monocytes-macrophages in several ways. Zinc is required for the development of monocytes-macrophages and regulates its functions such as phagocytosis and

pro-inflammatory cytokine production [50, 51]. LPS stimulation of zinc-sufficient monocytes results in downregulation of inflammatory cytokines such as TNF-α, IL-6, IL-1β, and IL-8. Zinc inhibits the cell membrane phosphodiesterase (PDE), which results in elevated levels of second messenger cGMP, which is followed by decreased activation of NF-κB and decreased generation of inflammatory cytokines [50, 51]. We have shown that zinc induces A-20, a zinc finger transcription factor protein, which inhibits NF-κB signaling via TNF receptor-associated pathways, resulting in downregulation of mRNAs of inflammatory cytokines [52, 53]. Based on these we propose that zinc is an important anti-inflammatory agent.

Infection or other stresses activate NADPH oxidase in monocytes-macrophages, which upregulates the generation of free radicals ($O\bullet^{-}_{2}$). Zinc is an inhibitor of NADPH oxidase. In the next step, the free radical is converted to H_2O_2, and the enzyme involved in this process is superoxide dismutase, a zinc- and copper-dependent enzyme. H_2O_2 generates •OH ions. Free radicals, H_2O_2 and •OH ions, are known as collectively reactive oxygen species (ROS). By the above mechanisms, zinc functions as an important antioxidant.

Zinc deficiency adversely affects Th1 functions [29]. Serum thymulin activity and generation of Th1 cytokine IL-2 and IFN-γ were adversely affected within 8–12 weeks of institution of zinc-restricted diet (3–5 mg daily) in human volunteers, whereas plasma zinc decreased later after 20–24 weeks of the institution of the experimental diet. These studies showed that Th1 cells are very sensitive to zinc restriction. Th2 cytokines were not affected in subjects who received experimental zinc-deficient diet. Thymulin is a zinc containing nonapeptide and is essential for the development and proliferation of Th cells.

Our studies also showed that several transcription factors such as NF-κB, AP-1, and SP-1 were zinc dependent and their binding to the DNA of IL-2 gene were decreased, resulting in decreased generation of IL-2 [24, 25]. A similar effect of zinc was also seen in generation of IFN-γ. Decreased IL-2 resulted in decreased NK and cytolytic T cell activity. Decreased IFN-γ along with decreased IL-12, another zinc-dependent cytokine generated by monocyte-macrophages, results in decreased phagocytosis by monocyte-macrophages.

In Th0, a human malignant lymphoblastoid cell line HUT-78, we showed that in zinc-sufficient cells, mRNA levels of IFN-γ, IL-12 Rβ2, and T-bet in PMA-/PHA-stimulated cells were increased in comparison to zinc-deficient cells [54]. Although intracellular free zinc increased only slightly in PMA-/PHA-stimulated cells, in Con-A stimulated cells in zinc-sufficient medium, there was an increased and sustained level of intracellular free zinc in comparison to the zinc-deficient cells [54]. We hypothesized that in stimulation of cells by Con-A via TCR, there was a release of intracellular free zinc which resulted in signal transduction for generation of IFN-γ and T-bet, IL-12 Rβ2, and STAT-4 mRNAs which participated in Th-1 cell differentiation.

8.10. Zinc transporters

Zinc transporters maintain intracellular zinc homeostasis very tightly. Ten ZnT (SLC 30) family of zinc transporters lower intracellular zinc concentration through export of zinc or by import into cellular compartment organelles such as Golgi, intracellular vesicles, mitochondria, or nucleus.

Fourteen ZIP (SLC 39) transporters are responsible for increasing intracellular zinc concentration through zinc import into cells or export from intracellular organelles.

In humans, ZIP 4 mutation in AE is known to result in severe deficiency of zinc, which is fatal if not recognized and properly treated with zinc. This entity has been discussed in a previous section.

A detailed study of Ehlers-Danlos syndrome characterized by progressive kyphoscoliosis, joint hypermobility, hyperelasticity of the skin, and severe hypotonicity of skeletal muscles has shown that these patients are homozygous for a 9 bp in-frame deletion of exon 4 of SLC 39 A 13 [55].

Bone morphogenetic protein (BMP)/transforming growth factor β (TGF-β) enters the mesenchymal cells, fibroblasts, osteoblasts, chondrocytes, etc. for generation of collagen, Max 2, hard connective tissue of bone, teeth, and cartilage, etc. Intracellularly BMP/TGF-β activates Smad which is then involved in gene expression of collagen, bone, and cartilage. Zinc is required for activation of Smad. ZIP-13 mutation, however, affects the activation of Smad by zinc, and these results in Ehlers-Danlos syndrome.

In prostate cancer cells, ZIP-1 and ZIP-2 are mutated, and this decreases the intracellular zinc concentration of prostate cancer cells. This results in activation of NF-κB, which leads to upregulation of anti-apoptotic genes and growth factors and proliferation of cancer cells, which leads to progression of prostate cancer. It is possible that proper supplementation of zinc in these patients may have a beneficial effect in patients with prostate cancer.

9. Conclusion

The essentiality of zinc for humans was established in 1963. The National Academy of Sciences and the US Congress established recommended dietary allowance for zinc in 1974, and the FDA made it mandatory to include zinc in the total parenteral nutrition in 1978.

The major clinical effects of zinc deficiency in humans include growth retardation in young ages, cell-mediated immune dysfunction, neurosensory disorders, delayed wound healing, and cognitive impairment. Currently it is the estimate of the WHO that nearly 2 billion subjects in the developing world may be zinc deficient. This is due to the fact that these populations mainly subsist on cereal proteins mainly and their diet has high levels of phytate which makes zinc unavailable for absorption.

Over 300 enzymes require zinc for their structure stability and activity, and over 2000 transcription factors are zinc dependent. Zinc is a molecular signal for immune and neuronal cells. Zinc is essential for thymulin, a thymic hormone which is required for T helper cell differentiation and proliferation. Zinc is essential for gene expression of Th1 cytokines. We have now documented that several transcription factors such as NF-κB, AP-1, SP-1, A20, T-bet, and STAT 4 are zinc dependent and are adversely affected by zinc deficiency. Zinc is required for DNA synthesis and cell proliferation, and we have shown that the gene expression of deoxy thymidine kinase (TK) is zinc dependent. Zinc not only improves cell-mediated immune functions, but it is also an antioxidant and anti-inflammatory agent.

Currently plasma zinc assay by AAS is used globally as a biomarker of zinc deficiency in humans. In our experience, this assay is not very sensitive nor specific for zinc deficiency. The immunological assays such as measurement of thymulin activity, assay of IL-2 mRNA and protein following PHA stimulation of mononuclear cells, and assay of lymphocyte ecto 5′ nucleotidase are very specific and sensitive biomarkers of zinc deficiency.

Therapeutic impact of zinc has been tremendous in several conditions. These include treatment of diarrhea in infants and children globally resulting in saving millions of lives, development of zinc therapy for Wilson's disease, common cold, prevention of blindness in elderly subjects with AMD, decreasing the incidence of infections and the incidences of adverse cardiovascular events in the elderly, successful treatment of carbon-monoxide poisoning, and decreasing the incidences of infection and pain crisis in patients with SCD.

Abbreviations

Con-A	concanavalin-A
dTMP	deoxythymidine phosphate
dTTP	deoxythymidine tetra phosphate
IGF-1	Insulin-like growth factor 1
IL-1β	Interleukin-1β
IL-6	Interleukin 6
IL-10	Interleukin-10
LPS	lipopolysaccharide
MHC	major histocompatibility complex
MMP	matrix metalloproteinases
MyD88	myeloid differentiation primary response 88 (human)
NADPH	nicotinamide adenine dinucleotide phosphate
TCR	T-cell receptor
TLR 4	toll-like receptor 4

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