ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 1, 2 SECTIO DDD 2010

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Biochemistry of zinc

Biochemia cynku

The essentiality of zinc for humans has been known for 40 years. The zinc-deficient patients had severe immune dysfunction mainly affecting T helper cells. In men a decreased serum testosterone level and oligospermia were documented. Besides, hyperammonemia and decreased lean body mass were reported. It appears that zinc deficiency is prevalent in the developing world and billions of people may be growth with zinc deficiency [15]. The human body contains 2–3 g zinc. Most zinc is bound to proteins [5]. In an adult organism 55% of the overall quantity of zinc is in muscles and about 30% in bones. Large quantities of the element are accumulated in brain structures, i.e. the hippocampus, amygdaloid nuclei and cerebral cortex [11]. Zinc affects sperm activity which basically influences reproduction [2]. Normal blood plasma zinc levels range from 0.7 mg/l to 1.3 mg/l [11].

ZINC AS A COFACTOR

Over 300 enzymes have been shown to contain zinc as a cofactor [5]. Zinc is the only metal encountered in each enzyme class, i.e. oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Two properties of zinc in biochemistry are important. First, unlike other metals from IIB series, zinc is nontoxic [24]. However, adverse symptoms in humans are observed on inhalation of zinc fumes, or accidental ingestion of unusually large amounts of zinc. High concentrations of zinc have been found to kill viruses, bacteria and cultured cells [26]. Secondly, its physical and chemical properties make it highly adaptable to bound to protein and enzymes, which carry out diverse biological functions. These properties cause that zinc also has extensive participation in nucleic acid, carbohydrate, lipid metabolism, in the control of gene transcription and other fundamental biological processes [24]. Enzymes containing zinc as a cofactor include superoxide dismutase, alcohol dehydrogenase, carbonic anhydrase. Superoxide dismutase catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. There are three major families of superoxide dismutase, depending on the metal cofactor: SOD1 (CuZnSOD), which binds both copper and zinc, is located in the cytoplasm, SOD2 (MnSOD), which binds manganese, is located in the mitochondria and SOD3 (ECSOD) is extracellular. SOD3 is found in lymph, plasma and synovial fluid [22]. Zinc

induction increases the activity of superoxide dismutase [19]. SOD1 (CuZnSOD) is localized in cytosol, nucleus, peroxisomes and mitochondrial intermembrane space of human cells. When SOD1 has mutated, it can also cause disease. Over 100 different mutations have been recognized in the sod1 genes of patients diagnosed with the familial form of amyotrophic lateral sclerosis (fALS) [23].

Carbonic anhydrase catalyzes the reversible hydration of carbon dioxide to bicarbonate. The active site of most carbonic anhydrases contains a zinc ion, which is coordinated by the imidazole rings of 3 histidine residues (His94, His96 and His119) [17].

EFFECTS OF ZINC DEFICIENCY

Severe zinc deficiency is characterized by skin lesions, growth retardation, impaired wound healing, anemia, mental retardation, and impaired visual and immunological function. Even mild zinc deficiency effects the immunity system [5]. Recent research proves that chronic heavy alcohol consumption causes disturbances of zinc homeostasis, leading to a decrease in its serum level by over 25% in comparison to concentrations shown by healthy people [11].

In humans, *acrodermatitis enteropathica* is a rare autosomal recessive inheritable disease that causes thymic artrophy and a high susceptibility to bacterial, fungal and viral infection. This disease is based on a mutation of the gene responsible for the intestinal zinc transport protein hZip4. It is a zinc-specific malabsorption syndrome. Fortunately, all symptoms can be reversed by nutritional supplementation of zinc [5].

Zinc and other trace elements play a principal role in Parkinson's disease (PD). Recent research demonstrates changes in the serum levels of trace elements. They reflect a variation in data among certain elements in early and severe PD compared to controls. Zinc concentration was significantly lower in severe PD compared to controls. The data revealed an imbalance in the inter-element relations and suggested a disturbance in the element homeostasis during the progression of PD [12]. Furthermore, it has been postulated that zinc plays a significant role in multiple sclerosis (MS) and Alzheimer's disease (AD). Similarly to Parkinson's disease (PD), some disturbances in the element homeostasis were observed [1]. In Alzheimer's disease (AD) an increased zinc concentration in brain was noticed [25].

There are a lot of similarities between immunological changes during aging and zinc deficiency. In both cases thymic arthropy occurs, a shift of the Th cell balance toward Th2, and a decreased response to vaccination. During aging a decreased zinc concentration in tissues and serum were observed. Consequently, oral zinc supplementation improves immunity and efficiently regulates chronic inflammatory responses in the elderly. Zinc is essential for cell proliferation and differentiation. Despite zinc homeostasis it is involved in signal transduction and apoptosis. Humans require the appropriate and regular supply of zinc [5,16]. Less than one percent of the total body content of zinc depends on regular distribution of zinc. Zinc stores are easily depleted. During infections pro-inflammatory cytokines induced variation in hepatic zinc homeostasis. These changes lead to sequestration of zinc into liver cells and hypozincemia. Additionally, changes in zinc uptake, retention, sequestration and secretion lead to zinc deficiency and affect immune system [5].

POSITIVE ROLE OF ZINC FOR HUMANS

Zinc is an antioxidant and it has anti-inflammatory effects [15]. Clinical studies suggest that a high dosage of zinc or a combination of antioxidants above normal daily requirements prevent humans from xenobiotic hepatotoxicity. Thus, it is recommended to consume food rich in zinc, which reduces the oxidative stress induced by xenobiotic [18]. Zinc has a significant role in the treatment of acute infantile diarrhea and common cold. Moreover, in HL-60 cells (promyelocytic leukemia cell line) zinc has a positive influence on the up- regulation of A20 m RNA. Besides, it diminishes NF-κB activation, which has an effect on decreasing gene expression and generation of tumor necrosis factor (TNF)- α, IL-1β and IL-8. Zinc reduces oxidative stress markers and generation of inflammatory cytokines [15].

Zinc treatment in Wilson's disease (WD) prevents patients from progression over the course of 10 years. Wilson's disease (WD) is a disorder of copper metabolism characterized by a failure of the liver to excrete copper. This dysfunction leads to its accumulation in the liver, brain, cornea, and kidney. As a result, chronic degenerative changes are observed. After oral zinc supplementation, excellent clinical results in all patients were observed. Especially in hepatic histologic copper concentrations were reduced by treatment. That suggests that zinc was able not only to prevent further accumulation of copper but also to support the effeteness of its stores [10].

Zinc is also known to be essential for the immune system. Positive effects of zinc on the immune system depend on its concentration. All kinds of immune cells show impaired function after zinc effeteness. In neutrophil granulocytes phagocytosis is reduced, while in natural killer cells cytotoxicity is decreased. The normal functions of T cells and monocytes are impaired, whereas B cells undergo apoptosis. During zinc deficiency, zinc treatment negative effects on immune cells are abolished [7]. In humans, about 1% of the total body zinc content is replenished daily by the diet [3]. Despite its important function, high zinc concentration evokes negative effects on the immune system. Besides, while peripheral blood mononuclear cells are incubated with zinc in vitro, cytokines such as interleukins, IL-1 and IL-6, tumor necrosis factor (TNF)- α, soluble IL-2R and interferon (INF)-gamma are released [7].

ZINC-PROTEIN CONNECTION

The number of genes coding for proteins with zinc-binding domains is conservatively estimated at >3% of the human genome. There is a possibility that the number of genes come to even 10%. Zinc transporter genes react to hormonal and cytokine stimulation. Coordination of intracellular zinc trafficking concentrates on the cysteine-rich protein metallothionein (MT) [3].

Many transcription factors contain zinc finger and similar structural motifs. From *in silico* studies it was evaluated that 10% of human genome encode zinc proteins [5]. Zinc finger motifs organize protein sub-domains for the interaction with DNA or other proteins [20]. They are structurally diverse and perform functions in various cellular processes, such as replication and repair, transcription and translation, metabolism and signaling, cell proliferation and apoptosis. Zinc finger motifs bind to a wide variety of compounds, such as nucleic acids, proteins and small molecules. In contrast to other transcription factors, in zinc finger motifs, zinc is structurally bound to DNA. Zinc in zinc finger

motifs is stabilized by free cellular zinc. Zinc finger motifs are classified into eight groups which are based on the structural properties in the vicinity of the zinc-binding site. Three of these groups contain the majority of zinc fingers, called C2H2-like finger, treble clef finger and the zinc ribbon [9].

ZINC IN SIGNALING ION

On the cellular level, zinc is essential for differentiation and proliferation. Besides, its important function is involvement in signal transduction [5]. Zinc ions are hydrophilic and do not get trough cell membranes by passive diffusion [20]. One principal mechanism by which zinc influences immunity is its role as a signaling ion (Fig. 1). Three mechanisms regulate the intracellular concentration of free zinc. The first is represented by transport through the plasma membrane. The second mechanism is involved in storage and release from zincosomes. Zincosomes are vesicles, in which zinc is stored as a complex with various ligands. In the last mechanism, zinc is bound to metallothionein (MT) [5]. Induction of metallothionein and changes in the concentration of copper and iron during zinc supplementation decrease the levels of lipid peroxidation [19].

Zinc signals act on immune cell signal transduction. Zinc homeostasis is controlled by three mechanisms, where the intracellular concentration of free zinc is modified. Zinc regulates the activity of the major signaling molecules, i.e., protein kinase C (PKC), which has been recognized as a molecular partner for zinc in T cells. Its structure contains N terminal regulatory domain, in which four Cys3His zinc binding motifs are located. Zinc treatment impels PKC activity as a consequence of its affinity to the plasma membrane and cytoskeleton. Another example is the lymphocyte protein tyrosine kinase (Lck), in which zinc takes part in signal transduction. Zinc ions enable activation

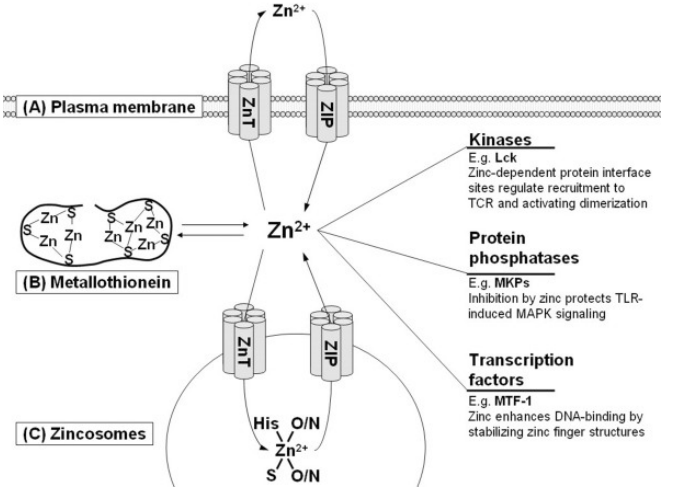

Fig. 1. Zinc as a signal ion for immune cells : (A) Transport through the plasma membrane by zinc transporters from the ZnT (SLC A30) or ZIP (SLC A39) families. (B) Buffering by metallothionein. (C) Reversible transport by ZnT and ZIP proteins into or out of zincosomes, and storage bound to ligands, which create a zinc sink. Zinc signals, i.e., changes in the intracellular concentration of free zinc, control immune cell signal transduction, including kinases, phosphatases, and transcription factors. (T cell receptor, MKP, MAPK phosphatase, metal-response element binding transcription factor-1) [5]

of Lck and its annexation to the T cell receptor complex by linking two protein. The N- terminal region of Lck is involved to the intracellular domains of the membrane proteins CD8 or CD4 by 'zinc clasp' structure.

Zinc signals regulate inflammatory signaling through the activity of cyclic nucleotide phosphodiesterases and MAPK phosphatases. Zinc transporters are also involved in signal transduction. Zrt/Irt-like protein (ZIP) 7 releases Zn from the ER, controlling tyrosine phosphorylation, whereas lysosomal ZIP 8 is required for zinc-mediated calcineurin inhibition and interferon (IFN)-γ expression in T cells. The metal-response element binding transcription factor (MTF)-1 controls the promoters of MT and several zinc transporters.

Zinc deficiency in the elderly might disturb zinc-dependent signaling and as a result, the immune system. Peripheral blood mononuclear cells (PBMC) from zinc-deficient elderly appeared impaired NF-κB activation and interleukin (IL)-2 production in response to stimulation with PHA [5]. Furthermore, zinc is involved in transmembrane gradients. Using fluorescent imaging, Na^{+}-dependent Zn^{2+} transport in HEK293 cells and cortical neurons, were monitored. With all probability, this mechanism plays a significant role, not only in generating the transmembrane zinc gradients, but also in protecting cells from the potentially toxic effects of this ion [13].

ZINC AS A NEUROMODULATOR

Zinc is a modulator of synaptic transmission in the central nervous system [11]. The mammalian forebrain contains a subset of glutamatergic neurons with zinc in their synaptic vesicles. This zinc may be released into the synaptic cleft and may act at neurons [14]. During synaptic transmission, zinc binds to receptors on the post- or presynaptic membrane affecting the activity of neuron [8]. Extracellular zinc has the potential to interact with many different synaptics. NMDA receptor subtypes contain allosteric sites sensitive to extracellular zinc [14]. Zinc is a strong inhibitor of ionotropic NMDA receptors in stimulating glutaminergic synapses [11].

IMPACT OF APOPTOSIS

Zinc is essential in various physiological and biochemical processes. The epithelial cells of prostate have the ability to accumulate zinc, which is essential to the production and secretion of citrate (Fig. 2). The production of citrate and its secretion into prostatic fluid is important for reproduction.

The lost ability of the malignant cells to accumulate zinc and citrate is an important factor in the development and progression of malignancy prostate cancer. This process is the result of decreased expression of specific zinc uptake transporters [4]. Zinc prevents prostate cells from growing by its induction of apoptosis. The accumulation of cellular zinc has an effect on the mitochondrial pore-forming process, releasing of cytochrome c. Then it initiates the caspase cascade, leading to apoptosis. Moreover, zinc increases the gene expression and cellular production of Bax, which initiates the caspase cascade (Fig. 3). In humans, a proper concentration of zinc induces apoptosis in prostate cells. The malignant cells in prostate cancer posses genetic adaptation that prevent the cellular accumulation of zinc [21].

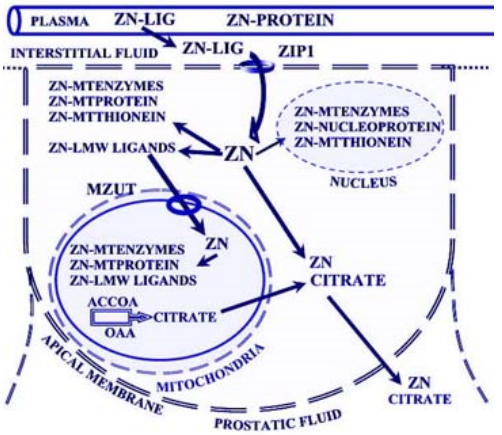

Fig. 2. The distribution of zinc in Prostate Epithelial Cells. Free zinc ions are absent in the represented compartments. Abbreviations: MT=Metallo-, LMW=low molecular weight, MZUT=mitochondrial zinc uptake transporter [4]

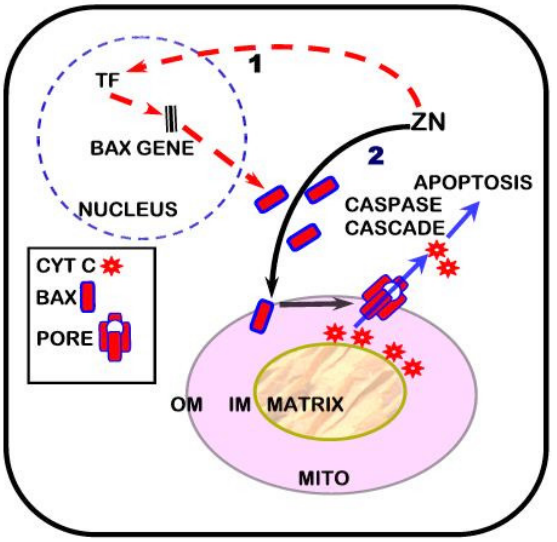

Fig. 3. A representation of the integrated apoptogenic effects of zinc [6]

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SUMMARY

Zinc is an essential trace element to physiological functions of human organism. Over 300 enzymes have been shown to contain zinc as a cofactor. The zinc-deficient patients have severe dysfunction, mainly affecting T helper cells. Severe zinc deficiency is characterized by skin lesion, growth retardation, impaired wound healing, anemia, and mental retardation. Fortunately, all symptoms can be reversed by zinc treatment. Zinc reduces oxidative stress markers and generation of inflammatory cytokines. Besides, zinc plays a significant role in Parkinson's disease (PD), multiple sclerosis (MS), Alzheimer's disease (AD) and Wilson's disease (WD). It is also involved in signal transduction and apoptosis, i.e. zinc prevents prostate cells from growing by its induction of apoptosis. Many transcription factors contain zinc finger and similar structural motifs. Zinc finger motifs bind to a wide variety of compounds, such as nucleic acids, proteins and small molecules. Besides, zinc is a modulator of synaptic transmission in the central nervous system.

STRESZCZENIE

Cynk jest mikroelementem niezbędnym do prawidłowego funkcjonowania organizmu człowieka. Ponad 300 enzymów zawierających cynk jako kofaktor jest bezpośrednio zaangażowanych w katalizie. Pacjenci z niedoborem cynku cierpią na poważne dysfunkcje immunologiczne. Niedobór cynku objawia się opóźnieniem wzrostu, zmianami skórnymi oraz zaburzeniem gojenia ran, anemią i opóźnieniem umysłowym. Objawy chorobowe ustępują w wyniku suplementacji nadmiarem cynku. Cynk powoduje zmniejszenie stężenia markerów stresu oksydacyjnego i stymuluje wytwarzanie cytokin. Cynk odgrywa również znaczącą rolę w chorobie Parkinsona (PD), w stwardnieniu rozsianym (MS), w chorobie Alzheimera i w chorobie Wilsona. Ponadto cynk jest zaangażowany w transdukcji sygnału i w apoptozie, np. prawidłowe stężenie cynku w organizmie indukuje apoptozę w komórkach gruczołu krokowego. Wśród czynników transkrypcyjnych wyróżniamy białka zawierające palce cynkowe i podobne motywy strukturalne. Typową funkcją palców cynkowych są interakcje i łączenie się z wieloma różnymi związkami, takimi jak kwasy nukleinowe, białka i małe cząsteczki. Poza tym cynk jest modulatorem synaptycznej transmisji w centralnym układzie nerwowym.