Review paper

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Inhibition of respiratory processes by overabundance of zinc in neuronal cells

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Abstract

Studies suggest that excessive amounts of free zinc ions can cause neuron death by interfering with the energy production process. The sites of the cell oxidation activity of zinc are the glycolytic enzymes, the Krebs cycle components and the respiratory chain. Further consequences of reduced access to energy are: increased production of reactive forms of oxygen, decrease of the mitochondrial membrane potential and decreased level of ATP. Also, the toxicity of zinc accelerates the supply of extra amounts of this element to the mitochondria, which results in their increased permeability.

Key words: *zinc, neurotoxicity, metallothionein, tricarboxylic acid cycle, electron transport chain.*

Introduction

Zinc (Zn) occurs in the brain at the level of ~ 200 µg per mg of protein. According to Frederickson [13], in the central nervous system (CNS) there are three pools of Zn:

a) ca 80% of zinc occurs as protein-bound zinc: a bound pool or so-called "inactivated" Zn;
b) another pool of zinc occurs in the synaptic vesicles – this pool can be exposed through histochemical staining and constitutes about 10% of overall zinc content in a cell. This Zn locally co-exists with glutaminic acid and, similar to glutaminic acid, is released into the synaptic space [24].
c) still another pool of zinc, so-called "free" zinc, not bound to proteins.

Studies confirm that the toxicity of zinc shows up when there is an increase in the third fraction or free zinc in a cell. The increase of Zn^{2+} level can be triggered by some factors that cause damage to the mechanisms maintaining the physiological values of zinc [3-5,18,30,31]. The authors suggest that 300 nM is a toxic value for cortical neurons.

Neurotoxicity of zinc was demonstrated in animal models, in which a stroke, ischaemia, Alzheimer's disease or convulsions were induced [4,9,11,25]. The detailed mechanism of the toxic activity of zinc is not known, but it seems that the main cause of neuronal death is low energy production. The question arises of how the mitochondria – the sub-cell entities specializing in energy production – contribute to the death of a neuron [14].

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Zinc level control in a cell

In order to prevent intracellular zinc from exceeding the critical values, it has to be chelated and its excess removed. Many cells, including neurons, have two ways in which to take up zinc: carrier-mediated transport, and through voltage-gated channels [16]. Neurons, like most cells, have several transporting proteins at their disposal: those within the membrane, responsible for the uptake and removal of excessive Zn, and transporting proteins in the membranes of intracellular organelles, responsible for its sequestration [16,29]. Inside the cell, on the other hand, metallothioneins are the proteins responsible for chelating most of Zn [12,23,26]. Metallothioneins not only bind Zn, but also mediate in passing it on to other proteins (zinc proteins) which require zinc ions to operate and which co-operate with the transporting proteins within the cell membranes [2].

Excessive amounts of zinc and the glycolytic process

Zinc blocks two enzymes of the glycolytic process: phosphofructokinase and glyceraldehyde 3-phosphate dehydrogenase (Fig. 1) [21]. Considering the

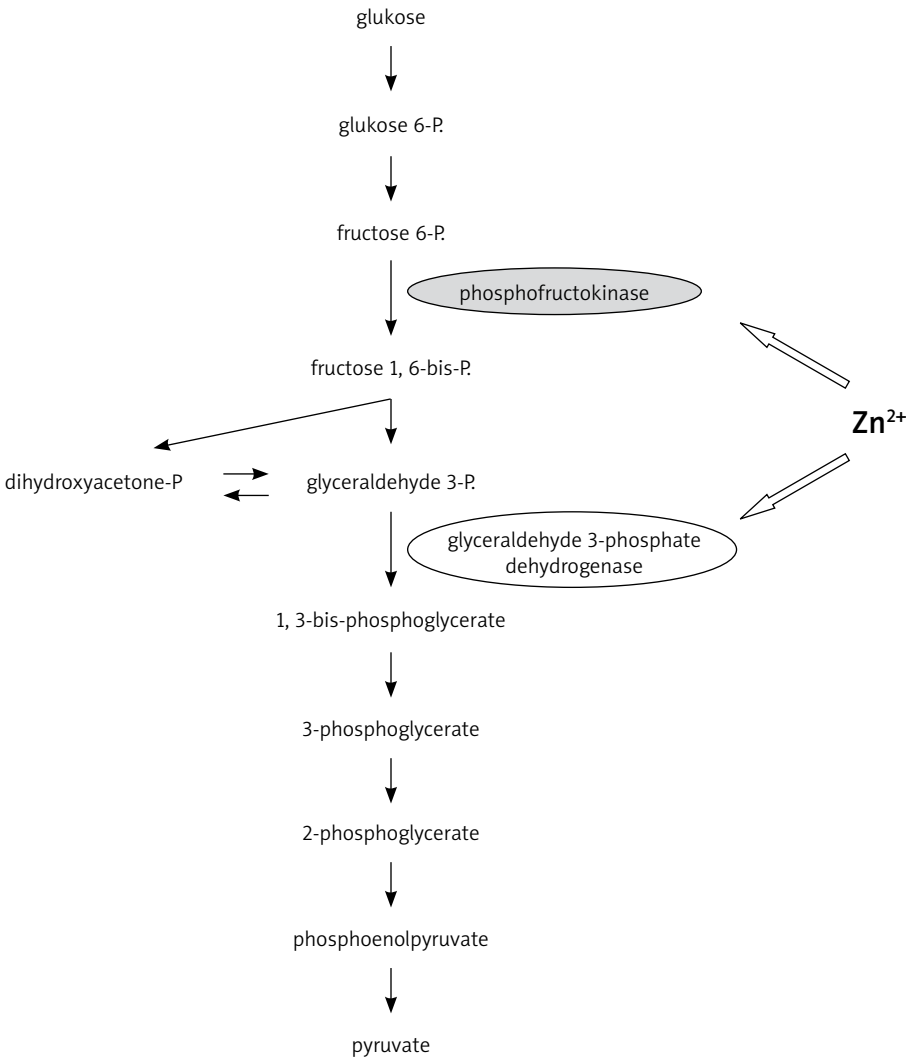

Fig. 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphofructokinase are two glycolytic enzymes that might be impaired when cells experience elevated Zn^{2+}.

special role that glyceraldehyde 3-phosphate dehydrogenase (GAPDH) plays in the glycolytic pathway, the inhibition of this enzyme can be highly unfavourable for a cell. GAPDH inhibition is accompanied by accumulation of metabolites above and their decrease below the site of activity of the enzyme. The enzyme, co-operating with co-enzyme NAD, controls GAPDH transformation into an energy-rich intermediary, i.e. 1,3-diphosphoglycerine acid. There are two stages in the process. In the first stage there is dehydrogenation (oxidation) of the substrate and transfer of the hydrogens to NAD (substrate oxidation is a prerequisite for the creation of a high-energy bond). NADH + H co-enzyme regeneration happens through the passage of hydrogen atoms along the respiratory chain. The second stage of the reaction consists in attaching the phosphatic remnant from the environment to the oxidated substrate and producing 1,3-diphosphoglyceric acid.

The evidence of zinc complicity in the process of GAPDH inhibition is partial normalization of glycolysis following the supply of extracellular pyruvate [27].

The influence of excessive amounts of zinc on the tricarboxylic acid cycle and on the respiratory chain

Studies by Brown showed that zinc inhibits a key enzyme in the TCA cycle, namely α-ketoglutarate dehydrogenase (KGDHC) (Fig. 2). Gazarin's team [15] has identified the site of the inhibition using an isolated KGDHC from animal hearts. It turned out to be a diphosphate binding in the enzymatic protein of lipoamide dehydrogenase (LADH) – an enzyme constituting the KGDHC complex. What is more, LADH inhibition by Zn was also associated with the production of reactive forms of oxygen (ROS).

The process of inhibition of the chain of electron transport by zinc was first described by Skulachev [28]. It was then that the site of activity of zinc was determined as cytochrome b and c_1. That initial discovery prompted further study of the importance of zinc for the respiratory chain [17]. Lorusso et al. [20] as well as Link and von Jagov in 1995 [19] confirmed the site of activity of zinc as being the complex of cytochromes bc_1. Link and von Jagov [19] suggested that zinc inhibits the Q-cycle (co-enzyme Q cycle) in the vicinity of Q_p. The binding of zinc and inhibition of bc1 cytochrome complex took place at Zn concentrations of 100-200 nM, which is close to pathophysiological values.

The latest studies suggest that zinc inhibits respiration in the mitochondria extracted from the brain [6]. The above-mentioned authors used 200 nM zinc concentration and demonstrated a decrease of oxygen consumption and lowered values of proton gradient in the mitochondria. In their studies they used substrates for complex I and II of the respiratory chain and a substrate for glyceraldehydes 3-phosphate dehydrogenase.

Zinc-mediated changes in mitochondrial permeability

In physiological conditions pores in the mitochondrial membrane allow the passage of molecules ca 1.5 kDa in size. Disturbed function of the mitochondria and their damage affect the permeability of the pores, which become non-selective. This is a critical situation leading to both apoptotic and necrotic death of a cell [10,22].

The regulation of pore permeability has not been fully explained yet; nevertheless there are a few known substances which affect the activity of the pores. Magnesium (Mg) ions, adenine nucleotides, low pH and cyclosporine A are known to block the pores, whereas a low proton gradient ($\Delta\psi_m$ energy), high level of calcium in the mitochondrial matrix and oxidative stress contribute to increased permeability of the mitochondrial membranes.

The consequence of pore selectivity loss is, among other things, swelling of the mitochondria, leakage of calcium from the storage places, and the outflow of many molecules including glutathione, cytochrome c and the apoptosis-inducing factor (AIF) [33].

Studies concerning the role of zinc in the changes of mitochondrial permeability found swelling of the mitochondria and the escape of glutathione (GSH) [1,32,34]. The effects caused by zinc could be reversed by adding magnesium ions.

Studies which used isolated mitochondria extracted from the brain found that there was a (200 nM concentration) zinc-induced change in the permeability, the swelling of the mitochondria, the efflux of cytochrome c and AIF, and a decrease of $\Delta\psi_m$ energy. The effects caused by zinc toxicity were reversed by adding EGTA [7,8].

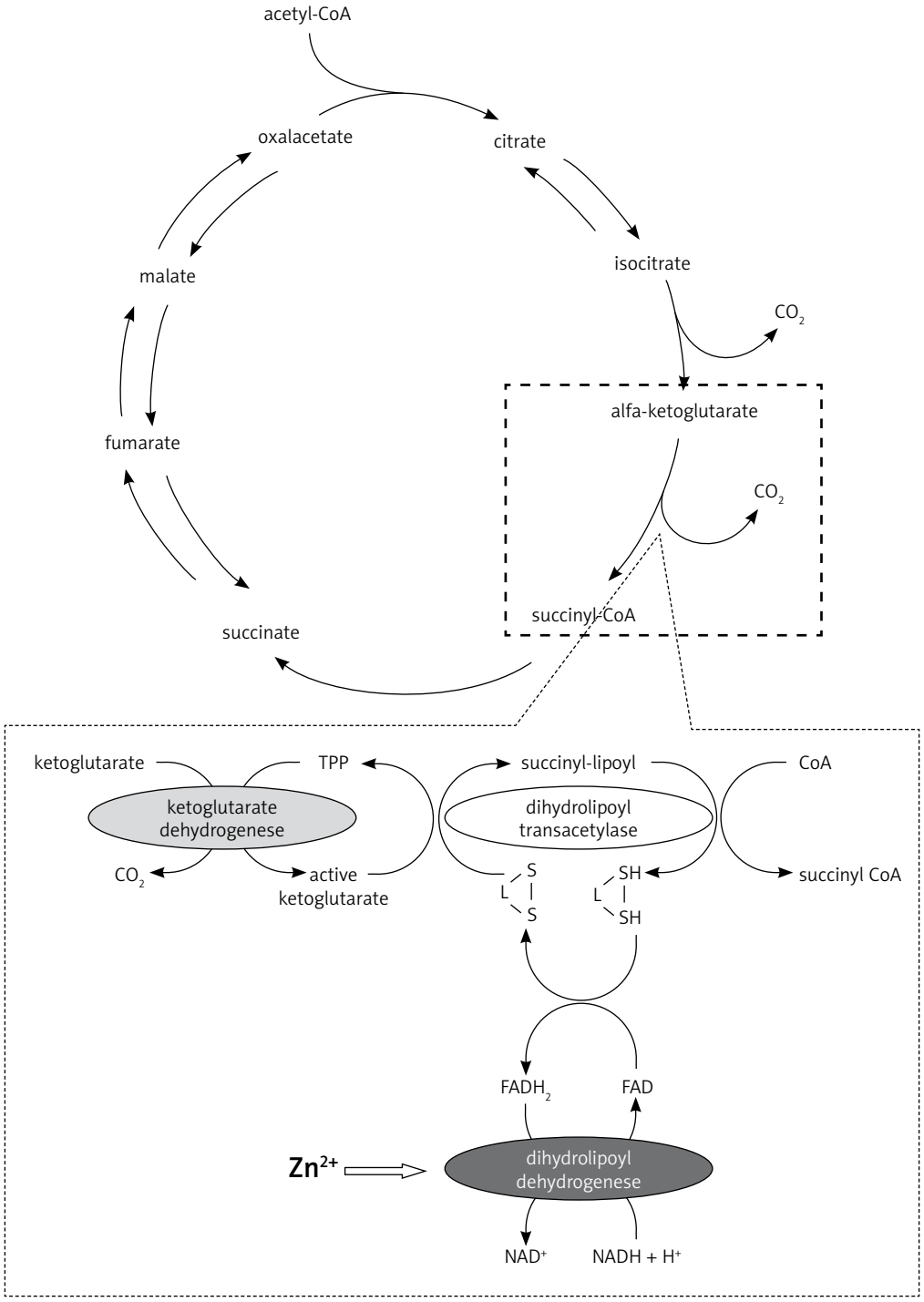

Fig. 2. The zinc inhibited the α-ketoglutarate dehydrogenase complex (KGDHC) of the TCA cycle. The alpha-ketoglutarate dehydrogenase complex is a multimolecular aggregate of three enzymes: ketoglutarate dehydrogenase (also called a decarboxylase), dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase. This complex converts alpha-ketoglutarate to succinyl CoA. The alpha-ketoglutarate complex contains five coenzymes that act as carriers or oxidants for the intermediates: thiamine pyrophosphate, lipoic acid, coenzyme A, FAD and NAD. Using KGDHC isolated from porcine heart, the site of inhibition was subsequently identified as a catalytic disulfide of the lipoamide dehydrogenase (LADH) subunit.

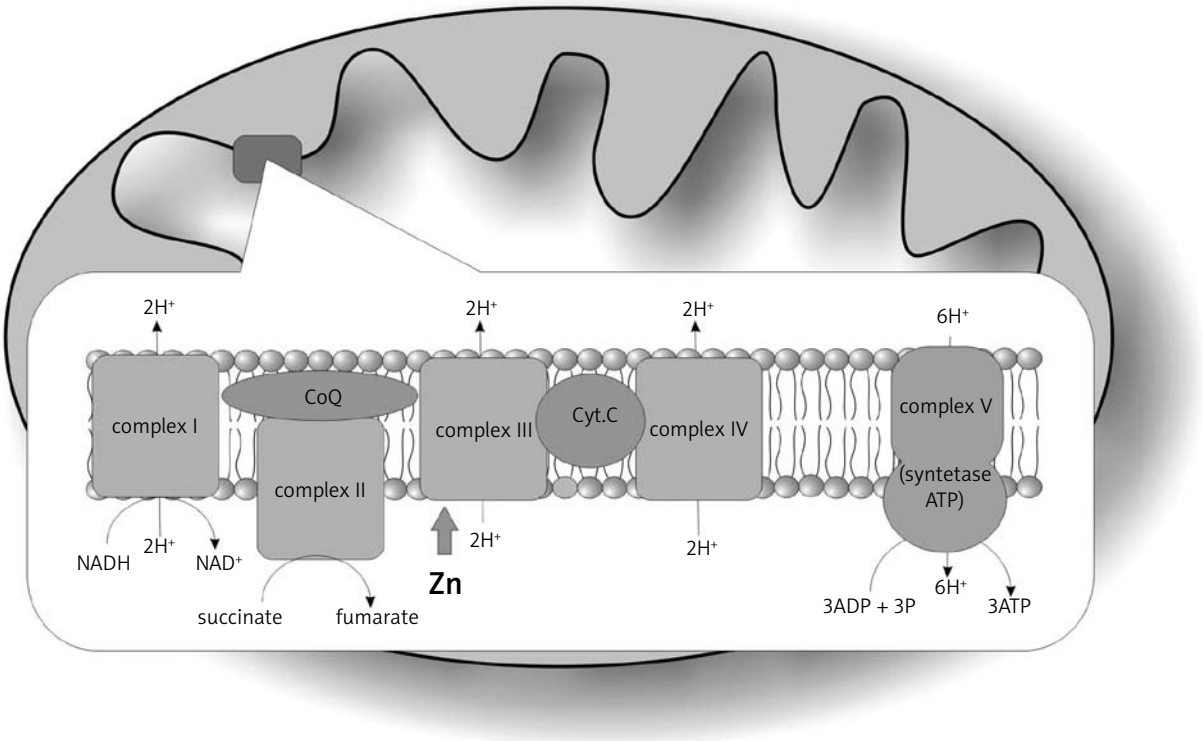

Fig. 3. Zn^{2+} inhibition of the electron transport chain between cytochromes *b* and c_1 (complex III). The main components of complex III are the cytochrome b, cytochrome c_1 and ferric-sulphuric proteins. Complex III of the respiratory chain is a place where the Q cycle takes place. At the level of complex III there is ionization of hydrogen atoms ($H = H^+ + electron$) with resulting hydrogen cations and electrons. The hydrogen cations are expelled into the intramembrane space and the electrons are passed on to the oxygen via a series of cytochromes.

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