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Reviews

Trace elements that act as antioxidants in parenteral micronutrition

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The trace elements — copper, manganese, selenium, and zinc — act as cofactors of antioxidant enzymes to protect the body from oxygen free radicals (OFR) that are produced during oxidative stress. It is necessary to maintain a balance between the harmful pro-oxidant components produced and the antioxidant compounds that counter these effects. A delicate balance also exists for the redox trace elements such as copper, which can initiate free radical reactions but is also a cofactor of Cu/Zn-superoxide dismutase, a free radical scavenging enzyme. Metal chelators such as ceruloplasmin play an important function to contain the reactive Cu ion. Similarly, transferrin and transferrin receptor maintain homeostatic control of iron, allowing little or no free iron to participate in formation of the reactive hydroxyl radical. Selenium is found to be most severely deficient in traumatized patients who need adequate supplementation during parenteral micronutrition to assist the free radical scavenging activity of glutathione peroxidase and the immune system. (J. Nutr. Biochem. 9:304–307, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Patients with compromised nutritional status who cannot have oral or enteral intake of nutrients are placed on parenteral feeding, which, in addition to providing the basic nutrients, is supplemented with essential trace elements.[1] These trace elements typically include chromium (Cr; 15 μg), copper (Cu; 1.5 mg), iodine (I; 120 μg), manganese (Mn; 2 mg), selenium (Se; 100 μg), and zinc (Zn; 5 mg) over a 24-hour period (amounts as recommended for a 70-kg adult).[2] A number of these elements play a primary role in the body's antioxidant process, protecting the tissues from harmful oxygen-free radicals (OFRs). Iron (Fe) in its ferrous complexed state is an essential component of hemoglobin, but in its ionized free state it can oxidatively work with hydrogen to produce peroxide toxic hydroxyl radicals. There are a number of physiologic and pathologic sources of OFRs, such as ischemia reperfusion injury (activation of xanthine oxidase from xanthine dehydrogenase), respiratory-burst (as from leukocyte and macrophage oxygen uptake/conversion into superoxide anion in bacteria and virus opsonization), autooxidation of molecules (such as catecholamines), and leaks of anion radicals from mitochondrial electron transport systems.[3] These OFRs, which are generated in physiologic amounts in living cells, are not allowed to accumulate, but are removed by the body's antioxidant defense system. Excessive amounts of OFRs that would be cytotoxic to the cells can exceed the bodies defense mechanism and result in oxidative stress or cellular necrosis.

This study describes the major trace elements — Cu, Mn, Zn, Fe, and Se — which play a role in the formation of OFRs or act to protect the body from these harmful products. These trace elements are redox catalysts, form part of the active site necessary for the antioxidant function, or act as cofactors in the regulation of antioxidant enzymes.

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Free Radical Scavenging Enzymes

Superoxide dismutase:

Cu/Zn-SOD $2O_2^{\cdot} + 2H^+ \rightarrow H_2O_2 + O_2$

Mn-SOD $Mn^{2+} + O_2^{\cdot} + 2H^+ \rightarrow Mn^{3+} + H_2O_2$

Glutathione peroxidase:

Se-GPx $ROOH + 2GSH \rightarrow ROH + H_2O + GSSG$

R = carbon group, GSH = reduced glutathione, GSSG = oxidized glutathione
$O_2^{\cdot}$ = oxygen free radical

Figure 1 Enzymatic reactions for the removal of oxygen free radicals and hydrogen peroxide.

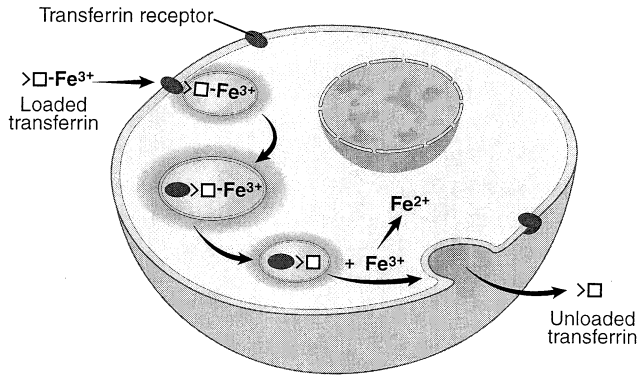

Figure 2 Uptake of iron from transferrin to a cell with transferrin receptor.

Copper, zinc, and manganese: Superoxide dismutases

Superoxide dismutase (SOD; EC 1.15.1.1) catalyzes the dismutation of superoxide radicals to oxygen and hydrogen perioxide.[4] It was previously known as cytocuprein (copper containing cytoplasm protein), which exists as a family of metalloproteins and is distributed in cells and tissues of erythrocytes (erythrocuprein), the liver (hepatocuprein), and the brain (cerebrocuprein).[5] Human tissue contains three different forms of SOD: Cu/Zn SOD, which is distributed in the cytoplasm, Mn SOD, which is present in the mitochondria,[6] and extracellular SOD (EC SOD) in the extracellular space, which also contains Cu and Zn.[7] EC SOD, a tetrameric high molecular weight enzyme, is found in several extracellular fluids including plasma, lymph, and synovial fluid.[8]

These free radical scavenging enzyme reactions are as shown in *Figure 1*. Cu, the transition metal, plays a key role as a cofactor regular in the transcription and posttranslation of Cu/Zn SOD, as illustrated in yeast. Lack of Cu^{2+} reduces the synthesis of Cu/Zn SOD mRNA as well as the insertion of this ion into the apoenzyme to form the active enzyme.[9] Diets deficient in Cu or the use of metal ion chelators have reduced the expression of Cu/Zn SOD activity in human tissues. Proteins such as metallothionein and ceruloplasmin bind Cu ions to prevent this transition metal from catalyzing hydroperoxide decomposition to free radicals.[9]

As shown in rats, a deficiency of Zn had only marginal effect on the expression of Cu/Zn SOD in erythrocytes.[10] Zn appears to have an indirect effect as an antioxidant, possibly by stabilizing sulfhydryl groups to prevent intramolecular disulfide formation, by competing with Cu and Fe binding sites that trigger electron transfer, and by reducing free radical production in neutrophils.[11] A deficiency of Zn also appears to compromise the alveolar-capillary membranes that protects against hyperoxia-induced lung damage in rats.[11]

Manganese is required for activity of the manganoenzyme Mn SOD to protect the mitochondrial membrane from free radical damage. Most of the Mn SOD is located in the mitochondrial matrix, but can occur in the liver cytosol as "extra" Mn SOD in cases of copper deficiency in which more of Mn-SOD is formed to compensate for less CuZn SOD.[12] Mammalian Mn SOD exists in human liver as a tetramer subunit with 4 moles Mn per mole enzymes.[12] Methods for the measurement of Mn SOD include immunoassay for human serum (not found in erythrocytes) and tissue,[13] and a copper blotting technique for tissues.[14]

Iron

Fe, the transition metal, acts as an important mediator in cell injury accompanying oxidative stress. The formation of extremely reactive hydroxyl radicals ($^{\cdot}OH$) occurs by means of the Fenton reaction: Fe (II) + $H_2O_2 \rightarrow$ Fe (III) + $^{\cdot}OH + OH^-$ in which ferrous Fe reduces peroxide. The hydroxyl radical can rapidly interact with DNA, protein, and lipids to induce oxidative injury.[15] In vivo, the transport protein transferrin binds Fe to keep it from participating in trace element-dependent reactions, and this chelation acts as an important antioxidant mechanism. Transferrin receptor (TR), a plasma protein receptor, allows control of the intracellular Fe homeostasis (*Figure 2*) to maintain little or no "free" Fe in the plasma.[16] For patients who require Fe supplementation to their total parenteral nutrition (TPN) solutions, free-Fe admixtures that can induce formation of free radicals should be replaced with bound-Fe such as Fe dextran to protect against spontaneous generation of hydroxyl radicals.[17]

Selenium

Key antioxidant enzymes contain selenium at their active sites, such as if found in selenocysteine, a pivotal prosthetic group component of glutathione peroxidase (EC 1.11.1.9, GPx). This selenoprotein breaks down harmful hydrogen peroxide, the end-product of the superoxide dismutase reaction, into oxygen and water.[18] After the discovery of this cellular enzyme (GPx1) in 1973,[19] several forms of selenocysteine containing selenoproteins have been reported, which include phospholipid hydroperoxide glutathione peroxidase (GPx4) in 1985,[20] extracellular glutathione peroxidase (GPx3) in 1986,[21] selenoprotein P in 1987,[22] type I iodothyronine deiodinase in 1991,[23] mitochondrial capsule selenoprotein in 1992,[24] gastrointestinal glutathione peroxidase (GPx2) in 1993,[25] selenoprotein W in 1993,[26] and type III iodothyronine deiodinase in 1995.[27] The biological roles of many of these selenoproteins, which include acting as a storage/transport form for Se or as an antioxidant enzyme, are under investigation.[28,29]

Some clinical changes associated with Se deficiencies include cardiomyopathy (Keshan and Kashin Beck diseases[30]; some of the effects of Se supplementation include its ability to decrease the incidence of cancer of the lung, colon/rectum, and prostate, although it has no effect on basal cell or squamous cell carcinoma of the skin on which the study was originally based.[31] The mechanisms for these conditions could include OFR, selenoprotein antioxidant activity, and viral diseases, as reported in 1996 at an international conference on Se in human viral diseases.[32] At this conference, studies were presented that link Keshan disease with deficiency of Se or vitamin E and infection with Coxsackie virus B3,[33] and Se deficiency with other diseases such as human immunodeficiency virus and acquired immunodeficiency disease[34] and liver cancer.[35]

The cofactor role of Se parallels the antioxidant and free radical scavenging action of vitamin E. Se can substitute for vitamin E to protect microsome and lysosome membranes from lipid peroxidation. Generally these nutrients support rather than replace their respective antioxidant functions because vitamin E is more lipophilic while Se is hydrophilic. Se supplementation is commonly used in parenteral nutrition to avoid deficiency symptoms as first reported in 1979.[36] It is important that infants on long-term TPN receive adequate supplementation that is based on protein intake such as at levels of 0.5 to 1.0 μg Se/g protein;[37] for short-term TPN, Se repletion in amounts of 3 μg of Se/g protein is required.[37] The Subcommittee on Pediatric Parenteral Nutrient Requirements (American Society for Clinical Nutrition) recommends that preterm and term infants and children be given 2 μg/kg/d Se up to a maximum of 30 μg/d only if the patients will be receiving TPN for longer than 4 weeks.[38] Adults on short-term TPN may be adequately maintained at 80 μg Se/d, but depending on the clinical condition, of the patient, such as in patients with inflammatory bowel disease or enterocutaneous fistulae, up to 120 μg/d or more may be needed to bring adult patients to an acceptable level.[39] In severely traumatized patients, Se, erythrocyte, and serum GPx are decreased.[40] Forms of Se supplementation of TPN include sodium selenite, selenious acid, and selenomethionine. Selenite in its sodium form or as selenious acid is rapidly incorporated into glutathione peroxidase.[41] Selenomethionine has caused concern that it may accumulate in the muscles because it is well absorbed, retained, and found to be evenly distributed into organs and tissues at higher levels (up to ten-fold) than the selenite form.[37] Toxic accumulations can be avoided by the use of controlled physiologic dosages and the monitoring of the serum and erythrocyte concentrations of Se and glutathione peroxidase both before and at periodic intervals following supplementation.

Other trace elements

Redox-active trace metals as cobalt,[42] chromium,[43] and nickel[44] have been proposed to involve OFR formation. Incubation of 1 mM Cr (III) with 10 mM hydrogen peroxide at physiologic pH can generate hydroxyl free radicals, which may have implications in chromium-induced carcinogenesis.[43] Cr in trace quantities is considered essential for glucose tolerance and lipid metabolism,[45] whereas its oxidative role requires further investigation. Molybdenum (Mo) is an essential cofactor of several enzymes such as aldehyde oxidase, sulfite oxidase, and xanthine dehydrogenase.[46] Xanthine dehydrogenase is converted into xanthine oxidase during ischemia, which results in microvascular injury following tissue reperfusion.[47] OFRs are generated from adenosine triphosphate as it is catabolized into uric acid. Deficiency of Mo also affects sulfite oxidase activity, which can result in multiple neurologic problems.[46]

Antioxidant potential

Total antioxidant levels have been found to be decreased in patients who have experienced critical clinical situations such as reperfusion injury following organ transplantation, cardiopulmonary bypass surgery, septic shock, and renal dialysis.[48–51] Decreased antioxidant status can result from deficiencies in trace elements that can be corrected by adequate supplementation with TPN solutions, and the activity of the antioxidant enzymes, SOD, and GPx can be monitored before and during therapy. Preanalytical factors such as age, gender, and smoking can affect the antioxidant enzyme reference intervals as described for CuZn SOD, GPx, catalase, and glutathione reductase in human erythrocytes.[52]

Summary

Trace elements such as Cu, Fe, Mn, Se, and Zn play key roles in the process of OFR formation and act as cofactors in the regulation of the antioxidant enzymes Cu/Zn SOD, Mn SOD, and GPx. These metal redox ions act rapidly at the pretranslational and posttranslational levels of the antioxidant enzymes in response to small and fleeting increases in OFR. Metal-sequestering proteins such as transferrin and ceruloplasmin safeguard the cells against free metal ions, which can trigger the formation of dangerous hydroxyl radicals such as by the Fenton reaction. In patients who require TPN, these trace elements must be provided in sufficient amounts to generate antioxidants that scavenge the accumulated pro-oxidants formed by the oxidative stress. Re-establishment of the balance between pro-oxidant and antioxidant elements allows the cells to regain their normal physiologic function.

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