Physiological and nutritional importance of selenium

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Summary. The essential trace element selenium has recently attracted attention because of its potentialities in the maintenance of human health. Selenium forms part of the active site of the peroxide-destroying enzyme glutathione peroxidase, and it also has other functions, for example in biotransformation, detoxification and the immune response. Functional and clinical consequences of selenium deficiency states have been described, and the selenium requirement, which is influenced by the usual selenium exposure, has been discussed. Wide variations have been found in selenium status in different parts of the world, and populations or groups of patients exposed to marginal deficiency are more numerous than was previously thought.

Current research activities in the field of human medicine and nutrition are devoted to the possibilities of using selenium for the prevention or treatment of degenerative or free radical diseases such as neurological disorders, inflammatory diseases or cancer. Pharmacological selenium doses are also recommended as an adjuvant in some treatments.

Key words. Selenium; glutathione peroxidase; free radicals; peroxidation; metabolic modulations; detoxification; immunity; degenerative diseases; cancer; supplementation.

Introduction

Selenium was first discovered in 1817 by the Swedish chemist J. J. Berzelius. It was considered for a long time to be a toxic element for humans and animals, causing severe poisoning in some regions. In 1957, Schwarz and Flotz[87] demonstrated that it is essential for animals, by using it to prevent nutritional deficiency diseases. This effect was influenced by vitamin E and sulfur containing amino acids. Some years later, the biochemical role of selenium in mammals was clearly established by the discovery that it is part of the active site of the peroxide-destroying enzyme glutathione peroxidase[78]. Finally, less than 10 years ago, its essentiality for man was proved by the successful prevention of an endemic fatal cardiomyopathy affecting children and young women in China, and called 'Keshan' disease[110] and, nearly simultaneously, by the treatment with selenium of a muscular dystrophy appearing in a patient on long-term parenteral nutrition[103]. From that time on, marginal or pronounced selenium deficiency states have been identified in many population groups, and the resulting clinical consequences have been described. An important role for selenium was established in the prevention as well as the treatment of chronic and/or degenerative diseases in humans.

Many books and reviews have recently been devoted to this intriguing element[27, 40, 41, 55, 57, 59]. The present contribution will deal particularly with two aspects: the established biological functions of selenium, and their implications in human medicine.

Its importance in nutrition will be underlined by an assessment of the adequacy of selenium status in various parts of the world, including European countries.

Essentiality and biological functions of selenium

The best-known biochemical role for selenium is as part of the active site of the enzyme glutathione peroxidase. This enzyme, present in the cytosol and mitochondrial matrix of cells, as well as in body fluids, is formed by four identical subunits each containing one atom of selenium as selenocysteine. Its incorporation in the enzyme is the result of a particular mechanism which was recently elucidated by Stadtman[93]: a serinyl t-RNA is first phosphorylated by a specific kinase; then the phosphate moiety is exchanged either spontaneously or enzymatically with selenide, giving rise to selenocysteine t-RNA, which is specifically incorporated into the peptide backbone, giving rise to glutathione peroxidase. The authors demonstrated in bacteria the existence in the genetic code of a codon specific for selenocysteinyl t-RNA, the UGA codon, which is different from the UGU codon for cysteine[93].

The metabolic functions of the selenoenzyme are vital for cells, as it is a part of the mechanism responsible for the metabolism and detoxification of oxygen. Reduction of oxygen in the form of active species such as the superoxide ($O_2^{\cdot-}$) or hydroxyl ($OH^{\cdot}$) free radicals, and of hydrogen or organic peroxides (R-OOH), is due to the activity of various enzymes such as the copper, zinc or manganese-containing superoxide dismutases, the iron-containing catalase, and glutathione peroxidase (GPx). These have specific functions and different subcellular localizations; they act in a complementary manner but cannot substitute one for another[18, 59]. The various active oxygen species are used by the organism in numerous physiological reactions, such as the oxidation of fatty acids and alcohols, hydroxylation of molecules, formation of metabolites of arachidonic acid (conversion of 12-HPETE to 12-HETE in blood platelets), and bio-

synthesis of thyroxine, and also during phagocytosis[11,15,31,59,104]. Their intracellular concentration must, however, be controlled as beside having these beneficial effects, the active species may be harmful to cell structures and essential constituents, leading to oxidative damage (e.g. lipid peroxidation, enzyme inactivation, denaturation of nucleic acids).

Homeostasis is maintained by the activity of the metalloenzymes as well as of scavengers of free radicals such as the lipophilic vitamin E (tocopherols) and other small molecules like vitamin C (ascorbate) and reduced glutathione[59]. Any perturbation of this system, for example due to selenium deficiency leading to a loss of GPx activity, will first have biochemical consequences that, if not compensated by other defense mechanisms, will result in clinically identifiable diseases. These, generally called 'peroxidative', 'oxidative' or 'free radical' diseases[33], will be described later. Such a perturbation can be assessed by a number of parameters: the increase in serum malondialdehyde, which reacts with thiobarbituric acid, and is produced by the oxidative degradation of polyunsaturated fatty acids; the modification in concentration of exhaled hydrocarbons (ethane, pentane, hexane) that are liberated by β-scission of some lipids; the direct measurement of H_2O_2 or reduced glutathione; and finally by the occurrence in serum of large quantities of chloroform-soluble fluorescent compounds, mainly ceroid and lipofuscin pigments, that are direct products of in vivo lipid autoxidation processes[59,64].

As has already been indicated, GPx activity is largely dependent on selenium status unless intake drops below a certain level. A low selenium status is frequently associated with an impairment in GPx function, which can be measured in various body fluids or cells. Normal activity can be restored by selenium supplementation. However, above a certain level that can be considered as optimal for GPx function the enzyme activity does not further increase, which indicates that the selenium requirement is satisfied. Such a criterion has been used to assess the adequacy of selenium intake in some population groups[58].

Beside this essential activity, other important biochemical roles of selenium have been identified. The metabolism of some exogenous compounds, both organic and inorganic, is influenced by selenium status. Selenium deficiency exacerbates the toxicity of several xenobiotics, for example drugs, insecticides or halogenated hydrocarbons, that act by the production of toxic oxygen derivatives in the organism. Similarly, selenium supplementation alleviates the toxic effects of drugs and antibiotics as well as of many experimental chemical carcinogens[22]. Such an effect may be mediated both by GPx or by a more general activity on xenobiotic-metabolizing enzymes in the liver, particularly the thiol-containing ones, as well as by a regulatory effect on glutathione metabolism[22,108]. The interaction with minerals was thoroughly investigated after it had been proved that the element is very effective in the prevention of toxic manifestations due to exposure to cadmium, mercury, lead, arsenic or cis-platinum derivatives[24,65,86]. Different mechanisms of action were demonstrated. For example, biologically inactive selenides may be formed (Cd, Pb, Pt, Ag, Hg), which accumulate as granules in some organs. Selenium may divert toxic metals from binding with some vital proteins to binding to less important ones after the formation of active selenotrisulfide centers (Hg, Cd); or it may act by metabolic interference (with As)[24,65]. Such interactions can occur with selenium at physiological levels, and therefore be significant as defense mechanisms against some heavy metals, except under certain exposure conditions[48].

Another biochemical activity that has recently attracted attention is the modulation by selenium of inflammatory and immune responses in animals and, less frequently, in humans[5,38,70,92]. Some properties of phagocytic cells, such as chemotaxis, migration, ingestion and fungicidal activity, are indeed clearly dependent on the selenium level in the phagocyte[38,70]. Incubation of human neutrophils with selenium stimulates phagocytosis and bactericidal activity[7,101] but large doses are inhibitory. The effects are explained either through effects on GPx activity (generation of increased amounts of oxygen species during phagocyte simulation) or by modulation of leukotriene synthesis[38,70]. The element can also affect all components of the immune system. Selenium-deficient animals are more prone to infections, and their antibody titers are low[38,70]. Retarded immunity in deficient animals is improved by supplementation, and so are lymphocyte reactivity, and graft rejection is stimulated[38,70]. In deficient subjects, the proliferative response of lymphocytes to mitogens and antigens is decreased, together with lymphokine production and also cytotoxic and NK (natural killer) functions[7,70]. Moreover, selenium at certain levels has an antitumor activity, which is considered in cancer prevention and treatment[88,89,92]. This property is again mediated by GPx or by the maintenance of the intracellular pool of reduced glutathione[38,70].

Finally, other roles for selenium have been identified in some metabolic pathways or physiological functions. As has already been suggested, the element is implicated at different levels in the metabolism of arachidonic acid derivatives. In the cyclooxygenase pathway, it regulates the balance between the proaggregatory and vasoconstrictory metabolite, thromboxane A_2, and the antiaggregatory and vasodilatatory prostacyclin PGI_2, with important consequences for platelet aggregation[25,31,49,100]. A selenoprotein of 10,000 Da has been characterized in human muscle and considered to be connected with the muscular dystrophy appearing in selenium-deficient subjects[103]. Keratinoid selenoproteins have also been identified in human spermatozoa. Their responsibility in maintaining the integrity of flagella has been proved by the demonstration of cases of infertility

due to selenium deficiency[14]. Finally, considerable work has been devoted to the antiproliferative action of selenium against tumoral cells. This was partly attributed to an inhibitory metabolite of protein synthesis, selenodiglutathione, formed when selenium is given in supranutritional (or pharmacological) doses[88,89,106].

Selenium status and requirement

Normal selenium status, as assessed by various biochemical indices[58], varies widely from one part of the world to another. This situation is the consequence of differences in selenium intake, which is dependent on the selenium content in soils and hence in food, as well as on its bioavailability[96]. Moreover, physiological influences such as age (below 20 and above 60 years), pregnancy or lactation, and also many pathological conditions, are liable to influence selenium indices[12,17,44,102]. Plasma selenium[2,19,96] is one of the most useful parameters, reflecting selenium intake under different exposure conditions quite well. Usual levels vary from 20–30 µg Se/l in Se-poor countries (China, New Zealand...) to 100–200 µg Se/l in Se-rich countries such as the USA, Canada, Norway or Japan[96]. Most European inhabitants have normal levels in the range 50–110 µg Se/l, but significant differences occur between different countries. Values lower than 30 µg Se/l are generally associated with severe clinical deficiency syndromes such as skeletal muscle dystrophy and cardiomyopathy[103,110]. Selenium dietary intakes in different parts of the world are in the range of 25–150 µg Se/day. The intake for European countries ranges from 30 to 60 µg Se/day[58].

Human selenium requirements are not precisely known. In 1980, the US National Academy of Sciences estimated as safe and adequate for adults a selenium intake in the range 50–200 µg Se/day[23]. This estimation, which was derived from animal studies, has been widely considered to be the 'recommended' intake. However, some limitations of this range have now become apparent. In a metabolic study using the 'balance' technique, Levander and Morris[43] calculated that North American adults need approximately 1 µg Se/kg b.wt/day to stay in balance (80 µg Se/day for males and 57 µg Se/day for females), and therefore suggested that the selenium requirement was dependent on lean body weight. Applying this technique to populations with a selenium exposure lower than in USA resulted in lower values: 33–35 µg in Great Britain, 24 µg in New Zealand and less than 10 µg in China[16,94]. These discrepancies indicate that the physiological selenium requirement is also dependent on the usual selenium exposure[43,63]. Mechanisms for homeostatic regulation of body selenium were recently identified: when the regular selenium intake is low, the organism maintains its body selenium at a constant level by decreasing urinary excretion[77].

A more recent approach to the estimation of selenium status and requirement, by the measurement of GPx activity[68], seems more satisfactory. Indeed, this selenium-dependent biochemical activity gives a direct indication of the selenium status of the organism from a functional point of view[41,42,58]. When selenium intake is low, GPx activity is directly correlated to selenium status (as estimated by plasma selenium or selenium dietary intake); with increasing selenium intake, the enzyme activity achieves saturation (showing a 'plateau of activity') for selenium levels that can be considered as adequate for the selenium-dependent function[41,53]. Measurement of the peroxidase activity in erythrocytes can be used as a long-term indicator of selenium status, owing to the relatively slow turnover of red blood cells in the circulation[72]. However, measurement in platelets, which have a shorter life time, can be considered as a very sensitive way of estimating the adequacy of selenium status in some population groups[42,63]. Using such techniques, the minimal selenium requirement was estimated to be about 40 µg Se/day in China, 50–70 µg Se/day in North America, and 70 µg Se/day in Belgium[41,45,46,56,61,62,95]. The optimal plasma selenium level in some European countries was demonstrated to be in the range 110–135 µg Se/l, while the usual concentrations in these countries are in the range 60–85 µg Se/l[32,58,60,96]. These data demonstrate that the physiological selenium requirement is not met by the usual food intake of the inhabitants. To solve this problem, since lack of selenium can have a negative influence on people's health, some countries are now systematically introducing selenium supplements at various levels in the food chain[1,39,105].

Importance of selenium in human medicine

'True' or pronounced selenium deficiency has been partly described already. This state occurs in subjects from very low-selenium areas (e.g. some parts of China and New Zealand), or with a poor selenium intake, for instance patients on total parenteral nutrition, or subjects with malnutrition. Biochemical perturbations include a reduction in the indices of selenium status (selenium levels and GPx activity), and also an inability of some cells to metabolize H_2O_2, macrocytosis, hemolysis, and changes in activity of the enzymes reflecting liver and muscle function (ASAT, ALAT, CK)[9,10,20,28,37,63]. Clinical symptoms are: skeletal muscle dystrophy, nail abnormalities, skin and hair depigmentation, and finally cardiomyopathy which is generally fatal[20,21,37,107]. The abnormalities, except for established cardiomyopathy, can be reversed by the administration of various forms of selenium at a level of 100–500 µg Se/day. Prevention of deficiency, particularly in patients with total parenteral nutrition or malnutrition, can be achieved by selenium supplementation with 50–100 µg Se/day[10,54]. 'Keshan' cardiomyopathy in China is nowadays prevented by providing 150–300 µg Se per week in subjects prone to selenium deficiency[110]. As was mentioned before, countries like Finland or Denmark give selenium

supplements to their whole population through adding it to animal feed or to fertilizers, or by foliar application [139].

Very few data are available from developing countries, and therefore it is quite impossible to obtain a clear picture of selenium status in a large part of the world and of the possible pathological consequences. A recent study from Africa suggests that selenium deficiency is a cofactor for neurological dysfunction accompanying iodine deficiency [30]. On the other hand, hemolytic anemia and immune dysfunction are clinical manifestations of selenium deficiency in the protein-calorie malnutrition occurring in these countries [28, 55, 57].

Marginal selenium deficiency states are more widespread than was previously thought, but the pathological implications of these states sometimes remain unclear. Low selenium status has been found in some neurological disorders as well as in aging. The element could be active in the prevention of the progressive failure of the immune system and the accumulation of peroxides and free radicals that are common features of *human aging* [64, 74]. Administration of selenium, together with other antioxidants, to elderly patients, had beneficial effects on the general condition as estimated by the Geriatric Scale [64, 97, 99]. In a similar study, a decrease in serum malondialdehyde concentration was observed with this treatment, as well as a significant inverse correlation between this lipid peroxidation by-product and blood selenium [64, 74, 97, 99]. In *neuronal ceroid lipofuscinosis*, a rare encephalopathy caused by the accumulation in the central nervous system of pigments that are products of lipid peroxidation, selenium deficiency was identified, as well as a marked negative correlation between glutathione peroxidase activity and neurological dysfunction. Treatment with selenium together with other antioxidants had some positive beneficial effects [85]. Rapid aging and cerebral degeneration are features of *trisomy 21*. They are related to increased peroxidative attack due to the hyperproduction of H_2O_2 by superoxide dismutase as a consequence of a gene dosage effect [52, 90, 91]. Increased GPx activity is observed in this condition, to deal with aggressive peroxides, but a marginal selenium deficiency simultaneously occurs, limitating peroxidase activity [91]. The link with the worsening of the clinical symptoms seems to be established by the relationship existing between GPx activity and the intelligence quotient of individuals [91].

Many patients with other 'peroxidative' or 'free radical' diseases [33] have been investigated for their selenium status, which has generally been found to be lower than normal [55, 57]. Relation of perturbed selenium status with biochemical or clinical parameters of disease activity were sometimes attempted, and significant relationships demonstrated. However, the effects of selenium supplementation were seldom considered. In hepatic diseases, especially *alcoholic cirrhosis*, blood and liver selenium are low, and peroxidative damage to the organ is apparent [3, 36]. Decreased selenium content has been related to an increase in prothrombin time, one index of hepatic function [25].

The pathogenic role of free radicals and other oxidative species has also been clearly established in *inflammatory rheumatic diseases*. Low blood selenium was significantly correlated with the number of affected joints and mobility in rheumatoid arthritis patients [89]. Peretz et al. [69, 71] found the lowest blood selenium levels in their functionally more affected patients, who were also those treated by corticosteroids. Administration of selenium (100–150 μg Se/day) to some rheumatoid arthritis patients had beneficial clinical effects in 40–45% of subjects [69]. Selenium deficiency is also a frequent feature of *cystic fibrosis*. Increased peroxidation products in these patients could be related to pulmonary insufficiency and frequency of infections [76]. *Renal insufficiency* with dialysis therapy is similarly characterized by the accumulation of various peroxidation products. Richard [75] observed in these patients a negative correlation between blood selenium and malondialdehyde, which suggested a protective role for the element. Finally, peroxidation is also present in some *muscular dystrophies*. Myotonic dystrophy was treated with success by selenium and vitamin E [66]. Possibilities were also explored by some authors in Duchenne muscular dystrophy [8].

Cardiovascular diseases have also been another area for research since 'Keshan' cardiomyopathy was characterized in China [110]. In non-obstructive cardiomyopathies and in coronary diseases, Oster [67] reported a positive correlation between plasma selenium and the left ventricular ejection fraction, confirming several studies in animals on the properties of selenium in cardiac function [6]. An inverse correlation was also demonstrated between plasma selenium and the severity of coronary atherosclerosis established by arteriography [4, 51]. In a group of people living in a low-selenium area, a relationship was established between plasma selenium and HDL cholesterol concentration [47]. An intervention study with selenium in these subjects resulted in a slight elevation of the fraction: HDL cholesterol/cholesterol. This indicated that the element can reduce the risk of coronary disease [47]. Several prospective epidemiological studies have been carried out, most of which showed that low plasma selenium (less than 45 μg Se/l in Finland) can be considered as a significant risk factor for cardiovascular disease [34, 50, 81–84].

A last fascinating field for selenium research is *cancer* prevention and treatment. The element has definitively proved to be a very potent anticarcinogenic agent in different models, with spontaneous, chemically induced or transplanted tumors, or in culture [80, 88, 89]. Large epidemiological studies recently confirmed the activity of selenium in the field of cancer prevention [80, 88, 89], and several intervention studies resulted in encouraging results. A trial using Se in chemotherapeutic prevention of viral hepatitis B in China, associated with liver cancer, achieved success: the addition of 15 ppm to kitchen salt

resulted in a reduction of disease incidence [111]. In cancer treatment, the element is nowadays recommended as an adjuvant of breast cancer therapy at a level of several hundred µg Se/day [35, 89]. This level must be considered as a pharmacological dose. Although Se has revealed some antiproliferative properties at nutritional doses (70–150 µg Se/day), it shows activity in many systems at levels higher than those required to optimize glutathione peroxidase activity [106].

Practical recommendations for the use of selenium in nutrition and medicine

Selenium supplementation with moderate doses (50–100 µg/day) is beneficial for health and should be recommended to people exposed to marginal selenium deficiency, including inhabitants of many parts of Europe. If supplementation is not undertaken on a large scale via food enrichment, under the supervision of governmental Health Authorities [1, 39, 105], self-administration is advisable by selection of selenium-rich foods (which is not easy in practice) or by taking commercial selenium supplements. These should be carefully manufactured under the control of specialized analysts, and their biological potency certified by clinical studies in humans. These requirements have unfortunately not been fulfilled for some proposed products. Medical doctors and nutritionists should keep in mind the high incidence of selenium deficiency states in some groups of patients [40, 41, 55, 57], and provide adequate supplementation when necessary. Higher selenium doses (several hundred µg Se/day) have proven activity in the prevention and treatment of cancer and of some other free-radical diseases. Although not toxic for humans, they should only be used for prolonged periods under the supervision of an informed health practitioner. Under normal conditions, a chronic selenium intake of less than 1000 µg Se/day in adults does not cause toxicity [73, 79, 109]. However, the health status of the candidate for selenium supplementation has to be carefully monitored before the introduction of pharmacological selenium doses, and follow-up of the patient is advisable.

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