Selenium and Sulfur in Antioxidant Protective Systems: Relationships with Vitamin E and Malaria (43430)

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Abstract. **The metabolic relationships among the antioxidant nutrients selenium, sulfur, and vitamin E are particularly close. Selenium and vitamin E have long been known to spare one another in certain nutritional diseases of animals, and selenium has been considered to have a key antioxidant defense function as a component of glutathione peroxidase. However, the antioxidant role of glutathione peroxidase has been questioned and new proteins containing selenium have been identified: phospholipid hydroperoxide glutathione peroxidase, selenoprotein P, and iodothyronine deiodinase. Glutathione peroxidase activity independent of selenium resides in the glutathione S-transferases. Glutathione participates in both enzymatic and nonenzymatic antioxidant defense systems. Some low-molecular weight selenium compounds (e.g., ebselen) exhibit glutathione peroxidase-like action. Certain low molecular weight thiols decompose peroxides nonenzymatically (e.g., the ovothiols).**

Murine malaria appears to be a useful experimental model for investigating interrelationships of selenium and vitamin E. Vitamin E deficiency protects against the parasite, especially when the mice are concurrently fed peroxidizable fat such as fish or linseed oils. Selenium deficiency, on the other hand, has little or no protective effect against the parasite. Any practical utility of pro-oxidant diets in combating human malaria remains to be determined. [P.S.E.B.M. 1992, Vol 200]

The metabolic relationships among vitamin E, selenium, and the sulfur amino acids have long interested biochemists studying the antioxidant nutrients. Dietary necrotic liver degeneration in the rat proved to be a very useful model for investigating these interrelationships in mammals. Either selenium or vitamin E independently prevented this disease, whereas the sulfur amino acids delayed the disease but did not really protect against it (1). There was considerable debate as to whether selenium and vitamin E were functioning merely as general antioxidants *in vivo* or whether these substances had more specific metabolic roles. Moreover, nutritionists were puzzled by the ability of traces of an inorganic mineral element such as selenium to substitute so completely for a complex lipid-soluble organic antioxidant molecule like vitamin E.

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Glutathione Peroxidase. The discovery by Rotruck and colleagues in 1973 that selenium was a constituent of the peroxide-decomposing enzyme glutathione peroxidase provided a new way to view the relationship of this nutrient with vitamin E (for a personal account of this breakthrough research, see Ref. 2). According to the scheme proposed by Hoekstra (3), vitamin E would act as a free radical trap and would prevent the formation of lipid hydroperoxides in membranes, whereas selenium, via glutathione peroxidase, would destroy any lipid hydroperoxides that were formed by peroxidation of polyunsaturated fatty acids that escaped the vitamin E protective mechanism. This hypothesis was very appealing because it appeared to tie together a vast body of experimental data related to the antioxidant properties of selenium, sulfur amino acids, and vitamin E. This hypothesis also stimulated a great deal of research into the nutritional biochemistry of selenium.

Glutathione peroxidase provided a convenient tool to nutritionists for assessing selenium status, since the activity of the enzyme is, in general, closely related to the dietary intake of bioavailable selenium over the nutritional range (4). Maximization of plasma glutathione peroxidase activity was used in a very practical way as the basis for establishing the first recommended dietary allowance for selenium in 1989 (5). On a more fundamental level, clarification of the biochemical steps by which selenium as selenocysteine is incorporated into glutathione peroxidase via a cotranslational mechanism that depends on a specific UGA codon in mRNA has provided new insights not only into the metabolism of selenium specifically, but also into the general molecular biology of protein biosynthesis as well (6).

However, some observations indicate that the role of selenium in glutathione peroxidase as an antioxidant defense mechanism is more complex than that originally suggested by Hoekstra (3). For example, glutathione peroxidase was found to have no activity against membrane-bound lipid peroxides and could only act against such peroxides after they were cleaved from phospholipids by the action of phospholipase A_2 (7). Several groups have now reported the existence of glutathione-dependent cytosolic or microsomal factors that inhibit lipid peroxidation, but are clearly distinct from glutathione peroxidase (8–10). Furthermore, Burk and colleagues (11) have been unable to demonstrate any correlation between increased glutathione peroxidase activity and the protection against diquat-induced lipid peroxidation afforded by selenium injections into rats fed selenium-deficient diets (11). The inability to delineate a precise antioxidant role for the selenium-dependent glutathione peroxidase led Burk to question whether this enzyme in fact functions as an oxidant defense (12). He also suggested that the enzyme might act as a regulated storage form of selenium (13).

Phospholipid Hydroperoxide Glutathione Peroxidase. In 1982, Ursini and colleagues (14) described a glutathione peroxidase from pig heart which, in contrast to the "classical" glutathione peroxidase, specifically reduced membrane-bound lipid hydroperoxides esterified in intact phospholipids. The activity of this enzyme, called phospholipid hydroperoxide glutathione peroxidase, which contains 1 g-atom of selenium as selenocysteine per subunit, decreases in mouse liver with dietary selenium depletion (15). A three-way interaction of phospholipid hydroperoxide glutathione peroxidase, glutathione, and vitamin E has been proposed in which all components are needed to inhibit effectively lipid peroxidation in rat liver microsomes incubated with iron and ascorbate (16). According to this scheme, phospholipid hydroperoxide glutathione peroxidase would prevent the formation of alkoxyl radicals by destroying lipid hydroperoxides, thereby sparing vitamin E and allowing the tocopherol to exert its chain-breaking action by interacting with lipid peroxyl radicals.

Nonselenium-Dependent Glutathione Peroxidase. Lawrence and Burk (17) found two peaks of glutathione peroxidase activity in chromatograms of rat liver supernate, one of which persisted in severe selenium deficiency. The persistent peak had activity against organic hydroperoxides, but not against hydrogen peroxide. Later work showed that this selenium-independent glutathione peroxidase activity is due largely to glutathione S-transferase B (18). Because this activity increases during selenium deficiency, this enzyme may play a partial compensatory role for the loss of selenium-dependent glutathione peroxidase.

Selenoprotein P. Burk and colleagues (19) have recently characterized in detail a glycoprotein (called selenoprotein P) that accounts for more than 60% of the selenium in rat plasma. This protein contains 10 selenocysteines per polypeptide chain, a deduction based on the presence of 10 TGA codons in the open reading frame of its cDNA. The protein is also rich in histidine and cysteine. Selenoprotein P responds to slightly lower dietary selenium intakes than does glutathione peroxidase activity and, therefore, provides a new tool for assessing selenium status (20). Although the function of this protein is not known, it could be involved in selenium transport or in protection against free radicals and other oxidants.

Iodothyronine Deiodinase and Other Selenoproteins. The discovery of Type I iodothyronine 5′-deiodinase (ID-I) as the second selenoprotein with well-characterized enzymatic activity warrants attention here. This enzyme performs the conversion of the prohormone thyroxine into the more metabolically active 3,3′,5-triiodothyronine. Arthur and Beckett (21) have shown that this conversion is impaired in selenium deficiency. These workers then found that a microsomal selenoprotein from rat liver with the same molecular mass as ID-I could be tagged *in vitro* with an affinity label known to bind to ID-I (22). On this basis, Arthur and associates suggested that ID-I is a selenoenzyme. Berry *et al.* (23) observed that the mRNA for this enzyme contains a UGA codon for selenocysteine that is necessary for maximal enzyme activity. In as much as ID-I requires a thiol group or its equivalent at its active site (24), a selenocysteine moiety may well be involved. On a more practical level, combined selenium and iodine deficiency have been linked with an increased frequency of endemic myxedematous cretinism in Central Africa (25). Any health benefits of selenium supplementation under such conditions, however, must await further research.

Gel electrophoretic evidence has led Behne and coworkers (26) to suggest the existence of as many as 13 selenium-containing proteins in rat tissues. While many of the proteins were found in several different tissues,

there were some tissue-specific proteins as well. The function of these new selenoproteins is not known, with the exception of Protein 7, which apparently is the Type I iodothyronine 5′-deiodinase (27) discussed above. Thus, additional roles for selenium in antioxidant protective systems and other biochemical reactions cannot be ruled out.

Nonenzymatic Antioxidant Protection Systems. Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one, also known as PZ-51) is a member of a new class of selenium-containing anti-inflammatory agents that exhibits glutathione peroxidase-like activity (28). The chemical structure of ebselen is thought to resemble that of the active site of glutathione peroxidase. The compound has a protective effect against lipid peroxidation induced in rat liver microsomes by ascorbate-iron-ADP mixtures. The sulfur analog of ebselen had no antioxidant effect in such experiments. Ebselen or related seleno-organic compounds may have therapeutic value in a variety of hydroperoxide-linked pathological conditions (29).

A number of natural compounds can serve as nonenzymatic antioxidant protectants, including tocopherols (vitamin E), ascorbic acid (vitamin C), carotenoids, uric acid, bilirubin, and certain plasma proteins such as ceruloplasmin (30). Glutathione can function physiologically in a nonenzymatic manner as a free radical scavenger, as it does after exposure to ionizing radiation (31). However, its main activities as an antioxidant are usually regarded as enzyme-catalyzed (30). The ovothiols (1-methyl-4-mercaptohistidines) appear to protect sea urchin eggs nonenzymatically against oxidative stress during the respiratory burst of fertilization (32).

Comparative Effects of Selenium and Vitamin E in Malaria

Much of the early nutritional research concerning selenium focused on its relationship with vitamin E, since either of these two nutrients could protect fully against certain dietary diseases in animals, such as liver necrosis in rats or exudative diathesis in chicks (3). However, selenium could not substitute for vitamin E in some other conditions, such as fetal resorption in rats or encephalomalacia in chicks. It was thought that even under conditions of adequate dietary selenium intake, glutathione peroxidase could not sufficiently protect the target tissues (fetal rat placenta or chick brain) in those cases.

Because the malarial parasite is highly susceptible to oxidative stress (33), this organism would seem to be an ideal model for studying interactions among antioxidant nutrients, such as selenium and vitamin E. We found that vitamin E deficiency strongly potentiated the therapeutic efficacy of qinghaosu (34), a Chinese traditional antimalarial drug (35) that acts by generating free radicals (36). Selenium deficiency had little or no effect on the activity of the drug. Furthermore, fish oil fed to vitamin E-deficient mice exerted a strong antimalarial effect in itself, whereas fish oil feeding had no antimalarial action in selenium-deficient mice.

Selenium, Vitamin E, and Qinghaosu. With its sesquiterpene endoperoxide structure, qinghaosu represents a new class of antimalarial agents. Rapid development of this drug is being encouraged by the World Health Organization as a possible way of dealing with the problem of chloroquine resistance (37). Qinghaosu is thought to kill the parasite through the action of oxy free radicals (36). Because we had demonstrated earlier an interaction between selenium deficiency in rats and adriamycin (38), another free radical-generating drug (39), we reasoned that an interaction of selenium and qinghaosu seemed likely. However, selenium deficiency had little or no effect on the suppressive or curative potency of a variety of doses of qinghaosu against *Plasmodium yoelii* in mice (34). On the other hand, vitamin E deficiency strongly enhanced the antimalarial efficacy of qinghaosu; at certain doses of the drug, most of the mice survived in the E-deficient group, whereas all of the mice died in the E-supplemented group.

These studies indicate the need to monitor vitamin E status in persons treated with qinghaosu; otherwise, possible inconsistent results with the drug could go unexplained. This could be particularly true for Third World populations, some of which are known to be in a state of marginal vitamin E nutriture (40, 41). But perhaps the most important result of the qinghaosu studies was that it led us to test the effect of fish oil against malaria in vitamin E-deficient mice.

Selenium, Vitamin E, and Fish Oil. Our original purpose in testing the effect of fish oil against malaria was to demonstrate a synergistic effect between peroxidizable dietary fat and the pro-oxidant drug qinghaosu in vitamin E-deficient mice (34). However, much to our surprise, the fish oil in itself had a highly potent antimalarial action in the vitamin E-deficient mice. We consequently began to focus exclusively on the therapeutic potential of such a pro-oxidant diet.

A variety of fish oils, fish oil concentrates, and linseed oil products all had substantial antimalarial potency when fed to vitamin E-deficient mice (42, 43). Such dietary intervention worked against chloroquine-resistant as well as drug-sensitive lines of the murine parasite (44). Severe vitamin E deficiency was not needed to obtain a beneficial effect, since some protection was obtained even when the fish oil diets were fed for only 1 week before infection (45). Some protection also was observed in mice fed fish oil diets containing low levels of tocopherol, thereby suggesting that an absolute vitamin E deficiency was not required to see a positive response (46). Certain synthetic organic antioxidants (e.g., *N,N′*-diphenyl-*p*-phenylenediamine) were

quite effective in blocking the antimalarial action of the vitamin E deficiency, whereas others (e.g., *tert*-butylhydroquinone) were not (47). Prolonged feeding of a selenium-deficient diet containing fish oil to mice (3 months) had no protective effect, despite the fact that erythrocyte glutathione peroxidase activities in the deficient group were less than 3% of those in the control group (47). Thus, it appears that the mouse/plasmodium host/parasite system offers a unique comparative biochemical model for studying the molecular interrelationships of antioxidant nutrients, particularly selenium and vitamin E. In contrast to other tissues in which selenium does not substitute nutritionally for vitamin E (fetal rat placenta or chick brain), the erythocytic stage of the malarial parasite is easily accessible experimentally and resides in a cell that is known to undergo wide diet-dependent variations in both glutathione peroxidase activity and vitamin E content. Therefore, this model could be a valuable tool for clarifying the specific metabolic roles of various antioxidant defense systems.

We are also hopeful that the pro-oxidant fish oil-containing diet described here may eventually be shown to have some use either in the treatment or in the prevention of human malaria. That a severe vitamin E deficiency is not needed to obtain some protective effect is encouraging in this regard. Perhaps our work will lead to fresh approaches to the disease that the World Health Organization considers to be "probably the most important infectious disease in the world" (48).

Concluding Remarks

Selenium, sulfur amino acids, and vitamin E have overlapping yet independent roles in antioxidant protective systems. The murine malaria parasite appears to offer a convenient tool for exploring the relationships among these antioxidant nutrients. Dietary manipulation of pro-oxidant stress by feeding peroxidizable fat (fish or linseed oils) may provide a promising new avenue for control of human malaria. The comparative study of nutrition-malaria interactions could yield profitable results in the future.

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