Vol. 107, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1982 Pages 75-81

EFFECTS OF INADEQUATE VITAMIN E AND/OR SELENIUM NUTRITION ON ENZYMES ASSOCIATED WITH XENOBIOTIC METABOLISM

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Received May 24, 1982

SUMMARY: Effects of inadequate vitamin E (E) and/or selenium (Se) nutrition on the activities of cytochrome P-450 mixed function oxidase system (heme hydroperoxidase, *p*-nitroanisole O-demethylase), and epoxide hydrolase have been investigated. Heme hydroperoxidase activity of liver and lung microsomes was significantly decreased in E deficiency. In the liver, Se deficiency resulted in a significant increase in hydroperoxidase activity. In contrast to the peroxidase activity, liver demethylase activity was only marginally affected in E/Se deficiency states. However, kidney demethylase activity was increased two fold in Se deficient states. Liver microsomal epoxide hydrolase activity was significantly increased in both E and Se deficiency states.

INTRODUCTION

It is known that polyunsaturated fatty acids (PUFA), which contain highly labile allylic and doubly allylic hydrogen sites, exhibit marked vulnerability to peroxidative damage initiated by free radical processes (1). Most biological membranes are rich in PUFA rendering them particularly susceptible to oxidative attack. Peroxidation of membrane lipids involves a complex set of reactions which leads first to the production of lipid hydroperoxides followed by their subsequent breakdown to highly reactive secondary products such as peroxyl, alkoxyl and hydroxyl radicals (2). However, dietary vitamin E (E) and selenium (Se) have been implicated in the protection of membrane lipids from such oxidative damage (3,4). Vitamin E, as an integral part of cell membrane structure, is visualized as a biological antioxidant preventing the

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0006-291X/82/130075-07$01.00/0

formation of lipid hydroperoxides by sequestering free radicals that initiate lipid peroxidation (3) whereas Se, as an essential component of Se-dependent glutathione peroxidase (Se-GSH-Px), has been proposed to reduce the hydroperoxides to less reactive alcohols (4). Increased lipid peroxidation, as a consequence of inadequate E and Se nutrition, could result in membrane disruption. Since the majority of the enzymes involved in drug metabolism are membrane bound, disruption of membranes could have profound effects on drug metabolic and detoxication processes. Therefore, because of their importance in the detoxication of xenobiotics as well as endogenously produced toxic intermediates, we have investigated the effects of inadequate E and/or Se nutrition on the heme hydroperoxidase and O-demethylase activities associated with cytochrome P-450 mixed function oxidase system (cyt P-450 MFO) and epoxide hydrolase (EH) activity.

METHODS

Animals and Diets: Post-weanling male Long-Evans Hooded rats were purchased from Charles River (Wilmington, MA) and fed on chemically purified, torula yeast-based diets formulated to provide adequate amounts of all known nutrients except as indicated below. Four conditions of E/Se dietary status were investigated: +E, +Se; -E, +Se; +E, -Se; -E, -Se. Selenium was supplimented as sodium selenite, 0.5 mg/kg diet, and E as d-α-tocopherol acetate, 150 IU/kg diet. The Se-deficient diets contained less than 0.01 mg Se/kg as measured by the method of Whetter and Ullery (5). The diets with inadequate E had undetectable total tocopherol levels as determined by the procedure of Taylor et al. (6). Tocopherol-stripped corn oil and lard supplied 36% of the total calories in these diets. The diets were prepared approximately every two weeks and were stored under refrigeration. In all experiments described the animals had been fed the experimental diets for 5 weeks.

Preparation of subcellular fractions: Microsomal and cytosolic fractions were prepared as previously described (7) with the following exceptions. Tissues were homogenized in 10 mM Tris-HCl buffer pH 7.4, containing 0.25 M sucrose. Microsomal pellets were suspended in 10 mM Tris-HCl buffer, pH 7.4, containing 1.15% KCl to a concentration of 15-20 mg of protein per ml.

Enzyme assays: Epoxide hydrolase activity was measured by a modification of the method of Jerina et al. (8) with styrene oxide (0.54 μmoles containing 0.4 μCi of 7[^{3}H] in acetonitrile) as substrate. To eliminate high background radioactivity resulting from slow non-enzymatic hydrolysis on long storage, radioactive styrene oxide was separated from its glycol as described by DePierre et al. (9) prior to use.

Hydroperoxidase activity associated with cyt P-450 MFO system was determined by a modification of the procedure of O'Brien and Rahimthula (10) with 1.5 mM cumene hydroperoxide (COP) as substrate and 1.0 mM

Table I

Enzyme Activities Associated with Cyt P-450 MFO in Tissues of Rats Fed Diets Deficient in E and/or Se for 5 Weeks

	Diet			
Enzyme	+E, +Se	-E, +Se	+E, -Se	-E, -Se
Hydroperoxidase activity[1]:				
Liver	125.5 ± 5.5[3,a]	19.1 ± 1.1[b]	240.5 ± 7.3[c]	58.1 ± 4.1[d]
Lung	49.2 ± 3.2[a]	6.9 ± 1.2[b]	35.7 ± 3.0[c]	20.8 ± 2.2[d]
Demethylase activity[2]:				
Liver	15.1 ± 1.5[a]	11.0 ± 1.0[a,b]	13.3 ± 1.0[a,b]	8.4 ± 0.4[b]
Kidney	1.0 ± 0.1[a]	0.6 ± 0.1[b]	2.4 ± 0.5[c]	2.2 ± 0.4[c]

1 Hydroperoxidase activity is expressed as nmoles TMPD oxidized per min. per mg protein.

2 Demethylase activity is expressed as nmoles *p*-nitrophenol formed per 15 min. per mg protein.

3 Values are means ± SE for 5-7 animals. Within a horizontal row means with different lettered superscripts are significantly different ($P < 0.05$).

tetramethyl-*p*-phenylenediamine (TMPD) as an electron donor. Sodium azide was included in the reaction mixtures to eliminate the oxidation of TMPD due to possible mitochondrial contamination of the microsomal preparation.

p-Nitroanisole O-demethylase activity was monitored by the procedure of Jensen and Clausen, (11).

Protein determination: Protein concentration was determined by the method of Lowry et al. (12) with bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Several forms of cyt P-450 exist in the microsomes of different tissues and the activities of these different molecular species differ with respect to different substrates (13). Therefore, assessment of the effects of dietary manipulation on the cyt P-450 MFO system via measurement of a single catalytic activity may not be indicative of overall effects. In anticipation of this problem, we measured the effects of E and/or Se deficiency on both the heme hydroperoxidase and O-demethylase activities of the cyt P-450 MFO system. As shown in Table I, E

and Se deficiency states appear to have opposing effects on liver microsomal heme hydroperoxidase activity. In E deficiency, the hydroperoxidase activity of both liver and lung microsomes was decreased by approximately 85% (+E, +Se vs -E, +Se). However, in the Se deficient state, hydroperoxidase activity was increased nearly two fold in liver microsomes (compare the +E, +Se and +E, -Se groups). In the combined deficiency state, hydroperoxidase activity was reduced only by approximately 50% in both liver and lung microsomes (+E, +Se vs -E, -Se). This may be the resultant of the combined reducing effect of E deficiency and elevating effect of Se deficiency. It should be noted that to differentiate the heme hydroperoxidase activity from cytosolic GSH-Px activity, TMPD, not GSH, was used as the electron donor. In contrast to heme hydroperoxidase activity, Se deficiency had no significant ($P > 0.05$) effect on liver microsomal demethylase activity. Similarly demethylase activity was only slightly decreased in E deficiency. However, kidney microsomal demethylase activity was significantly ($P < 0.05$) increased in Se deficiency states. Lung microsomal preparations showed very low or non detectable demethylase activity.

Little is known about the effects of E deficiency on heme hydroperoxidase activity. Our results suggest that the heme moiety of the enzymes of the cyt P-450 MFO system (especially the cyt P-450 form which catalyzes the reduction of peroxides) may be sensitive to peroxidative damage exerted by lipid hydroperoxides or free radicals formed as a consequence of E deficiency. This hypothesis is based on the evidence that prostaglandin synthetase, which exhibits heme peroxidase activity, has been shown to undergo irreversible self deactivation by free radical intermediates during the oxygenation of arachidonic acid (14). It is also reported that the self destruction of this enzyme could be prevented to some extent by the addition of antioxidants. The results of our study of the effects of Se deficiency on heme hydroperoxidase activity are noteworthy in light of the fact that Se-GSH-Px, which has been implicated in

Table II

Epoxide Hydrolase Activity[1,2] in Subcellular Fractions of Tissues of Rats Fed Diets Deficient in E and/or Se for 5 Weeks

	Diet			
Tissue	+E, +Se	-E, +Se	+E, -Se	-E, -Se
Liver				
Microsomes	331.0 ± 15.2[3,a]	486.1 ± 29.7[b]	983.8 ± 50.2[c]	876.7 ± 59.8[c]
Cytosol	31.5 ± 3.7[a]	33.3 ± 3.7[a]	20.3 ± 2.5[b]	33.3 ± 2.4[a]
Lung				
Microsomes	198.6 ± 17.2[a]	188.8 ± 32.5[a]	244.8 ± 18.4[a]	202.2 ± 10.8[a]
Cytosol	20.4 ± 1.7[a]	27.8 ± 1.3[a]	41.4 ± 3.5[b]	14.5 ± 1.8[a]

[1] Microsomal activity is expressed as nmoles diol formed per min. per gram of tissue.

[2] Cytosolic activity is expressed as nmoles diol formed per min. per mg. of protein.

[3] Values are means ± SE for 5-7 animals. Means within a horizontal row with different lettered superscripts are significantly different ($P < 0.05$).

the protection of cell from peroxidative damage, is almost completely abolished in Se deficiency (15). The metabolism of hydrogen peroxide (H_2O_2) is equally as important as the metabolism of organic hydroperoxides. Hydrogen peroxide is produced in tissues by different enzymatic as well as nonenzymatic mechanisms. The reduction of H_2O_2 is catalyzed by both catalase and Se-GSH-Px, the latter being more important at low H_2O_2 concentrations. Since Se-independent GSH-Px associated with GSH-S-transferases acts only on organic hydroperoxides, the catalase, which is localized primarily in peroxisomes, has a high K_m value for H_2O_2, a drastic reduction in Se-GSH-Px activity could severely impair H_2O_2 metabolism. However, increased cyt P-450 hydroperoxidase (which can decompose both organic hydroperoxides and H_2O_2) activity in the Se deficient state may partially compensate for the decreased Se-GSH-Px activity and thus protect cells against peroxidative damage. But, heme peroxidase has been shown to generate highly reactive oxygen radicals during hydroperoxide metabolism. These radicals have been implicated in several pathological conditions

including carcinogenesis (16). Therefore, elevation of heme peroxidase levels in Se deficient states may have serious consequences.

The effects of dietary E and/or Se deficiency on microsomal and cytosolic EH activity are shown in Table II. In contrast to the component activities of the cyt P-450 MFO system, liver membrane associated EH activity was significantly ($P < 0.05$) increased in E deficiency. Also, liver microsomal EH activity was elevated approximately 3 fold in Se deficient states which is similar to the increased heme peroxidase activity in Se deficiency. The effects of combined E and Se deficiency were not additive. In contrast, lung microsomes showed no significant differences ($P > 0.05$) among the dietary groups. The low level of EH activity observed in both liver and lung cytosolic preparations was significantly ($P < 0.05$) altered only in the +E, -Se state.

ACKNOWLEDGEMENTS

This research was supported by a research grant (R807746) from the U.S. Environmental Protection Agency.

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