ACTA BIOL. SZEGED. 38. pp. 19–23 (1992)

THE EFFECT OF VITAMIN E AND SELENIUM OVERLOAD ON RATS ANTIOXIDANT ENZYMES

AMAL A. RADY[1], NABIL TAHA[1] and B. MATKOVICS[2]

[1]*Department of Biochemistry Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt.*

[2]*Biological Isotope Laboratory, "A. J." University H-6701, Szeged, P. O. B. 539, Hungary (Correspondence and reprint request)*

(Received: June 22, 1992)

Abstract

1. Rats were treated with two concentrations of vitamin E and sodium-selenite and antioxidant enzyme activities and lipid peroxidation, were determined. The following antioxidant enzymes were studied superoxide dismutase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase in serum and liver homogenates.

2. In general the two known antioxidants in low concentration can increase the activities of antioxidant enzymes studied and inhibited lipid peroxidation.

3. On the other hand, the treatment with higher concentrations of the antioxidants changed their original effects and become prooxidants.

Key words: Vit. E and sodium selenite treatments, rats, serum and liver, antioxidant enzyme activities, lipid peroxidation.

Introduction

Lipid peroxidation is a toxic process in biological system. One thoroughly studied agent of peroxidation is the superoxide free radical (O_2^-) which can be generated in several metabolic and enzyme catalyzed reaction and has been found to have detrimental effects on cells and cell constituents (TAPPEL, 1973; CSALLANY et al., 1992). One of the principal biological defense against lipid peroxidatin is α-tocopherol (vitamin E) which is located in biomemberanes and has the capacity to scavenge O_2^-, H_2O_2, HO·, $^1O_2$ and lipid free radicals in vitro. (FUKUZAWA et al., 1985).

Treatment with selenium and vitamin E, so-called antioxidant treatment is well known for elimination of harmful effect of free radicals in the selenium metabolism, to the oxidation of the selenium as reducing equivalents reduced glutathione (GSH) and NADPH are necessary. The reduction of selenite to hydrogen selenite is catalysed by glutathione reductase via the intermediary selenopersulphide step (THOMPSON et al., 1991).

The above statement means that these treatments delay atherosclerotic

processes, aging and in several cases are applied in antitumour therapy (CHOW, 1991; STADTMAN, 1990).

In the present experiments we studied how Se and vitamin E overloading influences antioxidant systems in rats kept on normal diet.

Materials and Methods

Adult Wistar rats of the same age and about the same weight (200—250 g) kept under identical conditions, were used for the experiments. They were randomly divided into five groups. Ten rats were in each group. Water and food was given ad libitum.

The first group which was considered as control, were injected with 0.2 ml saline solution three daily for 24 days and 0.2 ml cotton seed oil once a day. The second and third groups were injected subsequently with 20 mg and 100 mg of vitamin E, (DL- alfa- tocopherol acetate, Sigma-Chemical Co., U.S.A.), respectively, dissolved in 0.2 ml cotton seed oil once a day for 24 days. Other two groups were injected subcutaneously with 0.25 mg and 1.5 mg sodium selenite (Na_2SeO_3), (Sigma-Chemical Co, U.S.A.) respectively, also three daily for 24 days.

Sample collection and measurements: After the above mentioned treatments the rats were sacrificed by decapitation and the blood were collected and the serum were separated. For biochemical analysis only the liver was used after the homogenization of 1 g wet weight in 9 ml physiological saline solution.

In measurement of superoxide dismutase (SOD, EC 1.15.1.1) the weighed liver tissue was homogenized in 0.05 M K_2HPO_4 solution (pH 7.8). Enzyme activity was determined on the basis of inhibition, of epinephrine-adrenochrome autocatalitic transformation (MISRA et al., 1972; MATKOVICS et al., 1977.)

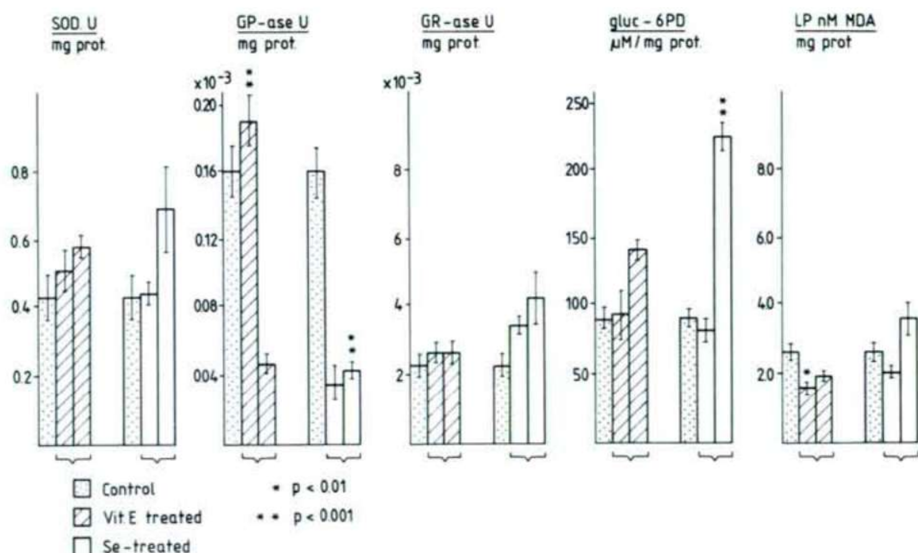

Fig. 1. Rat serum enzyme activities and lipid peroxidation of control, vitamin E and selenium treated animals. (the enzyme activities and LP are given in/mg protein values)

Glutathione peroxidase (GP-ase, EC 1.11.1.9) activity was determined with a chemical method using cumene hydroperoxide as co-substrate. Enzyme quantity was regarded 1 enzyme unit (IU) which transformed 1 micro mol substrate in 1 minute (CHIU et al., 1976; SEDLAK et al., 1968; MATKOVICS et al., 1988).

Glutathione reductase (GR-ase, EC 1.6.4.2) activity was measured with the method described by BERGMEYER et al. (1983) using NADPH (7.8). Unit is the amount of enzyme using 1 micromol NADPH in one minute.

Glucose-6 phosphate dehydrogenase (Gluc-6PD, EC 1.1.1.49) activity was measured with the method described by BERGMEYER et al. (1983) with using NADP-sodium salt as cosubstrate, 1 Unit was the enzyme quantity which could reduce 1 micromol NADP+ in 1 minute at 30 °C.

Lipid peroxidation (LP): Malondialdehyde (MDA) was used as an indicator for lipid peroxides. It was determined by the method described by PLACER et al. (1966). Calibration curve was prepared by using malondialdehyde diethyl acetate (Merck, Germany).

Protein content: Was measured by the method of LOWRY et al. (1951) using Folin-phenol reagent. Calibration curve was prepared with human serum albumin.

Measured data are the means of triplicate from 3 parallels each, which did not show higher deviation than 5% among them.

The results were statistically evaluated with Student's t−test and the correlation coefficients were also calculated.

Results

Results are demonstrated in two figures. Activities of SOD, GP−ase, GR−ase, Gluc−(PD and changes in LP in serum are shown in Fig. 1, while those measured in liver are in Fig. 2. compared to the control values.
The following observation can be made in Fig. 1.
(i) Activity of serum SOD shows a moderate increase upon both Vitamin E and sodium selenite treatment.
(ii) Interestingly, treatment with lower dose of vitamin E induced moderate increase in enzyme activity in case of GP-ase while, treatments with vitamin E in higher concentration showed a significant decrease.
(iii) GR−ase activity was increased by both substances tested, the increase though was more significant upon sodium-selenite treatment.
(iv) Increase of Glu-GPD was significant upon vitamin E as well as sodium selenite in higher concentration.
(v) LP value was decreased by vitamin E and increased by sodium selenite.
Variation in the order of magnitude can be observed in Fig. 2, because the activities were measured in liver homogenates.
(i) Total SOD activity increased in the function of antioxidant concentrations. Significant increase in total-SOD activity was induced by treatments with higher amount sodium selenite.
(ii) Low concentration of the substances increased, while higher concentrations decreased GP-ase activity.
(iii) GR-ase activity significantly increased upon the treatments with both substances.

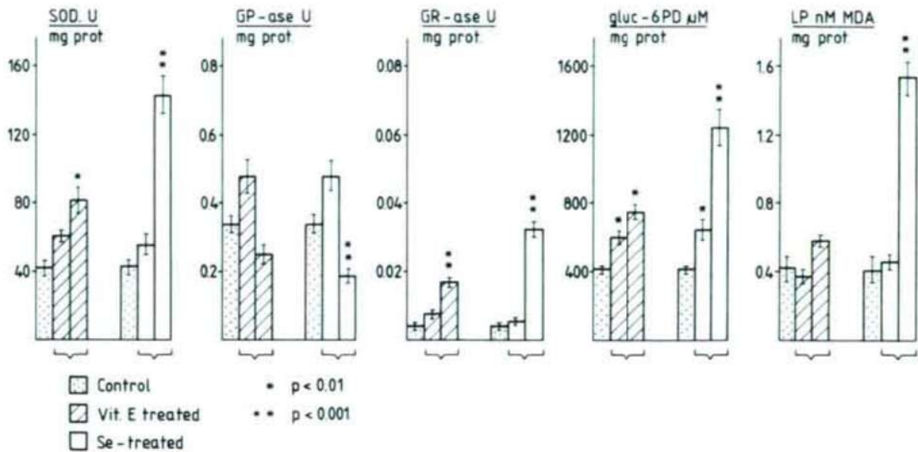

Fig. 2. Enzyme activities and lipid peroxidation of liver homogenisate of the vitamin E and selenium overloaded animals. (the enzyme activities and LP are given in/mg protein values)

(iv) Gluc-6PD activity slightly was increased by both treatment in a concentration dependent manner.
(v) LP value was increased by the higher concentration of vitamin E and sodium selenite overloading.

Discussion

Previous study showed that, vitamin E an effective antioxidant, inhibits lipid peroxidation via donation of a hydrogen atom to a lipid peroxyl radical, thus forming a lipid hydroproxide (LOOH), and reversibly oxidizes vitamin E (CHOW, 1991). This oxidised vitamin E is more rapidly reduced by glutathione in the presence of phospholipid hydroproxide glutathione peroxidase and glutathione (MAIORINO et al., 1989). In the present study we investigated whether low supplementation of vitamin E and selenium increases antioxidant enzyme activities and decreases lipid peroxidation. It has been shown that low levels of vitamin E and selenium can be maintained by glutathione in reduced form (LEEDLE et al., 1990; THOMPSON et al., 1991).

The results of present study are in agreement with HU et al. (1990) obtained from rats on vitamin E diet showed that supplementation of high concentration of vitamin E or selenium lead to significant decrease of glutathione peroxidase and significant increase in lipid peroxidation. It is possible that the level of glutathione in the cells used as cofactor for scavenging lipid peroxide and hydroperoxide, was not enough to reduce of reversibly oxidized vitamin E which resulted in its accumulation in cells (FUKUZAWA et al., 1985).

It can be concluded that vitamin E and selenium, in low concentration

increase the activity of antioxidant enzymes and decrease lipid peroxidation in the liver and serum, while high concentration of both antioxidant can turn over in the cells in oxidised form and cause alteration in the membrane proteins, which decrease the glutathione level. Due to the low level of GSH in the cell the vitamin E becomes prooxidant.

References

BERGMEYER H. U., BERGMEYER J. and GRASEL, M. (Editors) (1988): Methods of enzymatic analysis. Vol. II. Verlag Chemie, Wenheim (FRG).

CHING KAUNG CHOW. (1991): Vitamin E and oxidative stress. – Free Rad. Biol. Med. *11*, 215–232.

CHIU D. T. Y., STULTS F. H. and TAPPAL A. L. (1976): Purification and properties of rat lung soluble glutathione peroxidase. – Biochem. Biophys. Acta *445*, 558–566.

CSALANY, A. and YEONG, L. HA. (1992): α-Tocopherol oxidation mediated by superoxide anion (O_2^-) I-Reaaction in aprotic and protic condition. – Lipids *27*, 195–200.

FUKUZAWA, K., TAKASE, S. and TSUKATANI, H. (1985): The effect of concentration on the antioxidant effectiveness of α-Tocopherol in lipid peroxidation induced by superoxide free radicals. – Arch. Biochem. Biophys. *240*, 117–120.

HU, M. L., FRANKEL, E. N. and TRAPPEL, A. L. (1990): Effect of dietary menhaden oil and vitamin E on in vivo lipid peroxidation induced by iron. – Lipids *25*, 194–198.

LEEDLE, A. R., and AUST, D. S. (1990): The effect of glutathione on the vitamin E requairment for inhibition of liver microsomal lipid peroxidation. – Lipids *25*, 241–245.

LOWRY, O. H., ROSEBROUGH, N. I., FARR, A. L. and RANDALL, R. J. (1951): Protein measurement with the Folin phenol reagent. – J. biol. Chem. *193*, 265–275.

MAIORINO, M., COASSIN, M., ROVERI, A. and URSINI, F. (1989): Microsomal lipid peroxidation: Effect of vitamin E and its functional interaction with phospholipid hydroproxid glutathione peroxidase. – Lipids *24*, 721–726.

MATKOVICS, B., NOVÁK, R., HOANG DUC HAHN, SZABÓ L. and ZALESNA, G. (1977): A comparative study of some important animal peroxide metabolism enzymes. – Comp. Biochem. Physiol. *56B*, 31–34.

MATKOVICS, B., SZABÓ L. and VARGA, SZ. I. (1988): Determination of enzyme activities and lipid peroxidation and glutathione pathway. – Labor. Diagnosztika *15*, 248–250 (in Hungarian).

MISRA, H. P. and FRIDOVICH, I. (1972): The role of superoxide anion in the autoxidation of epinephrine and a simple assay of superoxide dismutase. – J. biol. Chem. *247*, 3170–3175.

PLACER, Z. A., CUSHMAN, L. and JOHNSON, B. C. (1966): Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical system. – Anal. Biochem. *16*, 359–364.

SEDLAK, I. and LINDSAY, R. H. (1968): Estimation of total protein-bond and nonprotein sulfhydryl groups in tissue with Ellman's reagent. – Anal. Biochem. *25*, 192–205.

STADTMAN, T. C. (1990): Selenium biochemistry. – Ann. Rev. Biochem. *59*, 111–127.

TAPPEL, A. L. (1973): Lipid peroxidation damage to cell components. – Fed. Proc. *32*, 1870–1874.

THOMPSON, H. J. and IP, C. (1991): Temporal changes in tissue glutathione in response to chemical form, dose, and duration of selenium treatment. – Biol. Trace. Element Res. *30*, 163–173.