Asian Journal of Chemistry Vol. 20, No. 2 (2008), 1068-1074

Investigation of Free Radical Scavenging Enzyme Activities and Lipid Peroxidation in Liver Tissue of Zinc Deficient Rats

HASAN KAYA†, SEYITHAN TAYSI*, ABDULLAH KAYA‡, ABDULLAH BOYUK††, HAKAN DURSUN‡‡, YAHYA ISLAMOGLU§, DIDEM TASTEKIN¶ and OZTEKIN CIKMAN**

Department of Biochemistry, Nenehatun Obstetric and Gynecology Hospital, Erzurum, Turkey

Fax: (90)(442)3172294; Tel: (90)(442)3172295; E-mail: seytaysi@hotmail.com

The aim of this study was to evaluate the lipid peroxidation and free radical scavenging enzyme activities in liver tissue of zinc (Zn)-deficient rats and investigate relationship among these parameters in either group. 16 Male rats with a weight of 35-40 g were used for the experiment. The rats were divided into control (n = 8) and Zn-deficient groups. After 4 weeks of feeding, the rats were killed by cervical dislocation and liver tissues were removed. Biochemical measurements in liver tissue were carried out using a spectrophotometer. Catalase, glutathione peroxidase, glutathione reductase, glutathione S transferase activities, total (enzymatic plus non-enzymatic) superoxide scavenger activity, superoxide dismutase, non-enzymatic superoxide scavenger activity, superoxide dismutase activities and Zn level in the Zn-deficient group were significantly lower than those of the control group, whereas malondialdehyde level was significantly higher than those of the control group. Slightly increased non-enzymatic superoxide scavenger activity was not significantly different from the controls. The results obtained in this study demonstrate that Zn-deficiency causes a decrease in antioxidant defence system and an increase in oxidative stress in liver tissue in rats.

Key Words: Lipid peroxidation, Malondialdehyde, Antioxidant enzymes, Zinc, Trace element, Free radicals.

†Mustafa Kemal University, Medical School, Department of Internal Medicine, Hatay, Turkey.

‡Department of Internal Medicine, 100 Yil University, Veterinary Faculty, Van, Turkey,

††Department of Surgery, Elazig Research and Educational Hospital, Elazig, Turkey.

‡‡Department of Internal Medicine, Ataturk University, Medical School, Erzurum, Turkey.

§Cardiology, Ataturk University, Medical School, Erzurum, Turkey.

¶Department of Internal Medicine, Medical School, Kafkas University, Kars, Turkey.

**Department of Surgery, Van State Hospital, Van, Turkey.

INTRODUCTION

Oxygen free radicals (OFRs), such as superoxide radical ($O^{\bullet -}_2$), hydroxyl radical ($^{\bullet}OH^-$), are highly reactive species generated by biochemical redox reactions as a part of normal cell metabolism. Oxygen metabolism in aerobic organisms has some advantages. In view of the generation of oxygen free radicals, certain adverse effects also occur. Practically all the essential biomolecules can undergo oxidative reactions mediated by oxygen free radicals. The study of oxygen free radicals and their proposed effects on biological systems has become an important area of biomedical research in recent years[1,2].

Oxygen free radicals and lipid peroxides have been implicated in the pathogenesis of a large number of diseases such as diabetes mellitus, cancer, rheumatoid arthritis, systemic lupus erythematosus, Behçet's disease, infectious diseases and atherosclerosis[3-6].

To prevent the damage caused by the oxygen free radicals, multiple defense systems, collectively called antioxidants, are present in various organs and tissues as well as in serum and erythrocytes. Antioxidants prevent the organism from the harmful effects of free radicals by scavenging or inhibiting their formation. Cells maintain their vital functions against oxidative damage with the help of a system that involves glutathione (GSH) and GSH-dependent enzymes (glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST), catalase (CAT), superoxide dismutases (SODs), vitamins A, E and C, some trace elements against oxidative damage[2,7]. However, after an increase in the production of free radicals or a decrease in the defence against toxic species or both, oxidative stress can occur. Oxidative stress reflects the consequences of a mismatch between the rate of formation of free radicals and the ability of the cell to transform them to less toxic species[8].

Studies on trace elements have become intensified within the last three decades. Since the levels of trace elements in human tissues and fluids play important roles in health and disease conditions, studies about them have been attracting great interest[9].

Zn is an essential trace element for animals, including humans and mammals. Zn has been shown to be essential to the structural and function of a large number of macromolecules and over 300 enzymic reactions. Zn is essential for the development and proper function of the immune system[9,10].

To our knowledge, there is simultaneously no available data study on liver total (enzymatic plus non-enzymatic) superoxide scavenger activity (TSSA), non-enzymatic superoxide scavenger activity (NSSA), GSH-Px, GST, CAT, SOD activities and glutathione (GSH) malondialdehyde (MDA) levels in Zn-deficient group. Therefore, in the present study, we aimed to investigate effects on lipid peroxidation and free radical scavenging enzyme activities in liver tissue of Zn-deficient rats.

EXPERIMENTAL

16 Male rats (4 weeks old, Sprague-Dawley strain) with a weight of 35-40 g were used for the experiment. All animals received human care compliance with the guidelines of Ataturk University Research Council's criteria. The composition of the diet was shown in Table-1[11].

TABLE-1
THE COMPOSITION OF THE EXPERIMENTAL DIET[12]

Diet (ingredients)	%	Control group (g/kg dry matter)	Zn-deficient group (g/kg dry matter)
Ovalbumin	70	700	700
DL-Methionine	1	10	10
Sucrose	12.3	123	123
Corn oil[a]	10	100	100
Cellulose powder	2	20	20
Cholin bitartrate	0.2	2	2
Vitamin mixture (AIN 76)	1	10	10
Mineral mixture (AIN 76)	3.5	35	–
Mineral mixture (AIN 76) (Zn-deficient)	3.5	–	35

[a]Ulker, Bizim corn oil (Ulker, Istanbul, Turkey). All compounds used in the study were analytical grade.

Biochemical analysis:

After 4 weeks of feeding, the blood was drawn from the heart under thiopenthal sodium anesthesia (50 mg kg^{-1}). The blood samples were centrifuged and the serum samples obtained were stored at -80 °C until the analysis date. The livers were homogenized 10-fold physiological saline solution by using homogenizer (Omni Accessory Pack International Homogenizer, USA). The homogenate was centrifuged at 10000 rpm for 1 h to remove debris. Supernatant was taken and all assays were carried out in this fraction.

TSSA, NSSA SOD assays were performed in the samples before and after TCA 20 % (w/v)[12]. GSH-Px, GRD, GST, CAT activities, MDA levels were measured as described, respectively[13-17]. The protein content was determined by using the Bradford method[18]. Results were expressed in U/mg protein for TSSA, NSSA, SOD, GRD and GST activities; IU/mg protein for GSH-Px activity; μmol/mg protein for GSH and nmol/mg protein for MDA levels. One unit of TSSA, NSSA and SOD was defined as the amount of enzyme protein causing 50 % inhibition in nitrobluetetrazolium reduction rate. Biochemical measurements were carried out using a spectrophotometer (CECIL CE 3041, Cambridge, UK).

For Zn assay, all of the materials (glass and plastic) used were thoroughly cleaned with hot solution of nitric acid (20 %, v/v) for 48 h and rinsed five times with deionised water. Serum samples were diluted with deionized, double distilled water for Zn, when required. Zn measurement was carried out with an atomic absorption spectrometer (Unicam 929, UK). Serum Zn level was expressed as μg/mL.

Statistical analysis: The findings were expressed as the mean ± SD. Statistical analysis was undertaken using the Mann-Whitney U-test. A p value < 0.05 was accepted statistically significant. Statistical analysis was performed with statistical package for the social sciences for Windows (SPSS, version 11.0, Chicago, IL, USA).

RESULTS AND DISCUSSION

All parameters are shown in Table-2. As seen from the Table, CAT, GSH-Px, GRD, GST, TSSA, SOD activities and Zn level in the Zn-deficient group were significantly lower than those of the control group ($p < 0.05$ for CAT, GRD and GST; $p < 0.01$ for GSH-Px, TSSA; $p < 0.0001$ for SOD, Zn), whereas MDA level was significantly higher than those of the control group ($p < 0.01$). Slightly increased NSSA was not significantly different from the controls.

TABLE-2
MEAN ± SD OF CAT, GSH-Px, GST, GRD, TSSA, NSSA, SOD ACTIVITIES AND MDA LEVELS IN CONTROL AND Zn-DEFICIENT GROUPS IN LIVER RATS

	Zn-deficient group (n = 8)	Control group (n = 8)
CAT (k/mg protein)	9.8 ± 0.7[a]	10.8 ± 0.8
GSH-Px (IU/mg protein)	1.6 ± 0.6[b]	2.5 ± 0.7
GRD (U/mg protein)	5.7 ± 1.7[a]	7.8 ± 1.4
GST (U/mg protein)	9.8 ± 0.9[a]	11.2 ± 1.4
TSSA (U/mg protein)	39.7 ± 3.2[b]	45.0 ± 3.4
NSSA (U/mg protein)	19.2 ± 1.6	18.6 ± 2.4
SOD (U/mg protein)	20.5 ± 2.1[c]	26.6 ± 3.8
MDA (nmol/mg protein)	6.2 ± 1.5[b]	3.8 ± 1.6
Serum Zn level (μg/mL)	1.2 ± 0.3[c]	4.2 ± 1.3

[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.0001$ *vs.* control group.

In humans and other mammals, Zn-deficiency has been associated to diseases such as Alzheimer's syndrome, tuberculosis, syphilis, rheumatoid arthritis, multiple myeloma, apoptosis, insulin resistance, immune dysfunction and night blindness[9,19,20]. In this work, the study of some oxidant/antioxidant parameters, in the liver tissue of Zn-deficient rats, has been carried out.

The present experimental data show that the activities of liver CAT, GSH-Px, GRD, GST, TSSA, SOD and serum Zn level are decreased and Liver MDA level is increased in the Zn-deficient group, compared with the control group.

Zn is an essential micronutrient for animals, including humans and mammals. This trace element has been found to be necessary for RNA polymerase, carbonic anhydrase, CuZn-SOD and zinc-finger proteins functioning. In addition, one of the most important biochemical roles of Zn is to maintain the structure and function of membranes. It has also been suggested that Zn can act as an antioxidant by displacing iron ions from their binding sites and inhibiting iron-dependent radical reactions. Antioxidants are both acute and chronic. Long-term deprivation of zinc renders an organism more susceptible to injury induced by a variety of oxidative stresses. The acute effects involve two mechanisms *viz.*, stabilization of sulfhydryls or reduction in the formation of hydroxyl radical ($OH^{\bullet -}$) from hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\bullet -}$) through the antagonism of redox-active transition metals such as iron and copper. The apparent stabilization of sulfhydryls, zinc protects some enzyme sulfhydryls from oxidation. The presence of Zn in the diet, along with vitamin A, E and C and other antioxidants, influences oxygen toxicity[9,19,20]. In fact, dietary Zn-deficiency promotes an increase in the susceptibility of rat liver microsomes to lipid peroxidatio (LP) *in vivo*[21] and *in vitro*[22,23]. In one study, it was also found that a Zn-deficient diet stimulated the free radical production in lung microsomes from rat[22]. In another study, it was shown that Zn-deficiency caused the release of H_2O_2 from the microsomal NADPH-dependent cytochrome P450 in the same mammal species[24]. Shaheen *et al.*[25] reported that liver MDA level was significantly elevated in Zn-deficient rats in comparison with the control group. We found that liver MDA level in Zn-deficient group was significantly higher than that of the control group. The present results for liver MDA level, which is an important indicator of oxidant stress, are in agreement with that obtained by Shaheen *et al.*[25]. These findings indicate that oxygen free radicals are increased in the liver of the Zn-deficient rats. Oxygen free radicals are shown to damage the microcirculatory of endothelia in all organs[26].

In some systems, both Zn and Mn are able to displace iron ions from their union sites in phospholipids and inhibit lipid peroxidase of membranes[26]. Therefore, zinc could be a stabilizer of membranes thus preventing the formation of reactive oxygen species (ROS) by means of a mechanism that could involve the protection of sulfhydryl groups against their oxidation and/or the inhibition of the production of ROS by transition metals[19,27]. Consequently, in rats fed with a Zn-deficient diet, lipid peroxidase of membranes is favoured since they lack this stabilizing ion. The

susceptibility of membranes to lipid peroxidase can be altered by changes in their lipid composition. Different studies have shown that the phospholipids and cholesterol ratio, the type of phospholipids and the fatty acids content, contribute to maintaining the structural integrity of membranes. Any change of these variables will make the membrane more susceptible to oxidative damage[23]. This could explain why in Zn-deficient rats also led to an increase in the lipid peroxidase levels. Zn-deficient group was fed with a deficient amount of food, which does not cover their energy requirements and they could therefore respond by mobilizing their lipidic pool. This mobilization probably implies a change in the ratio between lipidic components, including membranes, thus heightening their susceptibility to peroxidation.

Under Zn-deficiency, possibly low Zn levels inside the cell promote a post-translational modification of SOD isoenzymes, thus impeding Zn access to the quaternary structure of the mature protein. In fact, although Zn does not participate directly in the catalytic mechanism of CuZn-SOD, it is necessary for their synthesis and further enzyme activity[28].

Shaheen *et al.*[25] reported that both plasma and liver SOD was significantly lower in Zn-deficient rats in comparison with control group. Kraus *et al.*[29] reported that plasma SOD activity in Zn deficient rats was significantly low. Decreased CuZn-SOD, CAT, GSH-Px activities and increased liver MDA level were found in Zn-deficient group in the present study. CuZn-SOD dismutates the $O_2^{\bullet-}$ to H_2O_2. H_2O_2 is not a free radical; however, it can be converted to hypoclorous acid (HOCl) and $OH^{\bullet-}$ by myeloperoxidase in neutrophils. CAT and GSH-Px detoxifies H_2O_2 by consverting it to water and molecular oxygen. These antioxidant enzymes protect the cell constituents from damage by oxygen free radicals[4-6].

When SOD and TSSA activities are suppressed by increasing oxidant stress in Zn deficiency, $O_2^{\bullet-}$ radicals may be elevated in the liver tissues. SOD enzyme is the most important defense mechanism against the $O_2^{\bullet-}$ radicals producing in the cells. Increased $O_2^{\bullet-}$ might be responsible for the oxidative damage reflected as increased liver MDA levels in Zn-deficient group in present study.

We found that GRD and GST activities in liver of Zn-deficient rats were significantly lower than those of the control group. This indicates an increase in oxidative processes because activities of these antioxidants decreased in response to increased oxidative stress in rats. The reduction of GRD, GST and TSSA activities are supposed to be the effects of Zn deficiency. Depletion of these antioxidants may have contributed to the higher oxidative damage in liver of Zn deficient rats.

It has been suggested in the present study that Zn deficiency causes oxidant stress and peroxidation by means of excessive free radical production in liver tissue in rats and supplementation of Zn can eliminate the oxidant stress and prevent peroxidation reactions.

REFERENCES

1. J.P. Kamat and T.P.S. Devasagayam, *Toxicology*, **155**, 73 (2000).
2. B. Aktan, S. Taysi, K. Gumustekin, N. Bakan and Y. Sutbeyaz, *Ann. Clin. Lab. Sci.*, **33**, 232 (2003).
3. R. Memisogullari, S. Taysi, E. Bakan and I. Capoglu, *Cell. Biochem. Funct.*, **21**, 291 (2003).
4. S. Taysi, F. Polat, M. Gul, R.A. Sari and E. Bakan, *Rheumatol. Int.*, **21**, 200 (2002).
5. S. Taysi, M. Gul, R.A. Sari, F. Akcay and N. Bakan, *Clin. Chem. Lab. Med.*, **40**, 684 (2002).
6. S. Taysi, I. Kocer, R. Memisoullari and A. Kiziltunc, *Ann. Clin. Lab. Sci.*, **32**, 377 (2002).
7. M.F. Polat, S. Taysi, M. Gul, O. Cikman, I. Yilmaz, E. Bakan and F. Erdogan, *Cell. Biochem. Funct.*, **20**, 327 (2002).
8. F. Amicarelli, A.M. Ragnelli, P. Aimola, A. Bonfigli, S. Colafaria, C. DiIlio and M. Miranda, *Biochim. Biophys. Acta*, **1453**, 105 (1999).
9. S. Taysi, F. Akcay, C. Uslu, Y. Dogru and I. Gulcin, *Biol. Trace. Elem. Res.*, **91**, 11 (2003).
10. H. Tapiero, K.D. Tew, *Biomed. Pharmacother.*, **57**, 399 (2003).
11. J.G. Bieri, *J. Nutr.*, **109**, 925 (1979).
12. I. Durak, O. Canpolat, M. Kacmaz, G. Ozgen and H.S. Ozturk, *Clin. Chem. Lab. Med.*, **36**, 407 (1998).
13. D.E. Paglia and W.N. Valentina, *J. Lab. Clin. Med.*, **70**, 58 (1967).
14. R.E. Pinto and W. Bartley, *Biochem. J.*, **112**, 109 (1969).
15. C.K. Sen, E. Marin, M. Kretzschmar and O. Hänninen, *J. Appl. Physiol.*, **73**, 1265 (1992).
16. H. Aebi, Methods of Enzymatic Analysis, Weinheim, Verlag Chemie Chap, p. 673 (1974).
17. H. Ohkawa, N. Ohishi and K. Yagi, *J. Lipid. Res.*, **19**, 1053 (1978).
18. M.M. Bradford, *Anal. Biochem.*, **72**, 248 (1976).
19. M.C. Hidalgo, A. Exposito, J.M. Palma and M. de la Higuera, *Int. J. Biochem. Cell. Biol.*, **34**, 183 (2002).
20. B. Halliwel, Clarendon Press, Oxford (2000).
21. J.F. Sullivan, M.M. Jetton, H.K. Hahn and R.E. Burch, *Am. J. Clin. Nutr.*, **33**, 51 (1980).
22. J.P. Burke and M.R. Fenton, *Proc. Soc. Exp. Biol. Med.*,**179**, 187 (1985).
23. T.M. Bray, S. Kubow and W.J. Bettger, *J. Nutr.*, **116**, 1054 (1986).
24. J.D. Hammermueller, T.M. Bray and W.J. Bettger, *J. Nutr.*, **117**, 894 (1987).
25. A.A. Shaheen and A.A. El-Fattah, *Int. J. Biochem. Cell. Biol.*, **27**, 89 (1995).
26. C.E. Cross, B. Halliwell, E.T. Borish, W.A. Pryor, B.N. Ames, R.L. Saul, J.M. McCord and D. Harman, *Ann. Int. Med.*, **107**, 526 (1987).
27. J.M.C. Gutteridge and G.J. Quinlan, *Biochim. Biophys. Acta*, **1156**, 144 (1993).
28. T.M. Bray and W.J. Bettger, *Free Radic. Biol. Med.*, **8**, 281 (1990).
29. A. Kraus, H.P. Roth and M. Kirchgessner, *J. Nutr.*, **127**, 1290 (1997).

(*Received*: 25 January 2007; *Accepted*: 1 October 2007) AJC-5939