Physiol. Res. 46: 381–384, 1997

Superoxide Dismutase Activities in Different Tissues of Female Rats Treated with Olive Oil

S. B. PAJOVIC, J. KASAPOVIC, J. MARTINOVIC

Laboratory for Molecular Biology and Endocrinology, Vinča Institute for Nuclear Sciences, Belgrade, Yugoslavia

Received March 26, 1997
Accepted May 15, 1997

Summary
The activities of cytosol superoxide dismutase (CuZnSOD) and mitochondrial superoxide dismutase (MnSOD) were measured in subcellular fractions of homogenates prepared from the brain, thymus and liver of ovariectomized (OVX) female rats, non-treated or treated 24 h prior to sacrifice with a single s.c. dose of 0.1 ml olive oil. In the brain, neither MnSOD nor CuZnSOD were affected by olive oil, whereas in the thymus the olive oil injection elevated CuZnSOD and did not affect MnSOD activity. At the same time, the activity of CuZnSOD was reduced and that of MnSOD was elevated in the liver following oil treatment. These results suggest that olive oil has modulatory effects on the expression of CuZnSOD and MnSOD activity in the liver and of CuZnSOD in the thymus of female rats.

Key words
Superoxide dismutase – Olive oil – Brain – Thymus – Liver

Introduction

A delicate balance exists between oxidants, such as free radicals, on the one hand and protective enzymes and vitamins E and C as antioxidants on the other. Among antioxidative enzymes, the metalloenzymes superoxide dismutases (SOD) remove surplus of superoxide anion radicals, produced during oxygen metabolism, by catalyzing their dismutation to oxygen and hydrogen peroxide. CuZnSOD has been localized in the cytosol, as well as in the mitochondrial inter-membrane space (Taylor 1975), whereas MnSOD was found in mitochondria and peroxisomes (Dhaunsi *et al.* 1993, Del Rio *et al.* 1990) of all oxygen-metabolizing cells, providing a primary defense mechanism against oxidative damage (Singal *et al.* 1993). In recent years, growing evidence has been accumulating that nutrition plays an important role in maintaining the balance between oxidants and antioxidants. The SOD is contained in bovine milk and has been suggested to diminish the oxidative problems associated with the superoxide anion (Kiyosawa *et al.* 1993). In rabbits fed a hyperlipidic diet, SOD activity was reported to be significantly increased in atheromatous aortic tissue (Del Bocchio *et al.* 1990, Mantha *et al.* 1993). The effect of hypercholesterolaemia on MnSOD-containing macrophages in the myocardium was investigated in cholesterol-fed male New Zealand rabbits, in which the increase of MnSOD-containing macrophages was recorded and seemed to parallel that of lipoproteins (Kinscherf *et al.* 1995). D'Aquino *et al.* (1991) investigated the effects of fish oil and coconut oil on the antioxidant defense system and lipid peroxidation in microsomes isolated from the rat liver. These authors found that fish oil feeding in amounts comparable with human diet, while decreasing plasma lipids, actually challenge the antioxidant defense system. Data on the modulatory effects of vegetable oils on antioxidant activity are lacking. The rate of lipid peroxidation in isolated liver microsomes of rats fed coconut oil was shown to be three times lower than in rats fed fish oil (Kinscherf *et al.* 1995). In the present study, olive oil, which is rich in natural antioxidants (Visioli *et al.* 1995), was tested as a possible modulator of MnSOD and CuZnSOD activities in the brain, thymus and liver of female rats. Because of the sex differences in lipoprotein metabolism in the rat liver

(Luskey *et al.* 1974, Wilcox *et al.* 1974, Chan *et al.* 1976), the effects of olive oil on SOD activity in rat tissues were investigated using long-term ovariectomized (OVX) animals.

Methods

Female Wistar rats aged 3–3.5 months were used. Bilateral ovariectomy was performed under ether anaesthesia 3 weeks prior to olive oil treatment. OVX animals were housed in open colony cages under controlled conditions of temperature (23±2 °C) and illumination (lights on, from 05:00 to 17:00 h), and had free access to tap water and laboratory chow.

The OVX animals received an s.c. injection of a single dose of 0.1 ml oil. The controls were sham-injected. Twenty-four hours after the injection, all animals were sacrificed in the morning by decapitation with a guillotine (Harvard Apparatus) and fresh brains, livers and thymi were dissected for sample preparations.

Tissue homogenates were prepared by a slightly modified method of Rossi *et al.* (1983) and De Waziers and Albrecht (1987). Individual livers and thymi were homogenized in 0.05 M KH_2PO_4 buffer containing 0.1 mM EDTA, pH 7.8, whereas whole brains were homogenized in 0.25 M sucrose containing 0.05 M Tris-HCl and 1 mM EDTA, pH 7.4. The tissue homogenates were vortexed for 30 s several times with intermittent cooling on ice, and left frozen at −70 °C for 20 hours. The homogenates were then defrozen and centrifuged at 37 000 rpm for 65 min. Cytosols were kept at −20 °C until use. The protein concentration was determined by the method of Lowry *et al.* (1951).

The enzymatic activity of SOD was determined by the method of Misra and Fridovich (1972) before and after the inhibition of CuZnSOD with KCN (Geller and Winge 1983). This method is based on the ability of SOD to inhibit the autoxidation of adrenaline into adrenochrome at pH 10.2. The inhibition of autoxidation was monitored at 480 nm and enzyme activity was expressed in units per mg protein. One unit of SOD was defined as the amount of protein which caused 5 % inhibition of the conversion rate between the third and fourth minute of incubation. The results were analysed by Student's t-test at 5 % level of significance.

Results

CuZnSOD and MnSOD activities in the liver (Fig. 1)

Liver CuZnSOD activity in OVX animals was suppressed by 0.1 ml olive oil, the respective values for OVX controls and OVX treated with olive oil being 64.6±3.9 and 45.7±3.2 units/mg protein ($t_{(30)}=3.78$, $p<0.05$). On the other hand, the activity of MnSOD was significantly increased (30.6±4.2) in comparison to OVX controls (17.5±1.1) ($t_{(30)}=2.83$, $p<.05$).

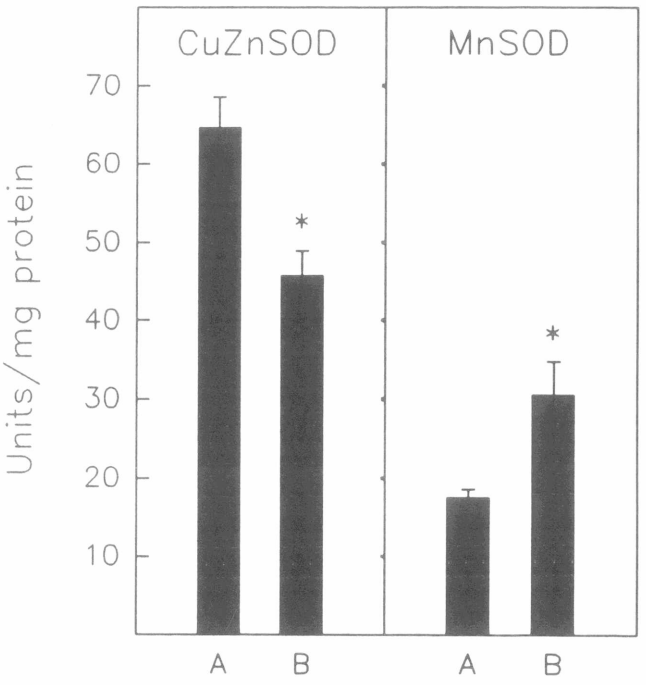

Fig. 1. *Activity of CuZnSOD and MnSOD in the liver of OVX rats. A – non-treated controls (n = 15); B – treated with olive oil (n = 17). Columns represent mean values and vertical bars are S.E.M. * p<0.05.*

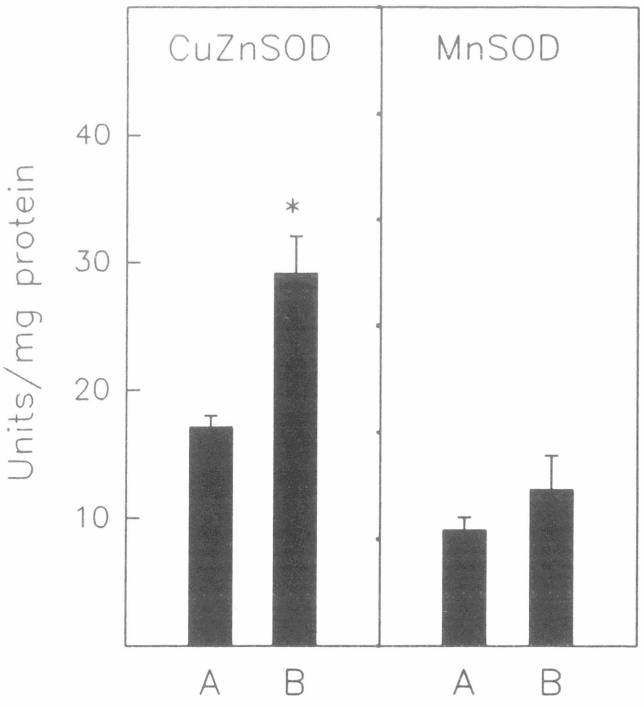

Fig. 2. *Activity of CuZnSOD and MnSOD in the thymus of OVX rats. A – non-treated controls (n = 15); B – treated with olive oil (n = 13).Columns represent mean values and vertical bars are S.E.M. * p<0.05.*

CuZnSOD and MnSOD activities in the thymus (Fig. 2)

Olive oil treatment 24 h before sacrifice significantly increased ($t_{(26)}=4.11$, $p<0.05$) the activity of thymus CuZnSOD (29.1±3.0) in comparison to control animals (17.1±0.9). At the same time, the activity of MnSOD was unchanged after the treatment (10.4±0.6 in OVX controls vs. 12.3±.6 in OVX treated with olive oil) ($t_{(26)}=1.17$, $p>0.05$).

CuZnSOD and MnSOD activities in the brain

Brain CuZnSOD activity in OVX animals did not change after the s.c. injection of 0.1 ml olive oil. The values for OVX controls and OVX animals treated with olive oil were 19.6±1.6 and 23.8±7.3 units/mg protein ($t_{(7)}=0.73$, $p>0.05$). Similarly, the activity of MnSOD appeared to be unchanged after administration of olive oil (14.7±0.6 in OVX vs. 11.7±2.5 in OVX treated with olive oil) ($t_{(7)}=1.49$, $p>0.05$).

Discussion

The present results have shown that the modulation of SOD activity by olive oil treatment was found in the thymus and liver of female rats. These changes of enzyme activities have been considered in relation to the functional state of the cells. The activity of both SODs in the rat brain was unchanged following olive oil treatment. In the thymus, only cytosol SOD activity was increased by olive oil. This is not unexpected if results of Mandola (1993) are taken into consideration. According to this author the biochemical analysis of calf thymus protein activity on cholesterol metabolism revealed an amino acid sequence which was found to be identical with bovine erythrocyte SOD (CuZnSOD). However, the olive oil treatment modulated the cytosol and mitochondrial SOD in the rat liver. The liver plays a central role in many metabolic processes, including lipoprotein metabolism and plasma protein synthesis. The up-regulation of MnSOD in mitochondria appears to be a protective mechanism against the effects of superoxide anions which might be generated during the metabolism of modified lipoproteins. MnSOD may be necessary for the maintenance of normal function of mitochondria after oxidative stress (Janssen *et al.* 1993). At the same time, the down-regulation of CuZnSOD might be a consequence of depleted levels of superoxide anions in the cytosol.

Recently, we have shown that brain MnSOD activity is suppressed by ovarian hormones (Pajovic *et al.* 1993). This appears to be a phenomenon common to rats of both sexes (Pajovic *et al.* 1996). On the other hand, the presence of sexual differences in lipoprotein metabolism of the rat liver has been proposed by several authors (Luskey *et al.* 1974, Wilcox *et al.* 1974, Chan *et al.* 1976). Therefore, we investigated the effects of olive oil on SOD activity in some tissues of OVX rats.

In conclusion, our results suggest that olive oil treatment especially challenges the antioxidant enzyme systems in the thymus and liver, but not in the brain, thus modulating the susceptibility of tissues to free radical oxidative damage.

Acknowledgment

This research was supported by the Serbian Research Foundation Grant No. 263. The authors are grateful to Dr. Miroslav M. Kopečni for careful reading of the manuscript.

References

DEL BOCCHIO G., LAPENNA D., PORRECCA E., PENNELLI A., SAVINI F., FELICIANI P., RICCI G., CUCCURULLO F.: Aortic antioxidant defence mechanisms: time-related changes in cholesterol-fed rabbits. *Atherosclerosis* **81:** 127–135, 1990.

CHAN L., JACKSON R.L., O'MALLEY B.W., MEANS A.R.: Synthesis of very low density lipoproteins in the cockerel. Effects of estrogen. *J. Clin. Invest.* **58:** 368–379, 1976.

D'AQUINO M., BENEDETTI P.C., DI FELICE M., GENTILI V., TOMASSI G., MAIORINO M. URSINI F.: Effects of fish oil and coconut oil on antioxidant defence system and lipid peroxidation in rat liver. *Free Radic. Res. Commun.* **12-13:** 147–152, 1991.

DEL RIO L.A., SANDALIO L.M., PALMA J.M.: A new cellular function for peroxisomes related to oxygen free radicals? *Experientia* **46:** 989–992, 1990.

DE WAZIERS I., ALBRECHT R.: The effects of vitamin nutritional status on glutathione, glutathione transferase and glutathione peroxidase activities in rat intestine. *Experientia* **43:** 394–395, 1987.

DHAUNSI G.S., SINGH I., HANEVOLD C.D.: Peroxisomal participation in the cellular response to the oxidative stress of endotoxin. *Mol. Cell. Biochem.* **126:** 25–35,1993.

GELLER B.L., WINGE D.R.: A method for distinguishing Cu,Zn- and Mn-containing superoxide dismutases. *Anal. Biochem.* **128:** 86–92, 1983.

JANSSEN Y.M., VAN HOUTEN B., BORM P.J., MOSSMAN B.T.: Cell and tissue responses to oxidative damage. *Lab. Invest.* **69:** 261–274, 1993.

KINSCHERF R., KOHLER C., KREUTER C., PILL J., METZ J.: Hypercholesterolemia increases manganese superoxide dismutase immunoreactive macrophages in myocardium. *Histochem. Cell Biol.* **104:** 295–300, 1995.

KIYOSAWA I., MATUYAMA J., NYUI S., YOSHIDA K.: Cu,Zn- and Mn-superoxide dismutase concentration in human colostrum and mature milk. *Biosci. Biotech. Biochem.* **57:** 676–677, 1993.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J.J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 235–241, 1951.

LUSKEY K.L., BROWN M.S., GOLDSTEIN J.L.: Stimulation of the synthesis of very low density lipoproteins in rooster liver by estradiol. *J. Biol. Chem.* **249:** 5939–5947, 1974.

MANDOLA P.: The calf thymus superoxide dismutase: a protein active on cholesterol metabolism. *Comp. Biochem. Physiol. B.* **105:** 457–464, 1993.

MANTHA S.V., PRASAD M., KALRA J., PRASAD K.: Antioxidant hypercholesterolemia and effects of vitamin E in rabbits. *Atherosclerosis* **101:** 135–144, 1993.

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

PAJOVIC S., NIKEZIC G., MARTINOVIC J.V.: Effects of ovarian steroids on superoxide dismutase activity in the rat brain. *Experientia* **49:** 73–75, 1993.

PAJOVIC S., SAICIC Z.S., SPASIC M.B., PETROVIC V.M., MARTINOVIC J.V.: Effects of progesterone and estradiol benzoate on superoxide dismutase activity in the brain of male rats. *Experientia* **52:** 221–224, 1996.

ROSSI M.A., CECCHINI G., DIANZINI M.M.: Glutathione peroxidase, glutathione reductase and glutathione transferase in two different hepatomas and in normal liver. *Med. Sci.* **11:** 805, 1983.

SINGAL P.K., DHALLA A.K., HILL M., THOMAS T.P.: Endogenous antioxidant changes in the myocardium in response to acute and chronic stress conditions. *Mol. Cell. Biochem.* **129:** 179–186, 1993.

TAYLOR D.D.: Polarographic assay and intracellular distribution of superoxide dismutase in rat liver. *Biochem. J.* **147:** 493–504, 1975.

VISIOLI F., VINCERI F.F., GALLI C.: Waste waters from olive oil production are rich in natural antioxidant. *Experientia* **51:** 32–34, 1995.

WILCOX H.G., WOODSIDE W.F., BREEN K.J., KNAPP H.R.Jr., HEIMBERG M.: The effects of sex on certain properties of the very low density lipoprotein secreted by the liver. *Biochem. Biophys. Res. Commun.* **58:** 919–926, 1974.

Reprint requests

S.B. Pajovic, Laboratory for Molecular Biology and Endocrinology, Vinča Institute for Nuclear Sciences, P.O.Box 522, 11001 Belgrade, Yugoslavia.