Physiol. Res. 48: 297-301, 1999

Effects of Olive Oil on Superoxide Dismutase Activity in the Brain of Newborn and Young Female Rats

S. PEJIĆ, J. KASAPOVIĆ, S.B. PAJOVIĆ

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

Received October 13, 1998
Accepted February 25, 1999

Summary

Changes in the activity of brain antioxidant superoxide dismutases (SOD) were followed in newborn and young female rats 8, 15, 30, 45, 60 and 75 days after birth treated with olive oil. In newborn rats, the content of brain cytosol SOD (CuZnSOD) and mitochondrial SOD (MnSOD) decreased after treatment with olive oil. However, in the brain of rats aged 8 days this effect was lost. The suppressive effect of olive oil on these enzymes reappeared again in 15-day-old rats. In rats aged one month, only the activity of CuZnSOD was reduced after olive oil treatment. In the brain of rats aged 45, 60 and 75 days, neither MnSOD nor CuZnSOD were affected by olive oil. The different effects of olive oil on the brain SOD, during ontogeny suggest that profound changes in the susceptibility of nervous tissue antioxidant enzymes to olive oil take place during sexual maturation.

Key words

Superoxide dismutase • Olive oil • Brain

Introduction

It is well known that the antioxidant defense system is very complex because of two components: a) enzyme systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and b) non-enzymatic, low-molecular components such as vitamins C, A and E (Cadenas 1989). The SODs are very important because they catalyze dismutation of superoxide in H_2O_2 and H_2O without oxidation of other molecules in the intracellular environment. If the surplus superoxide is not removed, very reactive and toxic OH radicals are produced as a consequence (Halliwell and Gutteridge 1985). Free radicals attack biomolecules such as DNA, proteins and lipids. Furthermore, they initiate lipid peroxidation and the release of hydrolytic enzymes. After the destruction of cellular components, the cells are condemned to die (Feeney and Berman 1976, Kong and Davidson 1980, Brawn and Fridovich 1980). Hence, free radicals are the cause of a large number of pathological processes (Reilly *et al.* 1991).

We have genetic predisposition for antioxidant defense but we can modulate it by food rich in antioxidants. Antioxidants are needed to prevent the formation and oppose the actions of reactive oxygen (Halliwell 1996). Hence, in recent years, there has been a growing evidence that a healthy balance should exist between oxidants and protective antioxidant enzymes. The role of antioxidants in nutrition is an area arousing

Fax +420 2 293 894
E-mail physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

increasing interest. Many dietary compounds have been suggested to be important antioxidants. DeDeckere and Korver (1996) investigated the minor constituents of rice bran oil as functional foods. Eder and Kirchgessner (1997) studied concentrations of lipids in plasma and lipoproteins and oxidative susceptibility of low-density lipoproteins (LDL) in zinc-deficient rats fed on linseed oil or olive oil. In general, their study showed that the effects of zinc deficiency on the concentrations of plasma lipids and the susceptibility of LDL to lipid peroxidation depend on the type of fat. In our previous study we showed that neither MnSOD nor CuZnSOD were affected by olive oil in the brain of female Wistar rats aged 3.5 months (Pajovic *et al.* 1997). In the present study, we investigated the physiological changes in the activity of SOD in newborn and young female rat brains induced by olive oil.

Methods

The activities of CuZnSOD and MnSOD were measured in subcellular fractions of homogenates prepared from brains of newborn, 8, 15, 30, 45, 60 and 75-day-old female Wistar rats, treated 24 h and 48 h prior to sacrifice with a subcutaneous injection of 0.1 ml olive oil / 100 g body weight. The controls were sham-injected. The pregnant rat mother was treated with olive oil 24 h and 48 h before delivery (on the 19^{th} and 20^{th} day of pregnancy). All animals were sacrificed in the morning by decapitation with a guillotine (Harvard Apparatus) and the fresh brains 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 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 h. 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).

SOD activity was measured by the method of Misra and Fridovich (1972). The reaction of autoxidation of norepinephrine to adrenochrome was performed in 3 ml of 0.05 M Na_2C0_3 at pH 10.2 and 26 °C. The inhibition of autoxidation was monitored at 480 nm. After assaying total SOD activity, the samples were treated with 4 mM KCN in order to inhibit cytosol SOD (Geller and Winge 1983) and subjected again to the enzyme assay as described above. The values thus obtained and the differences between the two measurements were considered as MnSOD and CuZnSOD activities, respectively. The results were expressed in units of enzyme activity. One unit of SOD was defined as the amount of protein which causes 50 % inhibition of the conversion rate between the 3rd and the 4th min of incubation.

The results were analyzed by ANOVA in combination with Scheffe's test, and by Student's t-test. Differences between means ± S.E.M. were considered significant at $p<0.05$.

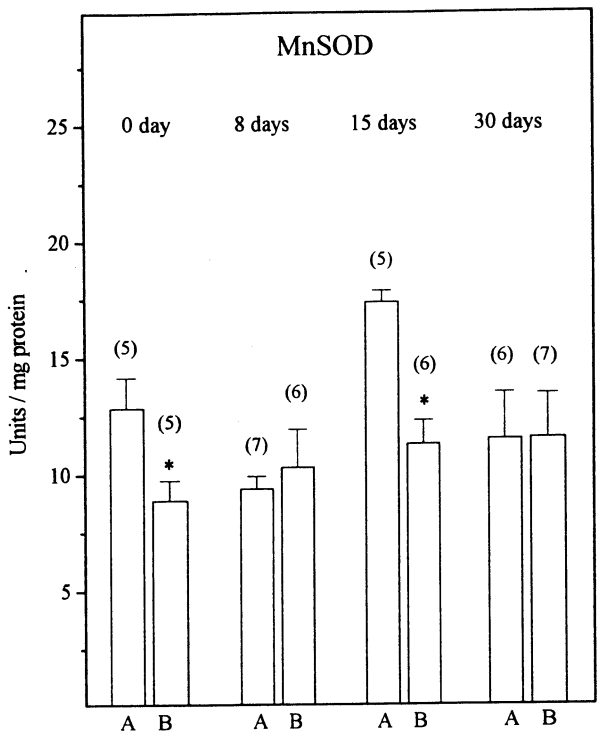

Fig. 1. *Activity of MnSOD in the brain of rats aged 0, 8 15 and 30 days. A - non-treated controls; B - treated with olive oil. Columns represent the mean values of the samples and vertical bars represent S.E.M. * $p<0.05$*

Results

MnSOD activities in the brain (Figs 1 and 2)

Brain MnSOD activity in newborn animals was suppressed by 0.1 ml olive oil, the respective values for the controls and those treated with olive oil being 12.9±1.3 and 8.9±0.9 units/mg protein ($F_{2,12}=4.3$ $p<0.05$), respectively. In contrast, this effect was lost in

brains of rats aged 8 days ($F_{2,17}$=0.14; p>0.05). However, the activity of MnSOD was significantly decreased again in the brains of 15-day-old rats (11.3±1.0) in comparison to the controls (17.4±0.5) (t_9=5.2; p<0.05). Olive oil treatment 24 h and 48 h before sacrifice in the brains of the rats aged 30 days (11.6±1.9), 45 days (12.7±3.2), 60 days (9.6±1.9) and 75 days (16.9±1.2) had no effect on the activity of MnSOD in comparison to the control animals (11.53±2; 10.8±1.6; 11.9±2.7; 16.4±1.2 units/mg protein) (p<0.05).

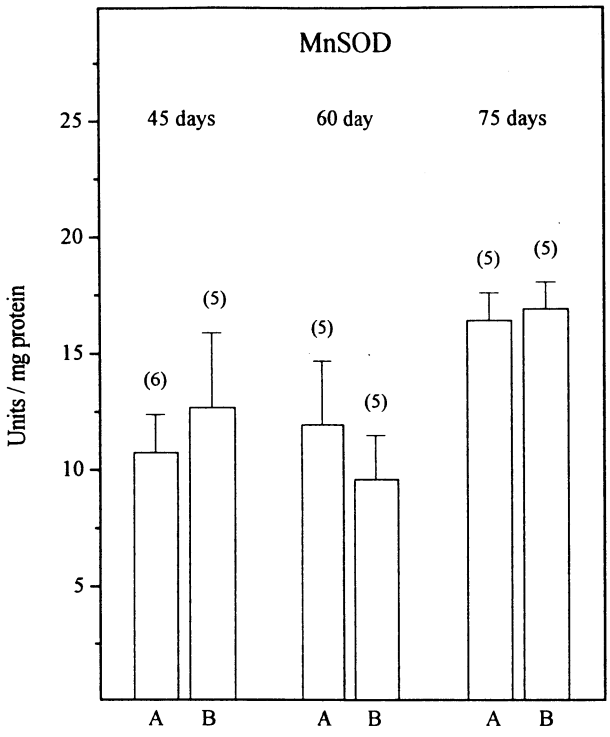

Fig. 2. *Activity of MnSOD in the brain of rats aged 45, 60 and 75 days. For legend see Fig. 1.*

CuZnSOD activities in the brain (Figs 3 and 4)

The activity of brain CuZnSOD was significantly decreased in newborn rats (20.1±0.7 units /mg protein in control vs 16.2±1.4 in treated with olive oil; $F_{2,12}$=4.5, p<0.05). The same was true for the brains of the rats aged 15 days (50.7±4.5 in controls vs 33.5±3.8 in those treated with olive oil; $F_{2,15}$=3.7, p<0.05) and 30 days (39.1±3.6 in controls vs 32.2±1.6 in animals treated with olive oil, $t_{(11)}$=1.84, p<0.05). CuZnSOD activity in brain homogenates of 8-day-old female rats was not affected by olive oil ($t_{(11)}$=0.36, p>0.05). On the other hand, the activity of CuZnSOD in the of rats aged 45 days (44.1±4.7), 60 days (41.03±1.5) and 75 days (44.2±4.4) remained unchanged in comparison to the controls (37.8±4.2; 51.0±5.8; 36.3±1.4 units/mg protein; p>0.05).

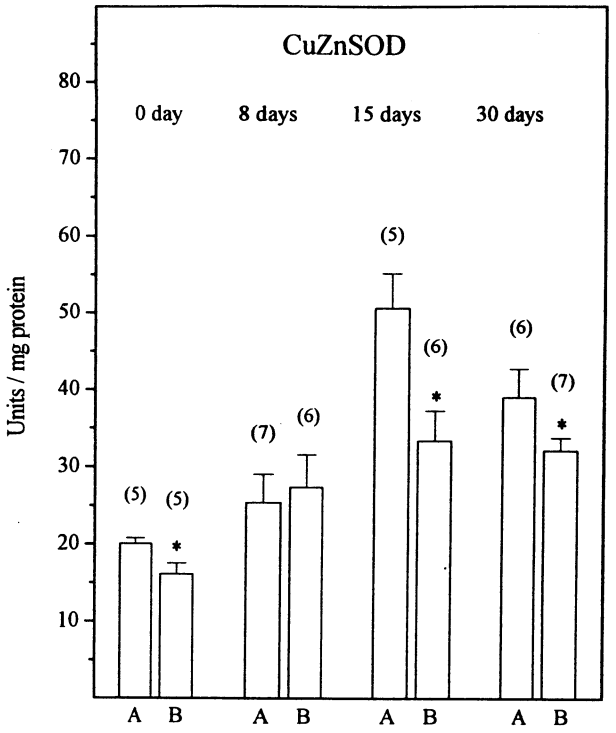

Fig. 3. *Activity of CuZnSOD in the brain of rats aged 0, 8, 15 and 30 days. For legend see Fig. 1.*

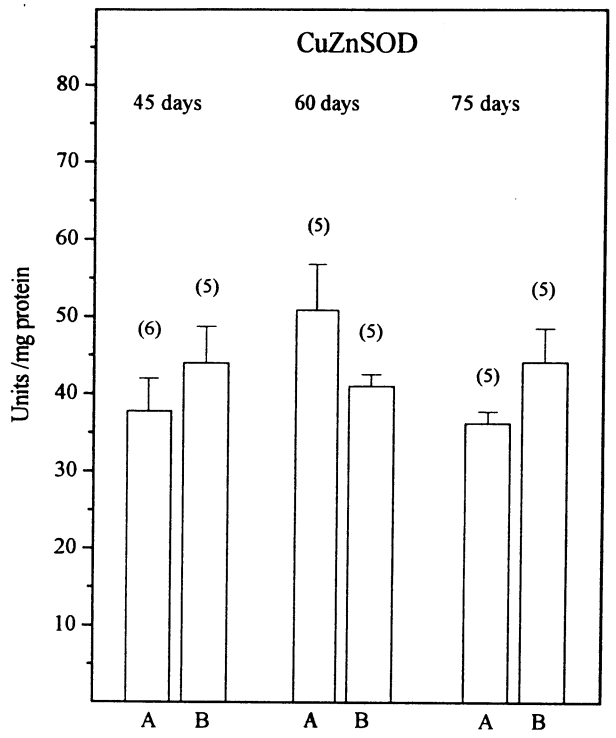

Fig. 4. *Activity of CuZnSOD in the brain of rats aged 45, 60 and 75 days. For legend see Fig. 1.*

Discussion

Free radicals and antioxidants are widely discussed in clinical and nutritional literature (Halliwell 1996). Antioxidants are used for prolonging shelf life, to

maintain the nutritional quality of lipid containing foods and to mitigate the consequences of oxidative damage in the human body (Mantha *et al.* 1993, Kinscherf *et al.* 1995). Jarvinen *et al.* (1997) studied the association between dietary antioxidant vitamins, dietary fibers, selected foods and risk of breast cancer. There was a significant inverse relationship between milk consumption and the occurrence of breast cancer, whereas higher consumption of fried meat was associated with increased risk of breast cancer. No significant relationship was found between the intakes of vitamin E, vitamin C, beta-carotene, lycopene, lutein or dietary fiber and the occurrence of breast cancer. Experimental approaches to the optimization of antioxidant nutrient intake are being proposed.

Our experimental results suggest that olive oil, which is rich in natural antioxidants, primarily challenges the antioxidant enzyme defense in the brain during sexual maturation, thus modulating the susceptibility of nerve cells to free radical oxidative damage. These changes of SOD activity have been considered in relation to the morphological and biochemical changes during differentiation of rat neurons and the blood-brain barrier. Peroxidation of phospholipid unsaturated fatty acids is accompanied by structural and functional changes of nerve cell membranes. These changes during sexual maturation have been correlated with certain physiological roles of cellular activity such as enzyme activation, inactivation and modifications of some membrane properties such as fluidity, permeability and surface potential. During the fetal-neonatal transition, dramatic changes in partial O_2 pressure in blood cells occur, due both to cardiovascular changes and to high O_2 pressure in the atmospheric air. The development of aerobic metabolism is very intensive. These changes in O_2 availability may result in oxidative stress to cells (Sies 1986). It is well known that the greater susceptibility of mammalian neonatal membranes to peroxidation is quite probably due to alterations in their lipid composition and to antioxidant differences between adults and newborns (Gonzalez *et al.* 1995, Allen and Balin 1989). On the other hand, this study describes the physiological changes in the activities of superoxide dismutases in the lipid peroxidation levels in the newborn and neonatal female rat brain (8, 15, 30, 45, 60, and 75 days after birth). The superoxide dismutase activities decreased after postnatal olive oil treatment and between 15 and 30 days after birth and then remained stable. It is known, that the newborn rat brain exhibits the highest susceptibility to lipid peroxidation on the first day of life. Finally, our results suggest that the changes in SOD activity could be mainly related to the development of aerobic metabolism, as well as to lipid peroxidation levels. Our current research considers the possibility that other factors may cause changes in the susceptibility of nervous tissue antioxidant enzymes to olive oil during sexual maturation.

References

ALLEN RG,. BALIN AK: Oxidative influence on development and differentiation: an overview of a free radical theory of development. *Free Radical Biol Med* **6**: 631-661, 1989.

BRAWN K. FRIDOVICH I: Superoxide radical and superoxide dismutases: threat and defense. *Acta Physiol Scand* Suppl 492: 9-18, 1980.

CADENAS E: Biochemistry of oxygen toxicity. *Annu Rev Biochem* **58**: 79-110, 1989.

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.

DEDECKERE E.M, KORVER O: Minor constituents of rice bran oil as functional foods. *Nutr Rev* **54**: S120-S126, 1996.

EDER K, KIRCHGESSNER M: Concentrations of lipids in plasma and lipoproteins and oxidative susceptibility of low-density lipoproteins in zinc-deficient rats fed linseed oil or olive oil. *J Nutr Biochem* **8**: 461-468, 1997.

FEENEY L, BERMAN ER: Oxygen toxicity: membrane damage by free radicals. *Invest Ophthalmol* **15**: 789-792, 1976.

GELLER BL. WINGE DR: A method for distinguishing Cu,Zn- and Mn-containing superoxide dismutases. *Anal Biochem* **128**: 86-92, 1983.

GONZALEZ MM, MADRID R, ARAHUETES M.R: Physiological changes in antioxidant defences in fetal and neonatal rat liver. *Reprod Fertil Dev* **7**: 1375-1380, 1995.

HALLIWELL B: Antioxidants in human health and disease. *Annu Rev Nutr* **16:** 33-50, 1996.

HALLIWELL B, GUTTERIDGE JMC: Oxygen radicals and the nervous system. *Trends Neurosci* **1:** 22-26, 1985.

JARVINEN R. KNEKT P, SEPPANEN R. TEPPO L: Diet and breast cancer risk in a cohort of Finnish women. *Canc Lett* **114**: 251-253, 1997.

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.

KONG S, DAVIDSON AJ: The role of interactions between O_2, H_2O_2, $^{\cdot}OH$, e^- and O_2^- in free radical damage to biological systems. *Arch Biochem Biophys* **204**: 18-29, 1980.

LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 235-241, 1951.

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

MISRA HP, 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.

PAJOVIĆ SB, KASAPOVIĆ J, MARTINOVIĆ J: Superoxide dismutase activities in different tissues of female rats treated with olive oil. *Physiol Res* **46**: 381-384, 1997.

REILLY PM, SCHILLER HJ, BULKLEY GB: Pharmacologic approach to tissue injury mediated by free radicals and other reactive oxygen metabolites. *Am J Surg* **161**: 488-503, 1991.

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

SIES H: Biochemistry of oxidative stress. *Angew Chem* **25**: 1058-1071, 1986.

Reprint requests

Dr Snežana B. Pajović, Laboratory for Molecular Biology and Endocrinology (090), Vinča Institute for Nuclear Sciences, P.O.Box.522, 11001 Belgrade, Yugoslavia.