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Cadmium Induced Changes in Lipid Peroxidation and Antioxidant Status in Brain of Ovariectomized Rats

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The study was designed to estimate the influence of chronic cadmium administration on lipid peroxidation and antioxidant enzymes in brain tissues of ovariectomized female rats. Rats were divided into four groups as follows: control group, ovariectomy group (Ovx group), cadmium group (Cd group) and ovariectomy-cadmium group (Ovx-Cd group). Twelve weeks after the ovariectomy, isotonic cadmium chloride solution was administered to cadmium and ovariectomy-cadmium group rats as intraperitoneal injection of 0.5 mg/kg three times a week for 18 weeks. The levels of malondialdehyde and superoxide dismutase activity showed a significant increase in the Cd and Ovx-Cd groups and a decrease in the Ovx group when compared to the control group. Catalase activity decreased in the Cd group while no change was observed in the Ovx and Ovx-Cd groups in comparison to the control group. The results of the present study may suggest that chronic Cd administration induces oxidative stress in the ovariectomized rat brain as evidenced by the increasing lipid peroxidation upon alteration in the antioxidant status of the brain tissue.

Key Words: Cadmium, Ovariectomy, Brain, Antioxidant, Lipid peroxidation.

INTRODUCTION

Cadmium is a widespread environmental pollutant, characterized by its toxicity in various organs[1]. It is used as a colour pigment in paints, in electroplating and galvanizing and in batteries[2]. Sources of human exposure to this metal include food (vegetables, grains and cereals), water, tobacco leaves, cigarette smoke and alcoholic beverages[3-5]. Cadmium concentration in cigarette smoke has been shown to be above 1 μg per cigarette[6]. Once absorbed, cadmium associates with

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cysteine residues of the low molecular weight protein, metallothionein and accumulates in the kidneys, lungs, brain, liver, heart and the testes[7]. In the human body, cadmium has a biological half-life of more than 20 years[8].

Cadmium and other heavy metals such as thallium, nickel, and cobalt are known to stimulate free radicals production and enhance lipid peroxidation[9–12]. In the brain, lipid peroxidation assumes significant because it is rich in polyunsaturated fatty acids that are liable to peroxidative damage and also because of relatively low concentrations of antioxidant enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase[13]. In addition, the brain consumes a large quantity of oxygen, making it particularly susceptible to oxidative stress[14].

In literature, there is a lot of information about the effects of cadmium and ovariectomy on superoxide dismutase, catalase activities and malondialdehyde (MDA) levels[15–19]. In contrast, limited researchers studied the combined effects of cadmium and ovariectomy on those parameters.

In a previous study[20], the acute effect of cadmium on lipid peroxidation and antioxidant enzymes in liver and kidney of ovariectomized rats has been studied. The present study was designed to assess the effects of chronic cadmium administration in ovariectomized rats on malondialdehyde levels and superoxide dismutase, catalase activities in the brain tissues of rats.

EXPERIMENTAL

Female albino Wistar rats weighing 230–250 g were maintained at standard temperature conditions (22°C) on a 12 : 12 h light/dark cycle and were provided food and water *ad libitum*. The rats were randomly divided into four groups as follows: control group ($n = 6$), cadmium group ($n = 8$), ovariectomized group ($n = 6$) and ovariectomized-cadmium group ($n = 9$). The rats in the control group were not operated or treated with cadmium, only distilled water was given *via* intraperitoneal (ip) route for 18 weeks. All procedures concerning animals were in strict accordance with the *National Institute of Health Guidelines on the Care and Use of Laboratory Animals* and the approval of the Ethic Committee of Mersin University, School of Medicine was obtained prior to the study.

Twelve weeks after the ovariectomy, isotonic cadmium chloride solutions were administered to rats (Cd and Ovx-Cd groups) ip three times a week at a dose of 0.5 mg/kg for 18 weeks. On the other side, distilled water was given to control and Ovx groups *via* ip route for 18 weeks. At the end of the treatment period, the animals were fasted overnight and anaesthetized with ketamine (Ketalar, Eczacibasi Pharmaceutical Co.). The experimental rats were sacrificed by decapitation and their brains were immediately removed and placed on ice. Brain tissues were homogenized with homogenizator in cold 0.175 M KCl/25 mM Tris HCl (pH = 7.4). The tissue homogenates were centrifuged at 10,000 g for 15 min and the supernatants were used for biochemical measurements. Protein level was determined in diluted aliquots of the homogenates by the method of Lowry *et al.*[21] using bovine serum albumin as standard.

MDA levels, as an index of lipid peroxidation, were measured by thiobarbituric acid reaction using the method of Yagi *et al.*[22]. The activity of the enzyme catalase was analyzed according to Aebi[23], measuring the initial rate of H_2O_2 decomposition at 240 nm. The results were expressed in catalase units/mg protein, where one unit is the amount of enzyme that hydrolyzes 1 μmol of H_2O_2 per minute under specific conditions. Superoxide dismutase activity was determined as described by Sun *et al.*[24]. In this assay, SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O_2^- generated by the xanthine/xanthine oxidase system. The amount of protein causing 50% inhibition of the NBT reduction rate was defined as one unit of SOD activity.

SPSS 10.0 statistical program was used for all statistical evaluations. Reported data are presented as mean ± standard deviation. Differences in enzyme activities and MDA levels between the groups were analyzed using Mann-Whitney U test. Values having p value < 0.05 were considered to be significant. All the statistical results are summarized in Table-1.

TABLE-1
EFFECTS OF OVARIECTOMY, CADMIUM AND THEIR COMBINATION ON MDA CONCENTRATION, SOD AND CATALASE ACTIVITIES IN THE BRAIN OF RATS

Groups	Mean ± Standard deviation		
	MDA (nmol/mg protein)	SOD (U/mg protein)	Catalase (U/mg protein)
Control	0.73 ± 0.25	33.82 ± 7.2	174.62 ± 23.99
Cd	1.97 ± 1.3[a]	59.14 ± 8.0[c]	112.26 ± 26.81[b]
Ovx	0.32 ± 0.3[b]	19.44 ± 4.0[b]	173.33 ± 23.26
Ovx-Cd	1.83 ± 1.07[a]	82.76 ± 10.3[c]	184.25 ± 19.05

[a]Significant difference from control at $p < 0.05$.
[b]Significant difference from control at $p < 0.01$.
[c]Significant difference from control at $p < 0.001$.

RESULTS AND DISCUSSIONS

In the present study, we investigated lipid peroxidation and antioxidant enzymes in the brain of ovariectomized rats subject to chronic exposure of cadmium. MDA concentration and SOD and catalase activities were measured after 18 weeks of treatment with cadmium (0.5 mg/kg three times a week). The results of our experiments are shown in Figs. 1–3.

Lipid peroxidation has long been considered the primary mechanism for cadmium toxicity[25-27], despite its inability to directly generate free radicals under physiological conditions[28]. Thus, it is believed that antioxidants should be one of the important components of an effective treatment of cadmium poisoning[29, 30].

The present results have clearly demonstrated the ability of cadmium to induce oxidative stress in rat brain as evidenced by increased lipid peroxidation (MDA) after 18 weeks of cadmium treatment (Fig. 1). This finding is in agreement with several reports demonstrating that cadmium induces oxidative stress in tissues[9-11, 31].

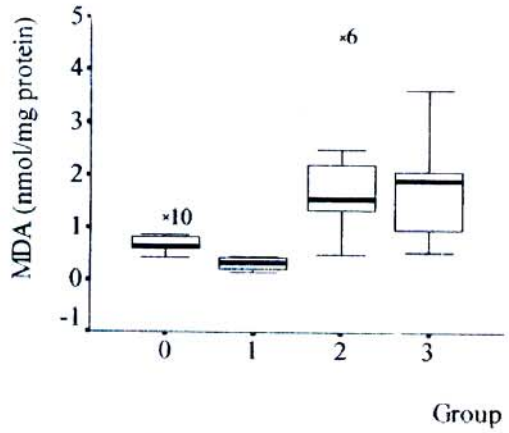

Fig. 1. Concentration of MDA levels in brain tissue of control, Ovx, cadmium and Ovx-Cd groups. Bars represent mean ± S.D. values. Groups: 0. Control group, 1. Ovx group, 2. cadmium group, 3. Cd-Ovx group

Cadmium is also known to exert an inhibitory effect on antioxidant enzymes, superoxide dismutase, peroxidase and catalase which are major scavenger enzymes of intracellular superoxides resulting in the accumulation of reactive oxygen species in cadmium-exposed cells[32]. SOD, a Cu-Zn containing enzyme, is responsible for the catalytic dismutation of potentially toxic superoxide radicals to H_2O_2. Some authors observed a significant decrease in brain SOD activity[13, 33, 34]. On the contrary, in our experiment, cadmium intoxication resulted in a significant increase in SOD activity when compared to the control group (59.14 ± 8.0 *vs.* 33.82 ± 7.2 U/mg protein, $p < 0.001$) (Fig. 2). It could be suggested that the enhancement of the antioxidant defence in experimental animals is a brain response to increase the detoxification process.

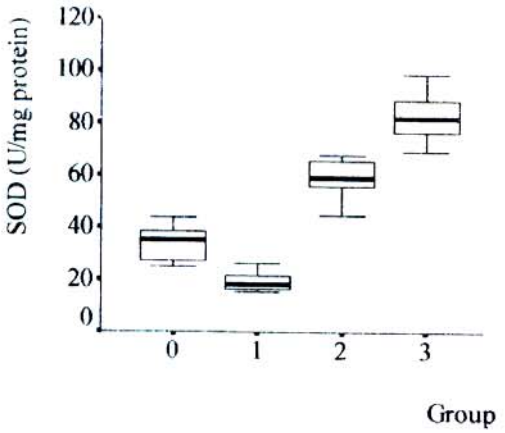

Fig. 2. Activity of SOD in brain tissue of control, Ovx, Cd and Ovx-Cd groups. Bars represent mean ± S.D. values. Groups: 0. Control group, 1. Ovx group, 2. cadmium group, 3. Cd-Ovx group

Catalase, although present in low concentrations in brain, helps in the removal of H_2O_2 to some extent. In the present study, a significant decrease in the activity of this enzyme is observed in cadmium treated group (112.26 ± 26.81 in cadmium group and 174.62 ± 23.99 U/mg protein in control, respectively, $p < 0.01$) (Fig. 3). The decrease in the catalase activity of the brain is in agreement with the findings of Raman *et al.*[13] and Gill *et al.*[33] who found that cadmium decreased the catalase activity in the rat brains. Animals exposed to this metal have shown various responses to catalase. Some studies observed decreased catalase activity and correlated this to the reduced absorption of iron, an essential trace element required for the activity of this enzyme, or the inhibition of heme biosynthesis caused by cadmium exposure[35, 36]. Cadmium induced depletion in iron content might also be the reason for the decrease in the enzyme activity of the brain. Differently, other investigators pointed to elevated enzyme activities, which suggested a significant role in protecting cells[37, 38].

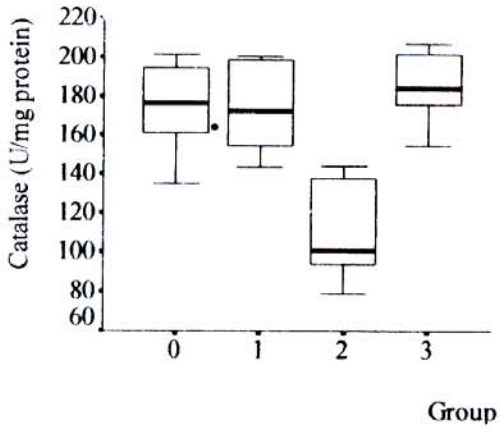

Fig. 3. Activity of catalase in brain tissue of control, Ovx, Cd and Ovx-Cd groups. Bars represent mean ± S.D. values. Groups: 0. Control group, 1. Ovx group, 2. cadmium group, 3. Cd-Ovx group

In our previous studies[39], we investigated the effect of free radicals and antioxidant enzymes on postmenopausal osteoporosis and we observed an increase in the MDA levels and no significant difference in the SOD activities in the osteoporotic group. In that study osteoporotic rat model was also used.

We determined a decrease in MDA levels in the brain of ovariectomized rats. In disagreement with this data, Ozgonul *et al.*[17] observed a significant increase in MDA levels. The reason for this decrease is still unclear to us but it may be related to properties of the brain.

In the present studies, SOD activities in brain showed a significant decrease following ovariectomy (19.44 ± 4.0 in Ovx group and 33.82 ± 7.2 U/mg protein in control, respectively, $p < 0.01$) (Fig. 2). Sreelathakumari *et al.*[18] reported a decreased SOD activity in the liver of ovariectomized rats. In disagreement with this data, Ozgonul *et al.*[17] observed no significant change in the brain SOD activity of ovariectomized rats. In present study, there was no significant change in the brain tissue catalase activity between the control and ovariectomy groups

(173.33 ± 23.26 and 174.62 ± 23.99 U/mg protein, respectively, $p = 0.924$). Our findings correspond with those of Ozgonul *et al.*[17], who found no difference in the activitity of catalase in brain tissue after ovariectomy. In short, the decrease in MDA level and SOD activity and no significant change in the catalase activity may suggest that ovariectomy has no effect on the oxidative stress in the brain.

MDA levels and SOD activities were found to be higher in the brain tissue of Cd-Ovx group as cadmium group compared to controls (1.83 ± 1.07 *vs.* 0.73 ± 0.25 nmol/mg protein, $p < 0.05$ for MDA; 82.76 ± 10.3 *vs.* 33.82 ± 7.2 U/mg protein, $p < 0.001$ for SOD). But the increase in those parameters was highest in the Cd-Ovx group. However, no significant changes were observed in catalase activity.

In conclusion, the results of present experiment showed that chronic cadmium administration induces oxidative stress in ovariectomized rat brain as evidenced by the increasing lipid peroxidation and by altering the antioxidant levels of the brain tissue.

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