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Effects of estrogen, estrogen/progesteron combination and genistein treatments on oxidant/antioxidant status in the brain of ovariectomized rats

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Abstract. – INTRODUCTION: The aim of this study was to investigate the antioxidative effects of estradiol (E), E plus progesteron (P) combination (E/P) and genistein (G) treatment in the brain of ovariectomized (OVX) rats.

MATERIALS AND METHODS: Adult female Sprague-Dawley rats were divided into five groups, with each group including ten rats. Rats were anesthetized and bilateral ovariectomy was performed under general anaesthesia in all groups except for the sham operation group. Groups included: Sham-operated, control (OVX), estrogen treated group (OVX+ E), E/P combination group (OVX+E/P) and G treated group (OVX+G). Treatments were applied for 8 weeks. The total anti-oxidant status (TAS), total oxidant status (TOS), nitric oxide level (NO), glutathione peroxidase activity (GSH-Px) and oxidative stress index (OSI) were analysed in the brain tissue of rats from each treatment category.

RESULTS: Ovariectomy lead to an increase in brain TOS and OSI levels compared to the sham group ($p < 0.05$). Also, ovariectomy resulted in a decrease in brain TAS levels compared to the sham group that approached statistical significance ($p = 0.078$). Significant decreases in TOS, OSI, GSH-Px and a significant increases in TAS and NO levels were observed in the E-treatment group compared to the control group ($p < 0.001$). The E/P combination group exhibited a significantly decreased TOS and OSI and significantly increased TAS and NO levels relative to the control group ($p < 0.05$). Genistein treatment resulted in a significant decrease in TOS and OSI compared to the control group ($p < 0.05$).

CONCLUSIONS: Oxidative stress markers increase in the brain tissue of OVX rats. Conversely, estradiol, E/P and G supplementation decreases oxidative stress markers and increases antioxidant activity. Using G may prevent neural pathologies result in menopause-related oxidative stress.

Key Words:
Rat, Brain, Oxidative stress, Menopause, Genistein.

Introduction

Menopause is a period marked by numerous significant changes resulting from the cessation of ovarian hormone secretion that has been linked to many pathophysiological complications. Estrogen (E) and progestogen (P) are used world-wide in varying regimens as hormone replacement therapy (HRT) to treat menopause-related symptoms[1]. Previous studies demonstrate that ovarian hormones (especially estrogen) are important for optimal brain function[2]. Epidemiological studies indicate that the incidence of Alzheimer's disease increases significantly in females after menopause at two to three times the rate of similarly aged males, supporting the hypothesis that E withdrawal plays a primary role in the onset of Alzheimer's disease in post-menopausal women[3,4]. Learning and memory may be impaired by the loss of E after menopause. These changes can be improved by E therapy or HRT[5]. Estrogen supplementation has neuroprotective effects and helps to maintain memory and cognition and also delays the onset of neurological disorders such as Alzheimer's disease[4]. However, long-term use of E in the postmenopausal period may increase the risk of endometrial and breast cancers[1]. Therefore, given the possible serious side effects of E, there has been a growing interest for an E substitute with fewer side effects[6]. Genistein (G) is a phytoestro-

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gen and isoflavone present in soybeans. It binds to E receptors, thereby exerting estrogenic effects and has been proposed as a natural alternative to estrogen replacement[7]. Dietary soy intake has been demonstrated to reduce plasma malondyaldehide and increase total plasma antioxidant capacity in postmenopausal women[8]. Ovariectomized female rats were studied as an animal model of the clinical features of the postmenopausal period, in which to test the function of G[7].

Oxidative stress is a disparity between the rate of free radical production and the rate of elimination, occuring when antioxidant mechanisms are overwhelmed. Oxidative stress increases in women after menopause[9]. Oxidative damage to the mitochondria is proposed to play a major role in aging and Alzheimer's disease. The ovarian hormones (E and P) have well-established neurotrophic, neuroprotective effects and support both reproductive function and cognitive health[10]. Estrogen acts as a free radical scavenger and degrades the reactive oxygen species (ROS) produced during membrane oxidation processes. Ovarian hormone deficiency also increases the production of ROS, which could result in oxidative stres and cell damage. Estrogen or phytoestrogens may up-regulate expression of antioxidant enzymes via the estrogen receptors[11]. Likewise, it has been indicated that antioxidant enzyme activities in the brain tissue of rats is dependent upon the concentration of E and P[12].

Bilateral ovariectomy of female rats is used to simulate the post-menopausal condition characterized by the absence of ovarian hormones. The novelty of this workis the comparison of the effects of E, E/P combination and G on oxidative stress parameters of the brain tissue of ovariectomized female rats.

Materials and Methods

Animals, Care and Nutrition

The study was approved by the Dicle University Animal Ethical Committee and was carried out at the Dicle University Health Sciences Practice and Research Center (DUSAM). Experiments were performed in accordance with the "Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Dicle University Animal Ethical Committee".

The study included 50 adult female rats of the Sprague-Dawley strain in the proestrous phase, weighing 250-300 g. The estrous cycle was verified by vaginal swabs 10 days prior the ovariectomy (OVX) procedure. The rats were kept at room temperature (22±2°C) with a photoperiod of 12 h light and 12 h dark cycle (07.00 am-07.00 pm). Throughout the experimental period a standard pellet diet and water ad libitum was given to the rats.

Experimental Treatment

The rats were randomly divided into five groups and each group included ten rats. Rats were anesthetized using intraperitoneal administration of 50 mg/kg ketamine (Ketalar®, Parke Davis, Eczacibasi, Istanbul, Turkey) + 5 mg/kg of xylasine (Rompun®, Bayer AG, Leverkusen, Germany). A midline abdominal incision was made at the pelvic level under general anaesthesia in all groups and as bilateral OVX was performed in all groups except the sham-operated group.

Group 1: (Sham group); The surgical procedure for the sham-operated rats, was the same except that the ovaries were not removed. Water was injected in each rat once daily for 8 weeks by orogastric catheter without any medication.

Group 2: (OVX; control group): ovariectomized rats received water by orogastric catheter once daily for 8 weeks without any medication.

Group 3: (OVX + E): rats received 0.014 mg/kg 17-β estradiol by orogastric catheter once daily for 8 weeks.

Group 4: (OVX+E/P): rats received 0.014 mg/kg 17-β estradiol plus 0.028 mg/kg drosperinone by orogastric catheter once daily for 8 weeks.

Group 5: (OVX+G): rats received 10 mg/kg per day Genistein by orogastric catheter.

All treatments were initiated exactly one week after surgery. At the end of study, the rats were euthanasied under general anesthesia (60 mg/kg ketamine). In ovariectomized rats, uterine atrophy was verified as indicative of successful removal of both ovaries. Rats were decapitated, and the whole brain, except the cerebellum, was removed rapidly. The single-brain was weighed, minced, homogenized and immediately stored at –50°C for later assays.

Biochemical Measurements

Assays were performed on the supernatant of the homogenate that was prepared by centrifugation at 14.000 rpm for 30 min at +4°C. The tissue protein concentration was measured using the Lowry method[13]. The assay for measuring the

brain nitric oxide (NO) level was based on the Griess reaction[14]. Total antioxidant status (TAS) was determined using the automated method, developed by Erel[15]. The results were expressed as micromol (μM) Trolox equiv./L. Total oxidant status (TOS) was determined using the automated measurement method developed by Erel[16]. The results were expressed in terms of μM hydrogen peroxide equivalent per litre (μM H_2O_2 equiv./L). The ratio of the total peroxide to the total antioxidant potential gives the oxidative stress index (OSI), a marker of the degree of oxidative stress[17]. The assay for measuring the activity of glutathione peroxidase (GSH-Px) was based on the method of Paglia and Valentine[18].

Statistical Analyses

Statistical analysis was carried out using the Statistical Package for the Social Sciences version 15.0 (SPSS Inc., 15.0 for Windows, Chicago, IL, USA). The results are expressed as mean±standard deviation. The One-way analysis of variance (ANOVA) and post hoc multiple comparison tests (LSD) were performed on the biochemical variables to examine the difference among groups. A p-value of <0.05 was considered statistically significant.

Results

The results of the analysis of brain tissue for NO, GSH-Px, TOS, TAS and OSI are presented in Table I. Ovariectomy resulted in an increase in brain TOS and OSI levels compared to the sham group ($p < 0.05$). In addition, ovariectomy resulted in a decrease in brain TAS levels compared to the sham group that approached statistical significance ($p = 0.078$). No statistically significant difference was observed between NO level and GSH-Px activity of OVX as compared to the sham group ($p > 0.05$).

The E-treated group exhibited a significant decrease in GSH-Px, TOS and OSI, and a significant increase in TAS and NO levels compared to the control group ($p < 0.001$). The E/P treated group had significantly decreased in TOS and OSI, and significantly increased TAS and NO levels compared to the control group ($p < 0.05$), but had no significant effect on GSH-Px activity ($p > 0.05$). Total antioxidant status was increased in the E/P treated group ($p = 0.056$), and GSH-Px activity was significantly increased compared to the E treated group ($p = 0.015$), but there was no significant difference in TOS, OSI and NO levels ($p > 0.05$).

The G treated group had significantly decreased TOS and OSI compared to the control group ($p > 0.05$), but no significant change in GSH-Px activity, TAS and NO levels ($p > 0.05$). The E treated group had significantly decreased OSI and GSH-Px, and significantly increased NO levels compared to the G treated group ($p > 0.05$), but no significant change in TOS ($p > 0.05$).

Discussion

The purpose of this study was to compare the antioxidant effects of G, E and E/P combination in an experimental model of menopause. Our results

Table I. Level of NO, activity of GSH-Px, TOS, TAS and OSI in brain of ovariectomized rats.

	NO (μM/g protein)	GSH-Px (U/g protein)	TOS (μM H_2O_2 Eq./g protein)	TAS (μM Trolox Eq./g protein)	OSI
Sham (I)	4.75 ± 1.01	0.81 ± 0.09	93.34 ± 7.81	12.62 ± 1.40	7.58 ± 1.14
Control (II)	4.21 ± 0.53	0.90 ± 0.19	106.1 ± 15.3	10.63 ± 2.47	10.71 ± 3.85
E (III)	6.46 ± 1.23	0.65 ± 0.13	79.79 ± 6.64	15.61 ± 3.14	5.11 ± 0.98
E/P (IV)	5.82 ± 1.30	0.83 ± 0.14	81.37 ± 11.23	13.45 ± 2.08	6.06 ± 0.99
G (V)	4.96 ± 2.22	0.99 ± 0.19	81.38 ± 11.23	11.17 ± 2.87	7.76 ± 2.35
p values					
I-II	NS	NS	0.009	0.078	0.002
II-III	0.001	0.001	0.001	0.001	0.001
II-IV	0.012	NS	0.001	0.014	0.001
II-V	NS	NS	0.001	NS	0.001
III-IV	NS	0.015	NS	NS	NS
III-V	0.018	0.001	NS	0.001	0.009

Data are presented as mean±standart deviation, μM: micromol, GSH-Px: glutathione peroxidase, NO: nitric oxide, TOS: total oxidant status, TAS: total anti-oxidant status, OSI: oxidative stress index, NS: not statistically significant.

showed that OVX rats exhibited elevated TOS and OSI as well as reduced TAS levels in the brain. These findings may indicate that ovariectomy leads to oxidative stress in brain tissue. Estrogen, E/P and G treatments decreased OSI and TOS levels in OVX rats. Moreover, E and E/P treatments abrogated the decreased TAS levels associated with OVX.

Increased oxidative stress in brain of OVX rats has been shown previously[19,20]. However previous studies did not examine TOS, TAS and OSI simultaneously in OVX rats. The measurement of anti-oxidants or oxidants alone in patients may not accurately reflect oxidative status[17,21]. In a previous study, TOS levels were significantly higher in the plasma and uterus of OVX rats compared to sham-operated rats[21]. Concerning the relation between OVX and oxidative stress this study may indicate that neurologic complications induced by OVX include a decrease in TAS and an increase in OSI and TOS levels in the rat brain. Increased generation of ROS together with decreased NO production has been reported in pathological conditions. Nitric oxide plays an important role in oxidative/nitrosative damage, and sex hormones influence NO production. The present study demonstrated that OVX leads to decreased NO in brain tissue. Treatment with E or E/P combination significantly increased NO levels in the brain tissue of OVX rats. Genistein increased NO but the difference is not statistically significant when compared to the control group[22].

The GSH-Px is found in all parts of the brain and increases with aging in both sexes and is elevated in females compared to male rats. In the brain of female animals, GSHPx activity is probably modulated directly by changes in estrogen[12]. As seen in the control group, ovariectomy increases GSH-Px activity. In the E treatment group GSH-Px activity is significantly decreased. However, the E/P and G groups were similar to the control group.

In this study, increased brain TOS levels may be due to decreased excretion or over-production of oxidants induced by OVX. In addition, decreased brain TAS levels may indicate that antioxidant compounds are consumed in OVX rats. Increased TOS and OSI and decreased TAS support the idea that increased oxidative stress may contribute to the pathogenesis of menopause related neurological condition.

Estrogen, E/P and G treatments were found to profoundly suppress oxidative stress by reducing TOS levels in the brains of OVX rats. In addition, E and E/P treatments increased TAS in the brains of OVX rats. The ratio of TOS to TAS is referred to as OSI. This has been used as a new marker of oxidative stress[22]. To our knowledge, no data are available in the literature regarding elevated OSI in the brain of OVX rats. In this report,we found an increase in OSI due to increased TOS and decreased TAS in the brains of OVX rats. On the contrary, E, E/P and G treatments reduced OSI levels in the brains of OVX rats.

The ovarian hormones (E and P); plays important neuromodulatory and neuroprotective roles, influencing cerebral blood flow, and neuronal survival. Ovarian hormone replacement induces mitochondrial alterations in the central nervous system reducing oxidative stress and attenuating endogenous oxidative damage, supporting efficient and balanced bioenergetics. The antioxidative effects of ovarian hormones have been demonstrated previously[9,23]. Hormone-treated mitochondria exhibit increased respiratory function and increased activity of the electron transport chain complex IV. This increased respiratory activity is coupled with a decreased rate of reactive oxygen leak and reduced lipid peroxidation, indicative of systematic enhancement of brain mitochondrial efficiency. Genistein, which has antioxidant properties similar to E, has therapeutic potential to reduce cognitive decline and neurodegenerative disease associated with menopause[24]. It has been suggested that G confers protection against OVX-induced neurodegeneration by attenuating oxidative stress, lipid peroxidation and mitochondria-mediated apoptosis in a regional and dose-dependent manner[20].

Conclusions

Oxidative stress markers increases in the brain tissue of OVX rats. These results suggest that surgical menopause leads to an increase in OSI in the rat brain. The administration of E, E/P or G to ovariectomized females is effective into reducing oxidant levels in the brain.

Using G may prevent oxidative-stress related neural pathologies associated with menopause. Additional studies will be required to examine the effect of G induced OSI increases in the brain on specific neurologic pathologies.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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