Original Article

Protection against brain tissues oxidative damage as a possible mechanism for improving effects of low doses of estradiol on scopolamine-induced learning and memory impairments in ovariectomized rats

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Abstract

Background: Regarding the anti-oxidative effects on the central nervous system, the possible protection against brain tissues oxidative damage as a possible mechanism for improving effects of low doses of estradiol on scopolamine-induced learning and memory impairments was investigated in ovariectomized (OVX) rats.

Materials and Methods: The OVX rats treated by (1) vehicle, (2) scopolamine, and (3–4) scopolamine plus estradiol (20 or 20 or 60 μg/kg). Estradiol was administered (20 or 60 μg/kg, intraperitoneally) daily for 6 weeks after ovariectomy. The rats were examined for learning and memory using passive avoidance test. Scopolamine (2 mg/kg) was injected 30 min after training in the test. The brains were then removed to determine malondialdehyde (MDA) and thiol contents.

Results: Scopolamine shortened the time latency to enter the dark compartment in ($P < 0.01$). Compared to scopolamine, pretreatment by both doses of estradiol prolonged the latency to enter the dark compartment ($P < 0.01$). The brain tissues MDA concentration as an index of lipid peroxidation was decreased ($P < 0.05$). Pretreatment by estradiol lowered the concentration of MDA, while it increased thiol content compared to scopolamine ($P < 0.05$ and $P < 0.01$).

Conclusions: These results allow us to suggest a protection against brain tissues oxidative damage as a possible mechanism for improving effects of low doses of estradiol on scopolamine-induced learning and memory impairments in OVX rats.

Key Words: Estradiol, learning, memory, oxidative stress, scopolamine

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INTRODUCTION

Alzheimer's disease (AD) has been known as the most widely recognized reason for sporadic dementia, afflicting almost 13 million individuals around the world.[1] Degeneration of cholinergic pathways in AD has been well documented.[2] A sex-dependent

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difference in cognition and neurogenesis implies that sex hormones are involved in AD.[3] A risk of AD in a female with an age above 65 years is suggested to be nearly two-fold more than males.[4] Hence, a sharp falling in estrogen levels during menopause could be an important contributor.[5] Interestingly, treatment of postmenopausal women by estrogen has been indicated to enhance several kinds of memory.[6-8] Using several kinds of animal models, 17β-estradiol has been able to influence neurogenesis in the hippocampus and affects on learning and memory of the rodents.[3,9-15]

Some studies suggest that depletion of ovarian hormones exacerbate the effects of cholinergic impairment on memory-related tasks.[16] Recent animal studies suggest that one way in which estrogen replacement may help to reduce cognitive deficits associated with aging and AD is by improving of cholinergic system functions.[17] It has also been demonstrated that ovariectomy and estrogen therapy fundamentally influence on basal forebrain cholinergic capacity.[18] Conversely, both medium and high injection doses of estradiol decreased the number of acetylcholine transferase-immunoreactive cells in the basal forebrain and other brain areas which are correlated with working memory.[19]

On the other hand, brain tissues oxidative damage has been well documented to have an important role in neurodegenerative illnesses such as AD.[20] A few studies explored the neuroprotective capability of estrogen and some of its derivatives against oxidative stress-induced neurodegeneration.[21] Surprisingly, in our previous study, chronic administration of a high dose of estradiol impaired learning and memory which was accompanied with brain tissues oxidative damage.[22] In the present study, estradiol was used in low doses and the possible protection against brain tissues oxidative damage as a possible mechanism for improving effects of low doses of estradiol on scopolamine-induced learning and memory impairments was investigated in ovariectomized (OVX) rats.

MATERIALS AND METHODS

Animals and drugs

Female 12-week-old Wistar rats with 200 ± 10 g in weight were obtained from the Laboratory Animal Center of School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. The animals were kept in standard conditions including 22 ± 2°C room temperature, a periodical 12 h light/dark, and a free access to food and water. All experimental procedures were carried out based on the rules set by the Mashhad Medical University Committee on Animal Research. The rats were OVX under ketamine and xylazine anesthesia and were then divided into four groups and treated by (1) OVX, (2) OVX-Scopolamine (OVX-Sco), (3) OVX-Sco-Estradiol 20 (OVX-Sco-Est 20), and (4) OVX-Sco-Est 60.

The animals in the OVX-Sco-Est groups (the Groups 3 and 4) were treated by daily injections of estradiol (20 or 60 μg/kg; intraperitoneally [IP]) for 6 weeks after ovariectomy. The animals of OVX group received 1 ml/kg of sesame oil instead of Est. Scopolamine (2 mg/kg) was injected after training in passive avoidance test.

Ketamine and xylazine were purchased from Alfasan Company, Woerden, Netherlands. Estradiol valerate was kindly provided by Iran Hormone Company, Tehran, Iran. Scopolamine was purchased from Sigma-Aldrich Company, St Louis, MO, USA. Other chemicals such as those which were used for biochemical assessments were purchased from Merck Company Darmstadt, Germany.

Surgery

The ovariectomy surgery was done under anesthesia condition induced by ketamine (100 and mg/kg, IP) and xylazine (15 mg/kg, IP). Anesthesia was confirmed by reduced respiratory rate and no response to gentle pinching of foot pad. An abdominal incision was carried out; the ovaries were appeared and were removed.[23]

Passive avoidance test

Passive avoidance is a well-known test which is used to evaluate nonsaptial learning and memory.[24,25] A plexiglass box containing a light and a dark compartment with a grid floor, adjoining to each other through a small gate, was used in the present study. The animals were placed into the apparatus to familiarize to it during a 5 min session. The rats were then placed into the light compartment and the latency to enter the dark compartment was recorded. On a training phase, an electric shock (1 mA, 2 s duration) was delivered to the rats when they entered into the dark. The rats were then injected by scopolamine and returned to their home cage. Three hours later, the rats were placed in the light compartment and the latency to enter the dark compartment, as well as the times spent in dark and light compartments, was recorded.[24]

Biochemical measurements

Finally, the rats were deeply anesthetized with urethane (1.5 g/kg), sacrificed, the brains were removed, and the cortical and hippocampal tissues were separated and submitted to determination of malondialdehyde (MDA) concentrations and total thiol (SH) contents.

MDA level is known to be as an index of lipid peroxidation and reacts with thiobarbituric acid (TBA) as a TBA-reactive substance to produce a red colored complex which has a peak absorbance at 535 nm. Briefly, 1 mL of the cortical or hippocampal homogenates was added to 2 mL of a complex solution containing TBA/trichloroacetic acid/hydrochloric acid. The complex solution was then placed in bath containing boiling water. After 40 min, the solution was allowed to reach the room temperature and centrifuged at 1000 g for 10 min. The absorbance was read at 535 nm. The MDA concentration was determined using an equation which has been previously used: C (M) = absorbance/1.56×10^5.[24,25]

Total thiol groups were measured using 2, 2'-dinitro-5, 5'-dithiodibenzoic acid (DTNB), a reagent that reacts with the SH groups, and produces a yellow-colored complex which has a peak absorbance at 412 nm. Briefly, 1 ml tris-EDTA buffer (pH = 8.6) was added to 50 μl of the cortical or hippocampal homogenates in 1 ml cuvettes and the absorbance was read at 412 nm against tris-EDTA buffer (A1). Then, 20 μl DTNB reagents (10 mM in methanol) were added to the mixture and after 15 min incubation at room temperature, the absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mM) was calculated based on an equation previously described.[24]

Statistical analysis

The data were expressed as a mean ± standard error of the mean. One-way ANOVA was run followed by Tukey's *post hoc* comparisons test. The criterion for the statistical significance was $P < 0.05$.

RESULTS

The results of passive avoidance test

In the present study, administration of scopolamine impaired learning and memory of OVX-Sco group using passive avoidance test which was reflected by decreasing of the latency to enter the dark compartment ($P < 0.001$). Treatment of the animals by low doses including 20 and 60 μg/kg of estradiol for 6 weeks after the removal of ovaries attenuated impairing effects of scopolamine which was presented by prolonging of latency to enter the dark compartment after the shock compared to scopolamine [$P < 0.01$, Figure 1]. The results also showed that the animals of OVX-Sco group had a higher number of entries to the dark compartment after the shock compared to OVX group ($P < 0.001$). Administration of scopolamine decreased the number of entries to the dark in both OVX-Sco-Est 20 and OVX-Sco-Est 60 groups compared to OVX-Sco group [$P < 0.001$ and $P < 0.01$, respectively, Figure 2]. In addition, the animals of OVX-Sco group had an increased level of the time spent in the dark compared to the OVX group ($P < 0.01$). The animals of OVX-Sco-Est 60 group spent a shorter time in the dark compartment where they had previously received a shock compared to the OVX-Sco group [$P < 0.05$, Figure 3]; however, there was no significant difference between the animals pretreated by a lower dose of estradiol compared to the Sco group [Figure 3]. Treatment of the OVX rats by scopolamine shortened the time spent in the light compartment compared to the OVX group ($P < 0.01$). Pretreatment of OVX rats by a higher dose of estradiol attenuated the effects of scopolamine which was reflected in a higher time spent in the light by the animals of OVX-Sco-Est 60 group compared to OVX-Sco group [$P < 0.05$, Figure 4]. No significant difference was observed when the time spent in the light was compared between OVX-Sco-Est 20 and OVX-Sco groups [Figure 4].

Biochemical results

To evaluate the brain tissues oxidative damage status, MDA concentration and thiol contents of both the hippocampal and cortical tissues were compared between the groups. Administration of scopolamine increased lipid peroxidation in the hippocampal tissues of OVX rats which was reflected in an increased level of MDA concentration in the hippocampus of OVX-Sco group compared to OVX group [$P < 0.05$, Figure 5]. Daily injection of 20 μg/kg of estradiol for 6 weeks which was started at the day after ovariectomy and was continued for 6 weeks was able to prevent from the effects of scopolamine. It was observed that the animals of OVX-Sco-Est 20 had a lower level of hippocampal MDA concentration compared to the OVX-Sco group [$P < 0.01$, Figure 5]. There was also a significant difference between the OVX-Sco-Est 60 and OVX-Sco groups [$P < 0.05$, Figure 5].

In contrast to MDA, total thiol contents was decreased in the hippocampal tissues of OVX-Sco group after administration of scopolamine compared to the OVX group ($P < 0.05$). Chronic treatment by both doses of estradiol prevented from lowering effects of scopolamine on hippocampal tissues thiol concentrations which was revealed by a low level of thiol groups in the hippocampal tissues of both OVX-Sco-Est 20 and OVX-Sco-Est 60 groups compared to OVX-Sco group [$P < 0.05$ and $P < 0.01$, respectively, Figure 6].

In addition, the cortical tissues of OVX- Sco showed a higher concentration of MDA compared to the OVX group [$P < 0.01$, Figure 7]. Interestingly, both doses including 20 and 60 μg/kg of estradiol lowered MDA concentrations in the cortical tissues of

OVX-Sco-Est 20 and OVX-Sco-Est 60 groups compared to OVX-Sco [both $P < 0.05$, Figure 7]. The total thiol concentrations in the cortical tissues of OVX-Sco group were lower than that of OVX group [$P < 0.05$, Figure 8].

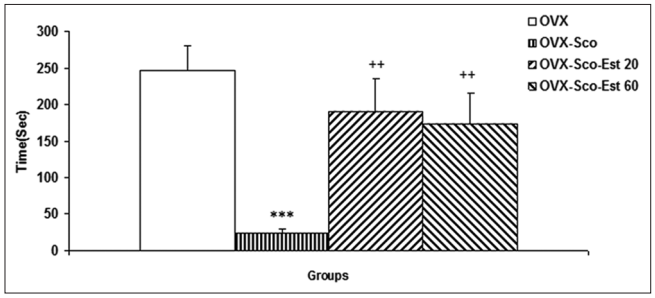

Figure 1: Comparison of time latency for entering the dark compartment. Data are presented as a mean ± standard error of the mean ($n = 9$–10 in each group). ***$P < 0.001$ compared to OVX group, ++$P < 0.01$ compared to OVX-Sco group

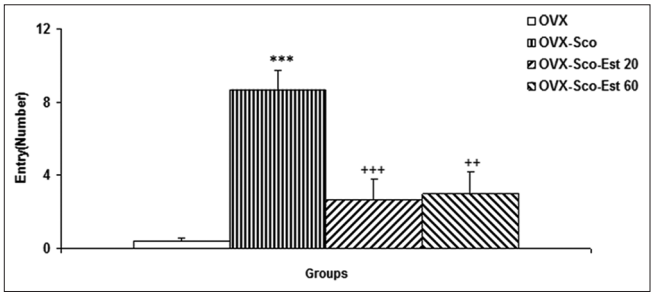

Figure 2: Comparison of the number of entries to the dark compartment. Data are presented as a mean ± standard error of the mean ($n = 9$-10 in each group). ***$P < 0.001$ compared to OVX group, ++$P < 0.01$, +++$P < 0.001$ compared to OVX-Sco group

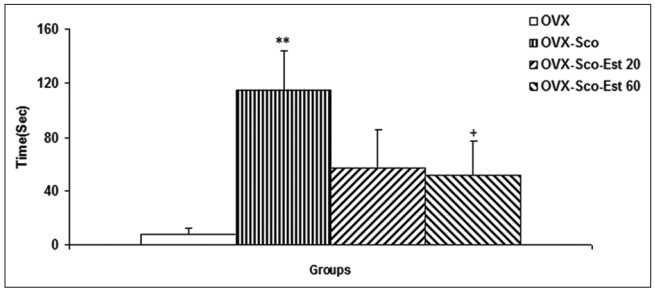

Figure 3: Comparison of the total time spent in the dark compartment. Data are presented as a mean ± standard error of the mean ($n = 9$–10 in each group). **$P < 0.01$ compared to OVX group, +$P < 0.05$ compared to OVX-Sco group

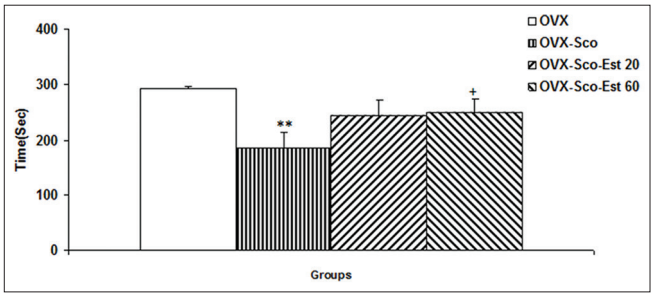

Figure 4: Comparison of the total time spent in the light compartment. Data are presented as a mean ± standard error of the mean ($n = 9$–10 in each group). **$P < 0.01$ compared to OVX group, +$P < 0.05$ compared to OVX-Sco group

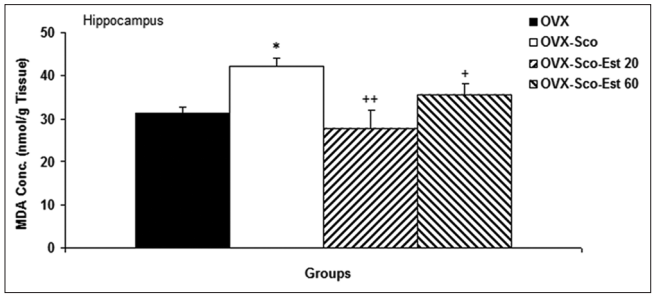

Figure 5: Comparison of hippocampal tissues malondialdehyde concentration. Data are presented as a mean ± standard error of the mean ($n = 6$–7 in each group). *$P < 0.05$ compared to OVX group, +$P < 0.05$ compared to OVX-Sco group

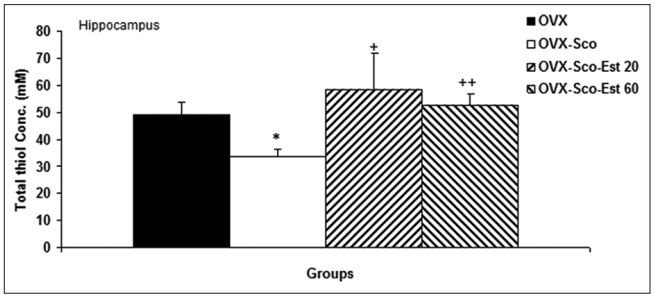

Figure 6: Comparison of hippocampal tissues total thiol content. Data are presented as a mean ± standard error of the mean ($n = 6$–7 in each group). *$P < 0.05$ compared to OVX group, +$P < 0.05$, ++$P < 0.01$ compared to OVX-Sco group

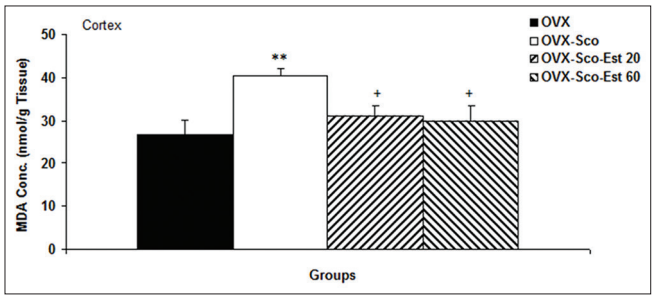

Figure 7: Comparison of cortical tissues malondialdehyde concentration. Data are presented as a mean ± standard error of the mean ($n = 6$–7 in each group). **$P < 0.01$ compared to OVX group, +$P < 0.05$ compared to OVX-Sco group

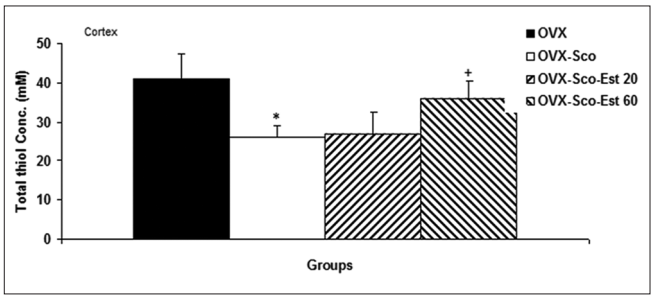

Figure 8: Comparison of cortical tissues total thiol content. Data are presented as a mean ± standard error of the mean ($n = 6$–7 in each group). *$P < 0.05$ compared to OVX group, +$P < 0.05$ compared to OVX-Sco group

The animals of OVX-Sco-Est 60 group had a higher total thiol contents in their cortical tissues compared to OVX-Sco group [$P < 0.05$, Figure 8]. There were no significant differences between OVX-Sco-Est 20 and OVX-Sco groups in the thiol cortical tissues [Figure 8].

DISCUSSION

The results of present study showed that low doses of estradiol improved learning and memory impairments induced by scopolamine in OVX rats accompanying with an improvement of the brain tissues oxidative damage. Scopolamine as a nonselective muscarinic antagonist has been frequently used to produce memory and cognitive dysfunctions.[26-30] In the present study, IP administration of scopolamine impaired memory of OVX rats in passive avoidance test. The animals of scopolamine-treated group showed a shorter latency to enter the dark while they spent a longer time in the dark compartment. Scopolamine also increased the number of entering to the dark where the animals had previously received a shock increased.

Besides disregulation of the cholinergic neuronal pathways, scopolamine has been suggested to be able to increase acetylcholine esterase activity.[29,31,32] In addition, inducing of an oxidative stress status in the brain is suggested to have a role in learning and memory impairment induced by scopolamine. In the present study, administration of scopolamine increased MDA concentrations in both hippocampal and cortical tissues while decreased thiol contents.[28,29,33] Regarding the results of present study, inducing of an oxidative stress status in the brain by scopolamine to induce learning and memory impairments in rats might be postulated. Supporting this idea, the results of our previous study also confirmed that scopolamine in impaired both spatial and nonspatial memory of OVX rats which was accompanied with brain tissues oxidative damage.[27] Degeneration of cholinergic neurons by oxidative stress outcomes is suggested to have a critical role in cognition and memory impairments.[34] In this respect, the recovery of cholinergic function together with the suppression of oxidative damage has been offered as a strategy for the treatment of AD.[35]

In our study we also understood that the animals pretreated by low doses of estradiol showed an increased delay time to enter the dark room. Estradiol also decreased the number of entries to and the total time spent in the dark compartment. The effects of estradiol on learning and memory impairments have been widely investigated.[11-16,18] Several researches have demonstrated that estrogens administered to young OVX rats and mice will upgrade performance on a variety of cognitive tasks.[36] It has also been reported that estradiol improves the cholinergic system in the brain.[37-41] Estrogen has been reported to stimulate choline acetylcholine transferase expression and activity and also acetylcholine release in rat hippocampus while it inhibits the activity of acetylcholine esterase.[37]

It has been documented that 10 days of estradiol treatment created a noteworthy increase in acetylcholine transferase action in the brain regions including frontal cortex and CA1 area of the hippocampus.[38] Some studies showed that decrements in T-maze and radial arm maze performance induced by either systemic or intrahippocampal administration of scopolamine can be diminished or anticipated by estradiol.[39,42] Packard[40] also demonstrated that memory-improving impacts created by estradiol infusing specifically into the hippocampus of the rats can be blocked by systemic injection of scopolamine. Marriott and Korol[41] confirmed the impacts of estradiol on acetylcholine discharge in the hippocampus. Both medium and high injection doses of estradiol decreased the number of acetylcholine transferase-immunoreactive cells in the basal forebrain and other brain areas which are correlated with working memory.[19] In the present study, low doses of estradiol improved learning and memory impairments induced by scopolamine. Considering these facts and the results of present study, an interaction between estrogen, when is used in low doses, and the cholinergic system to modulate learning and memory might be suggested.

It has been frequently reported that estradiol is able to change oxidative stress status in the brain.[20,43-49] On the other hand, protection against brain tissues oxidative damage as a possible mechanism for improving effects of the drugs or natural products on learning and memory has been well documented.[28,29] In our study, it was observed that estradiol in low doses increased the total thiol while it decreased MDA concentration in the brain tissues in comparison with scopolamine. Regarding the results of the present study and the evidence which was mentioned a protective effect against brain tissues oxidative damage as a possible mechanism for improving effect of low doses of estradiol on scopolamine-induced learning and memory impairments in OVX rats which were seen in the present study might be suggested. In supporting this idea, estradiol, progesterone, and their combination as well as genistein supplementation were able to decrease oxidative stress markers in the brain of OVX rats.[50] The results of present study were in contrast to the results of our previous study

in which chronic administration with a high dose of estradiol-impaired spatial learning and memory in Morris water maze which was accompanied with brain tissues oxidative damage.[22] It has also been reported that estradiol induces lipid peroxidation in a time-dependent manner.[51]

CONCLUSIONS

Finally, it is concluded that estradiol has an interaction with the cholinergic system to modulate learning and memory. Regarding the brain tissues oxidative damage associated with scopolamine memory impairment which has been previously reported and was also confirmed in the present study, it seems that beneficial effects of lower doses of estradiol on memory impairment induced by scopolamine that was seen in the present study may at least in part be due to the protection against the brain tissues oxidative damage.

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Conflicts of interest

There are no conflicts of interest.

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