Protective Effect of L-Arginine against Oxidative Damage as a Possible Mechanism of its Beneficial Properties on Spatial Learning in Ovariectomized Rats

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ABSTRACT

Introduction: The role of ovarian hormones and nitric oxide (NO) on oxidative damage in brain tissues as well as learning and memory has been widely investigated.

The present study was carried out to evaluate the effect of the precursor of NO, L-Arginine on learning and brain damage due to oxidative stress in ovariectomized (OVX) rats.

Methods: Thirty -two rats were divided into four groups: 1) Sham, 2) OVX, 3) Sham-L-Arginine (Sham-LA) and 4) OVX-L-Arginine(OVX-LA). The animals of sham- LA and OVX-LA were treated with 500 mg/kg of L-Arginine. The animals in Sham and OVX groups received 1 ml/kg saline. The animals were tested in Morris water maze and finally, the brains were removed and MDA and total thiol concentrations were measured.

Results: The escape latency and swimming path in OVX group were significantly higher than in Sham group ($p<0.01$). The animals in OVX-LA group had significantly lower swimming path length and escape latency compared to OVX group ($p<0.01$) while, there was no significant difference between Sham- LA and Sham groups. In OVX-LA group, the brain tissues total thiol concentration was significantly higher, and MDA concentration was lower than of OVX group ($p<0.001$).There was no significant difference between Sham-LA and Sham groups.

Discussion: It seems that the beneficial properties of L-Arginine on spatial learning of ovariectomized rats are in part due to its protective capacity against oxidative damage.

1. Introduction

High amount of lipids are found in the brain tissues(Halliwell, 1992) thus, central nervous system (CNS) is very vulnerable to oxidative damage (Bondy, 1995a; Frolich & Riederer, 1995). Numerous age related problems is closely connected with oxidative stress (Nicolle, et al., 2001). The effect of oxidative injury in cognitive disturbances including learning and memory loss and Alzheimer disease has been extensively investigated (Bondy, 1995b; Mecocci, Mariani, Cornacchiola, & Polidori, 2004).

Nitric oxide (NO), is a crucial neuromodulator and so, is entailed in learning, synaptic plasticity, long-term potentiation (LTP) and the consolidation of long-term memory (Monteiro, Matté, Bavaresco, Netto, & Wyse,

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2005; Prast & Philippu, 2001; Russell, et al., 2000). It is suggested that depletion of NO generation which occurs in aging may have a role in senile memory impairment (Kuiper, Visser, Bergmans, Scheltens, & Wolters, 1994; Law, O'donnell, Gauthier, & Quirion, 2002; Noda, Yamada, & Nabeshima, 1997). To confirm this, several studies reported the impairment of consolidation of memory and inhibition of LTP by nitric oxide syntahse (NOS) inhibitors (Blokland, et al., 1999; Bon & Garthwaite, 2003; Kopf, Benton, Kalfin, Giovannini, & Pepeu, 2001; Yildirim & Marangoz, 2004). NO also is a potential source of redox stress that can react with superoxide to produce peroxynitrite (ONOO-) which is an important oxidant (Malinski, 2007). Either protective or toxic effects of NO on nervous system is caused by several ways such as variable functions of NO isoforms (Goncharova, Oganyan, & Smelkova, 2006) and the level of reactive oxygen species (Tang, et al., 2006).

It has also been shown that L-arginine, the precursor of NO, enhances memory development and inverts the effects of NOS inhibitor (Majlessi, Choopani, Bozorgmehr, & Azizi, 2008; Plech, Klimkiewicz, & Maksym, 2003).

It has been documented that estrogen increases memory function and affects the morphology of neurons especially the number of dendrite spines in CA1 region of hippocampus, in OVX rats (Gould, Woolley, Frankfurt, & McEwen, 1990; Leuner, Mendolia-Loffredo, & Shors, 2004; Sato, et al., 2003; Warren, Humphreys, Juraska, & Greenough, 1995). Positive (Daniel, Winsauer, Brauner, & Moerschbaecher, 2002; Gibbs, 2002; Ping, Trieu, Wlodek, & Barrett, 2008), negative (Chesler & Juraska, 2000; Fugger, Cunningham, Rissman, & Foster, 1998) and no effect (Healy, Braham, & Braithwaite, 1999) of estrogen therapy on learning and memory in post-menopausal women and OVX animal models has also been reported.

Estrogen has been suggested to have antioxidant effects and increases NO production and consequently decreases coronary heart disease (Mendelsohn & Karas, 1999; White, 2002). Accordingly, some neuroprotective functions of estrogen can be ascribed to its antioxidant effects (Amantea, Russo, Bagetta, & Corasaniti, 2005; Behl, et al., 1997). Conversely, estrogen deprivation is associated with oxidative stress and memory impairment in Alzheimer disease (Tang, et al., 1996). It has also been shown that gonadectomy changes the activity of antioxidant system (Garcia-Navas, Munder, & Mollinedo, 2012).

Depending on the concentration or the rout of production, it seems that L-arginine may have cytoprotective of cytotoxic effects by conversing to NO or by other mechanism(s) (Albina, Caldwell, Henry, & Mills, 1989; Becker-Catania, et al., 2006; Garcia-Navas, et al., 2012; Sayan, Ugurlu, Babul, Take, & Erdogan, 2004; Takeuchi, Ohuchi, Kato, & Okabe, 1993). It has also been suggested that L-arginine may have prooxidant or antioxidant properties (Bast, Haenen, & Doelman, 1991; Joshi, Ponthier, & Lancaster, 1999; Tripathi, Chandra, & Misra, 2009).

In the previous study the effect of L-arginine (the precursor of nitric oxide) on spatial learning of ovariectomized rats was shown using Morris water maze (MWM) (Saffarzadeh, et al. 2010). Therefore, the aim of the present study was to evaluate the possible protective effects of L-arginine against oxidative damage in brain tissues of OVX and normal female rats.

2. Methods

Female Wistar rats, 16 weeks old (250±10 g) were used. The animals were housed in 4–5 per standard cages, at room temperature (24±1 °C) on a 12 h light/dark cycle. Food and water were available ad libitum properly. Animal handling and all related procedures were approved by the Mashhad Medical University Committee on Animal Research. L-arginine was purchased from Sigma Chemical Co. (USA) and dissolved in normal saline. Ketamine HCL and xylazine were purchased from Alfasan Company (Holland). The animal groups were 1) Sham, 2) OVX, 3) Sham-L-arginine (Sham-LA) and 4) OVX-L-arginine (OVX-LA). The animals in Sham-LA and OVX-LA were acutely treated by 500 mg/kg/day of LA before each MWM test (Hara, et al., 2004; Heinzen & Pollack, 2003; Saffarzadeh, et al.,2010; Yildiz Akar, et al., 2007). The animals of Sham and OVX groups received 1 ml/kg of normal saline instead of L-arginine. Finally, all animals were tested in Morris water maze.

2.1. Surgery

The animals were ovariectomized under xylazine and ketamine (24 and 120 mg/kg, respectively) anesthesia (Black, et al., 1994). Anesthesia was confirmed by reduced respiratory rate and no response to gentle pinching of foot pad. Abdominal incision was made through the skin of the flank of the rats and ovaries and ovarian fats were removed. Ovaries were isolated by ligation of the most proximal portion of the oviduct before removal. The same procedure was performed on the sham rats except that the wound was closed without removing

the ovaries (Hosseini, Sadeghnia, Salehabadi, Alavi, & Gorji, 2009).

2.2. Apparatus

The Morris water maze was a black circular pool with a diameter of 136 cm and a height of 60 cm, filled with 20±1 °C water to a depth of 30 cm. The maze was divided geographically into four equal quadrants and release points were designed at each quadrant as North (N), East (E), South (S), and West (W). A hidden circular platform (10 cm in diameter), made of plexiglass, was located in the center of the southeast quadrant, submerged 1.5 cm beneath the surface of the water. Fixed, outside of the maze visual cues were present at various locations around the maze (i.e. computer, hard wares, and posters). An infrared camera was mounted above the center of the maze. An infrared LED was attached to each rat as a probe so that the animal motion could be recorded and sent to the computer. Radiab software (Iran) was used to measure the escape latency, traveled path and swimming speed (Alaei, Moloudi, Sarkaki, Azizi-Malekabadi, & Hanninen, 2007; Hosseini, Hadjzadeh, et al.; Hosseini, Headari, et al. 2010; Monteiro, et al., 2005).

2.3. Behavioral Assessment

The Morris water maze task for testing spatial memory was assessed in a water tank as described previously. The animals received a block of four trials during five daily sessions. During 5 days, the platform, situated in the center of the southeast quadrant, was submerged 1.5 cm below the surface of water and therefore invisible. The platform position remained stable during 5 days. A trial was started by placing a rat into the pool, facing the wall of the tank. Each of four starting positions (N, E, S, W) was used once in a series of four trials and their order was randomized. Each trial was terminated as soon as the rat had climbed onto the platform or when 60 seconds had elapsed. The animal was allowed to stay on the platform for 15 seconds. Then it was taken from the platform and the next trial was started after 20 seconds. Rats that did not find the platform within 60 s, were put on the platform by the experimenter and were allowed to stay there for 15 seconds. At the end of the 4th trial, the animals were kept warm for an hour, and then returned to their home cage(Hosseini, Feizpour, et al. 2011; Hosseini, Hadjzadeh, et al. 2010; Hosseini, Headari, et al. 2010; Hosseini, Nemati Karimooy, Hadjzadeh, & Safari; Saffarzadeh, et al. 2011).The escape latency and traveled path calculated by a computer. All tests were conducted between 08:00 and 11:00 a.m.

2.4. Biochemical Assessment

After the last session of behavioral test, the animals were sacrificed. The brain tissues were removed and submitted to total thiol groups and MDA levels. Total SH groups were measured using DTNB (2, 2'-dinitro-5, 5'-dithiodibenzoic acid) as the reagent. This reagent reacts with the SH groups to produce 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 brain homogenate in 1 ml cuvettes and sample absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Then 20 µl DTNB reagents (10 mM in methanol) were added to the mixture and after 15 min (stored in laboratory temperature) the sample absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mM) was calculated from the following equation (Hosseinzadeh & Sadeghnia, 2005) :

Total thiol concentration (mM) = (A2-A1-B) × 1.07/0.05 × 13.6

Malondialdehyde (MDA) levels, as an index of lipid peroxidation, were measured. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to produce a red colored complex which has peak absorbance at 535 nm. Two ml from reagent of TBA/TCA/HCL was added to 1 ml of homogenate and the mixture was heated for 40 min in a boiling water bath. After cooling, the whole solutions were centrifuged within 1000g for 10 min. The absorbance was measured at 535 nm (Hall & Andrus, 2009; Janero, 1990). The MDA level was calculated as follows.

Absorbance C (m) = Absorbance/ (1.56 × 105)

2.5. Statistical Analysis

All data were expressed as means ± SEM. The data of different groups were evaluated by one-way ANOVA and post hoc test. Differences were considered statistically significant when $P<0.05$.

3. Results

Escape latency and traveled path in the OVX group were significantly higher than in the Sham group ($p<0.01$) (Figs. 1 and 2). The animals of the OVX-LA group had significantly lower latency and path length to reach the platform compared to OVX group ($p<0.01$ and $p<0.001$, respectively) (Figs. 1 and 2). There were no differences between the sham-LA and Sham groups in escape latency and path length (Figs. 1& 2).

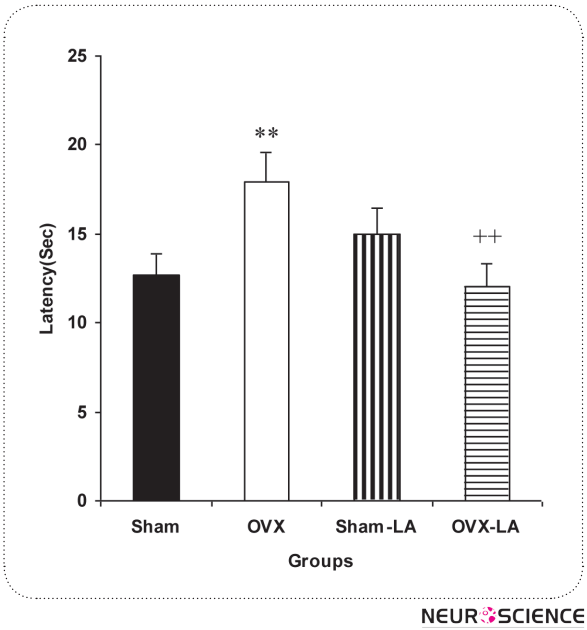

Figure 1. Comparison of the latencies among Sham, OVX, Sham-LA and OVX-LA groups. Data were presented as mean ± SEM. (n = 8 in each group). **P<0.01 compared to Sham group. ++P<0.01 compared to OVX group.

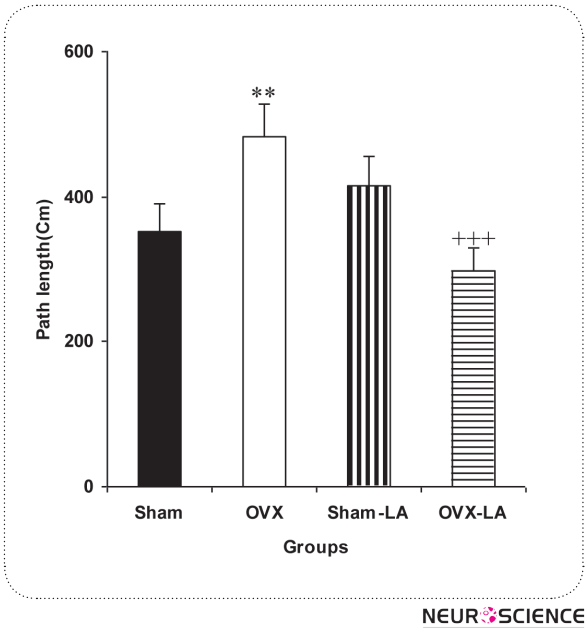

Figure 2. Comparison of path length among Sham, OVX, Sham-LA and OVX-LA groups. Data were presented as mean ± SEM. (n = 8 in each group). **P<0.01 compared to Sham group. +++P<0.001 compared to OVX group.

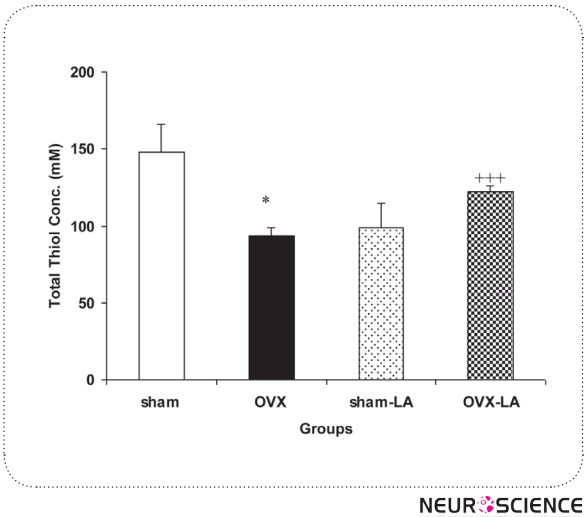

Figure 3. Comparison total thiol concentrations in brain tissues among Sham, OVX, Sham-LA and OVX-LA groups. Data were presented as mean ± SEM. (n = 8 in each group). *P<0.05 compared to Sham group. +++P<0.001 compared to OVX group.

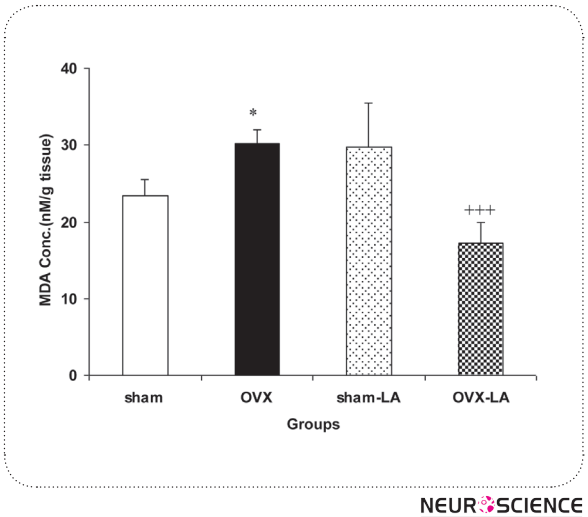

Figure 4. Comparison MDA concentrations in brain tissues among Sham, OVX, Sham-LA and OVX-LA groups. Data were presented as mean ± SEM. (n = 8 in each group). *P<0.05 compared to Sham group. +++P<0.001 compared to OVX group.

The total thiol groups in ovarictomized rats were significantly lower than sham animals (p<0.05). In OVX-LA group, the total thiol groups were significantly higher than OVX group (p<0.001). There was no significant difference between sham-LA and Sham groups in thiol group (Fig. 3).

MDA level in ovariectomized animals was higher than sham operated ones (p<0.05). Treatment of the ovariectomized rats by 500 mg/kg L-arginine attenuated the MDA concentrations in brain tissues in comparison with saline (p<0.001). However, there was no significant difference between Sham-LA and sham groups in brain tissues MDA levels (Fig. 4).

4. Discussion

In the current study, the antioxidant properties of L-arginine on thiol and MDA level in brain tissues of OVX rats with impaired spatial cognition in Morris water maze (MWM) was investigated. The OVX rats indicated impairments in MWM tasks which is consistent with previous studies revealing the deleterious effects of ovariectomy on learning and memory (Hosseini, Feizpour, et al. 2011; Monteiro, et al., 2005; Saffarzadeh, et al., 2010; Singh, Meyer, Millard, & Simpkins, 1994). In a study held by Monteiro and colleagues (2005) on rats, it was shown that hormone deprivation following ovariectomy increased the latency to find the platform in MWM test (Monteiro, et al., 2005). In some other studies, treatment with estrogen contributed to a considerable development in spatial learning performance in OVX rats (Ping, et al., 2008).

It is also reported that the dysfunction of cholinergic (Gibbs, 2000; Luine, 1985; Ping, et al., 2008) and monoaminergic systems (Holschneider, Kumazawa, Chen, & Shih, 1998) in the brain might be responsible for the deleterious effects of ovariectomy on cognitive impairments. On the other hand oxidative stress might be another responsible factor for this kind of damages following ovariectomy since the relationship between oxidative stress, neural damage and cognitive dysfunction has been well documented (de la Torre, 2008; Gustaw-Rothenberg, et al.; Ha, 2004; Head, 2009; Jellinger, 2009). MDA is one of the end-products of lipid peroxidation, the level of which has been shown to increase after ovariectomy due to the decline in the activity of antioxidative enzymes (Ha, 2004; Muthusami, et al., 2005). The learning and memory deficits observed in this study, after the ablation of the ovaries, can be partly because of the mechanisms mentioned above.

The results of previous studies showed that ovariectomy of rats impair MWM tasks which were reversible with L- arginine and was accompanied by low level of nitric oxide in that brain (Azizi-Malekabadi, Hosseini, Saffarzadeh, Karami, & Khodabandehloo, 2011; Azizi-Malekabadi, et al. 2012; Saffarzadeh, et al. 2010). From previous studies it can be inferred that some deleterious effects of ovairectomy may lead to low level of NO (Azizi-Malekabadi, Hosseini, Saffarzadeh, et al. 2011; Azizi-Malekabadi, Hosseini, Soukhtanloo, et al. 2012).

High level of endothelial nitric oxide synthase (eNOS) which is seen in cerebral microvessels of rats treated chronically with estrogen (McNeill, Zhang, Stanczyk, Duckles, & Krause, 2002) also confirm that estrogen increases production of NO (López-Jaramillo & Terán, 1999). In addition, estrogen in physiological concentrations increases the activity of nNOS which is presumed to have a role in learning impairment following ovariectomy (Hayashi, et al., 1994).

It has been suggested that different isoformas of NOS mediate the effects of L- arginine on learning and memory (Plech, et al., 2003; Saffarzadeh, et al. 2010). It is supposed that systemic administration of L-arginine as the substrate of all isoforms of nitric oxide synthases enhances NO production in the brain (Hara, et al., 2004; Plech, et al., 2003; Yamada & Nabeshima, 1998). In this study, an improvement in MWM task of OVX rats was observed after their treatment with L-arginine which was consistent with the results of previous study (Saffarzadeh, et al. 2010). It has also previously reported that both intracerebroventricularly (icv) and subcutaneously (sc) administrations of L- arginine significantly lead to prolongation of latency time in passive avoidance test (Plech, et al., 2003).

The results of present study also showed that cute treatment by L- arginine attenuated the MDA concentrations and increased the total thiol concentrations in brain tissues of ovareictomized rats. Other studies have also documented that L-arginine is firmly associated with protection against oxidative stress (Adawi, Kasravi, Molin, & Jeppsson, 1996; Lubec, Hayn, Kitzmuller, Vierhapper, & Lubec, 1997).

In addition, some of vitro studies have reported the potency of L-arginine in interacting with O2- and serving a protective function against reactive oxygen species (ROS) attacks which is probably mediated by NO (Lass, Suessenbacher, Wlkart, Mayer, & Brunner, 2002). Besides, a reduction in peroxynitrite formation in myocardial ischemia/reperfusion models is caused by L-arginine as well as prevention of the rise in thiobarbituric acid reactive substances (TBARS) after acute pulmonary embolism (Liang, et al., 2004; Souza-Costa, et al., 2005). It is also suggested that nitric oxide may mediate the superoxide (O2-) scavenging effects of L-arginine (Fukahori, Ichimori, Ishida, Nakagawa, & Okino, 1994; Kelly, Balligand, & Smith, 1996).

In this study, the decreased level of thiol groups and elevated MDA concentration in OVX rats were compensated by L-arginine treatment which may be caused by either NO production or the anti-oxidative stress function. Therefore it can be inferred that a long-term ovariectomy, can cause oxidative stress in rats' brain tissues which might be related to NO deficiency (Ejima, et

al., 1999). It is also considered that NO level in brain is modulated by estrogen. Therefore the effects of NO on learning and memory may be due to either its own direct effect on neurons or its influence in oxidative system (Öge, Sezer, Özgnül, Bayraktar, & Szmen, 2003).

Some other studies have mentioned the direct chemical interaction of L-arginine with superoxide anion which is a probable mechanism for its anti-oxidative stress function (Lanteri, et al., 2006; Lass, et al., 2002; Venditti & Di Meo, 1996). Besides, the reduction of pulmonary and cardiac oxidative stress during exhaustive exercise in either young or old rats, can be achieved by L-arginine administration (Lin, Yang, Chen, Huang, & Lee, 2005; Lin, Yang, Tsai, Huang, & Lee, 2006) conversely, some researches indicated that the excessive production of NO can be extremely toxic for the cells (Radi, Beckman, Bush, & Freeman, 1991). NO is a free radical and can combine with superoxide anions to form peroxynitrite, another highly destructive radical moiety(Eliasson, et al., 1999).The resultant reactive oxygen species (ROS) can induce significant oxidative stress that causes lipid peroxidation and produces functional alterations in proteins and DNA, eventually leading to neuronal death (Beckman, Chen, Crow, & Ye, 1994).

In conclusion, systemic administration of L-arginine has a significant effect on learning in ovariectomized rats. Furthermore, this effect might be related to antioxidant activity of L-arginine but it needs to be further investigated.

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