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The effect of l-arginine on estrogen level and ovarian cyst in aged rats with polycystic ovaries

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Abstract

Background and Objective: Menopause in women is associated with many complications that most of them are related to the decrease of estrogen levels in this period. Treatment with high doses of estrogen is common but has side effects. In this study, the effect of l-arginine administration on the level of this hormone in elderly rats was investigated.

Materials and Methods: Elderly Wistar rats were first studied with the help of Papanicolaou test to identify the stage of female sexual cycle. If confirmed to have diestrus phase, the rats were randomly classified into the following groups: control (saline 1 ml/kg, i.p.) and l-arginine group (5, 25, and 50 mg/kg). They were injected saline or l-arginine over a period of three to nine days. At the end, the rats were anesthetized by an i.p. injection of ketamine 100 mg/kg and xylazine 20 mg/kg and the blood samples were collected and the estrogen levels were measured with ELISA kit. The rats' ovaries and uteri were also biometrically examined and fixed in the formalin. They were stained by H&E method and the number of cysts in the ovaries were counted. Data were analyzed by the ANOVA.

Results: L-arginine at all doses (5-50 mg/kg) during all injection periods from three to nine days significantly increased the estrogen levels, but prominently reduced the ovarian cysts at the lowest dose (5 mg/kg).

Conclusion: Low doses of l-arginine over short periods of time can relieve menopausal problems including estrogen levels and ovarian status, probably by the modulator nitric oxide.

Keywords: Menopause, Nitric oxide, Elderly

1. Introduction

Menopause with permanent features occurs on average at the age of 51. Although menopause is associated with changes in pituitary and hypothalamic hormones, it is not caused by central nuclei failure but is an early ovarian lemon. During menopause, the ovary is unable to respond to the pituitary hormones FSH and LH. Therefore, serum FSH levels increase in response to ovarian letdown and the absence of negative feedback from the ovaries (1-4). Also, the production of estrogen and progesterone stops and serum estrogen levels decrease, so, estrogen therapy is common during postmenopausal, but can the effects of estrogen dose be monitored by measuring serum estradiol levels? Although estradiol levels in young women are significantly different from postmenopausal women, but, in relation to postmenopausal estrogen therapy, serum estradiol level is not directly proportional to estrogen dose, and thus, the estradiol levels can not reflect the effects of estrogen dose (5, 6).

According to the above criteria, a major problem in menopause is estrogen depletion; decreased estrogen leads to menopausal consequences (7), such as hot flashes (8), osteoporosis (9), cystic ovaries (10, 11), etc.

One of the ways in which estrogen acts on target tissues is by regulating the activity of the nitric oxide system (12, 13). The NO is a gaseous molecule that acts as an endogenous mediator in many physiological reactions in the body. It is produced by the conversion of the amino acid L-arginine to L-citrulline during a reaction catalyzed by the enzyme NO synthase (NOS)

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(14). The range of activity of the NOS enzyme is wide. Some hormones play a role in regulating its activity, and thus, participate in NO production (15). Estrogen is effective in regulating NO production in the female sex, and at least one of the pathways for the effect of sex hormones is to involve the NO system (16). Studies have shown that during menopause, plasma NO levels decrease, which may be a reason for a decrease in estrogen levels during this period (17). In this study, l-arginine (a precursor of NO) is injected to female Wistar rats to stimulate the production of NO and we want to examine whether this substance could affect the level of estrogen and eliminate ovarian cysts.

2. Materials and Methods

In this study, 60 elderly female Wistar rats weighing approximately 250 g from 36 weeks of age were obtained from the Animal Center of Pasteur Institute of Iran and adapted for one week at 21°C and 12 h of light. They had free access to water and food *ad libitum*. Smears were prepared from the vaginas of rats to determine the female cycle. The procedures were done under the principles of the Helsinki Declaration and approved by the Local Ethics Committee (IR.SHAHED.REC.1399.106).

2.1. Smear preparation

First, using pasteurized sterilized pipette, some physiological serum along with the washed cells were collected from the uterine wall and smeared on a 15 x 45 mm slide. After drying the vaginal sample on the slide, the samples were fixed on the slide with 95% ethanol. After this step, the stabilized samples were washed with running water for 5 sec and then stained with Hematoxylin & Eosin for about 2 min. After this time, the samples were immediately rinsed with running water and stained with Orange G dye to differentiate the cytoplasm color after exposure to 3% ethanol (3 sec). After this step, the samples were immersed in 3% ethanol. Then, the clarification was performed in 4 steps with xylene and at the end, the samples were glued and fixed on the slide with Entellan (Merck, Germany) and examined with a light microscope. Animals in the diestrus cycle were selected according to sources, because older animals are in the diestrous stage (18).

2.2. Animal categories

Animals were classified into these groups: 1- Control group that received saline (physiological serum); this group received the placebo (1 ml/kg) for a few (3, 5, and 9) days by intraperitoneal (i.p.) injection. 2- Experimental group that i.p. received l-arginine (treatment) at doses of 5, 25, and 50 mg/kg in a few days (3, 5, and 9) (once a day). First, three-day injections were performed with the lowest dose of the drug and followed to the highest dose, then 5-day and 9-day injections were performed with the lowest dose to the highest dose of the drug, respectively.

2.3. Blood sampling from rat hearts

Twenty-four h after the last injection, blood samples were taken from the hearts of rats (under anesthesia with ketamine-xylazine) using a 5 ml syringe.

2.4. Serum preparation and hormone measurement by ELISA method

First, blood samples were kept in a test tube in the laboratory for a while to allow blood to clot. The tubes containing the blood samples were then centrifuged at 3000 rpm for 5 min. The serum sample was collected and stored in a -80°C freezer and during 2 weeks, the serum estrogen level was measured by ELISA kit.

2.5. Surgery and stabilization of ovarian and uterine tissue

At the end of blood sampling, a longitudinal incision was made in the abdominal surface of rats and the ovaries were studied from the abdominal cavity separately. These tissues were then isolated and fixed in 10% formalin. Using a Leica model microtome (Germany), 4 µm sections were taken from these tissues and stained by Hematoxylin & Eosin and examined for cyst number.

2.6. Statistical analysis

All data were primarily checked with homogeneity test in the SPSS program, and if passed, the analysis of variance (ANOVA) was performed under α=0.05. To compare different groups with each other, in case of significant results, Tukey's post hoc was followed.

3. Results

3.1. Estradiol levels

In the 3 to 9-day injection groups, in all doses of l-arginine (5, 25, and 50 mg/kg), estrogen levels showed a significant increase as compared to the control group (Fig. 1). Based on statistical results in all doses during all three treatments (3 to 9 days), there was an increase in estrogen levels but in longer periods this effect declined.

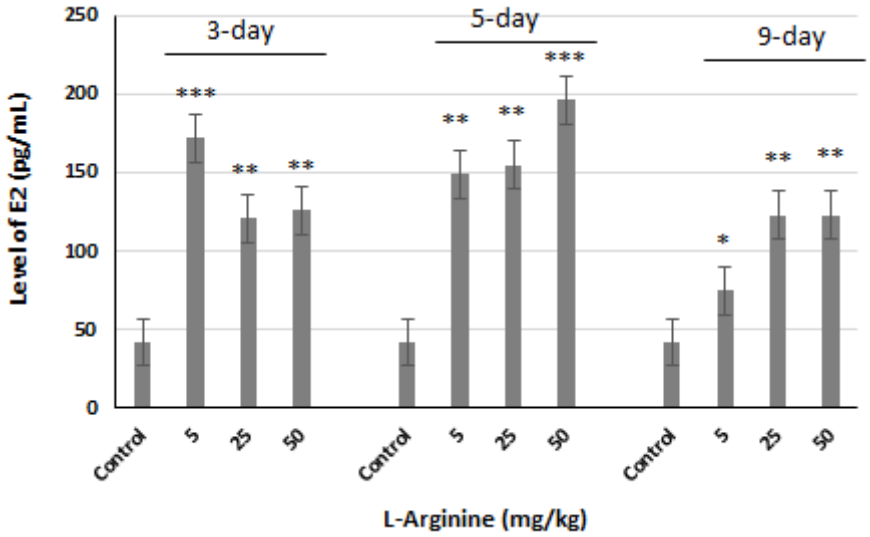

Figure 1: The rats were injected saline or l-arginine (5, 25, and 50 mg/kg) over a period of three to nine days. The estrogen levels showed a significant increase compared to the control group except for the longer periods of injections, which weakened. The between groups' differences are based on Tukey's Post hoc test (*P<0.05, **P<0.01 and *** P< 0.001).

3.2. Number of cyst

In the 3 to 9-day injection groups, at lower doses of l-arginine (5 and 25 mg/kg), the number of cysts significantly decreased compared to the control group (Fig. 2). Statistical results show that at lower doses in all three treatments (3 to 9 days), the reduction of cyst is much more noticeable, and in longer periods, the reduction of cyst is weakened at some doses.

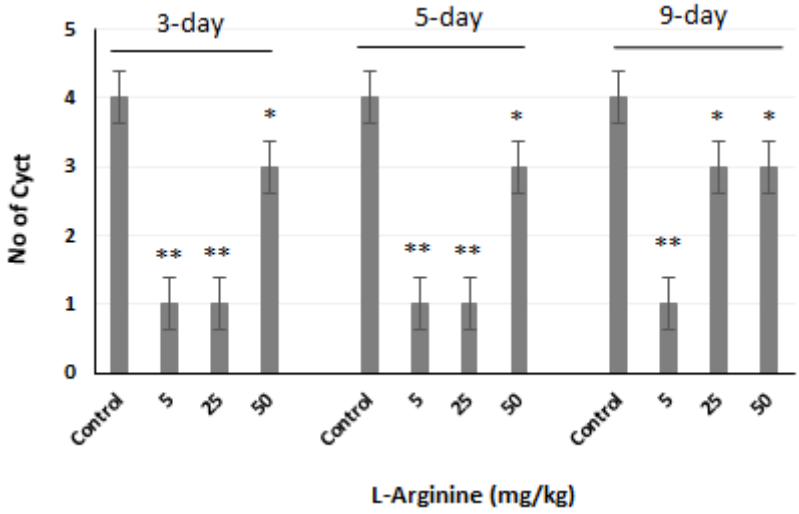

Figure 2: The animals were administered saline or l-arginine (5, 25, and 50 mg/kg) over a period of three to nine days. The number of cysts significantly decreased compared to the control group, but in longer periods, the reduction of cyst is declined at some doses. The between groups' differences are based on Tukey's Post hoc test (* P<0.05 and ** P< 0.01).

3.3. Number of Growing and Gravid follicles

In all groups, the number of growing and mature follicles increased except in more repetitions and higher doses of l-arginine (25 and 50 mg/kg) (Fig. 3).

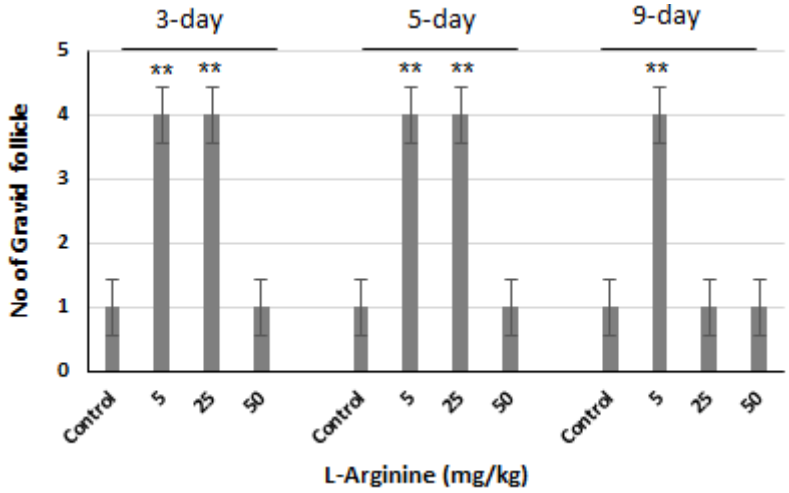

Figure 3. Saline or l-arginine (5, 25, and 50 mg/kg) were administered to rats over a period of three to nine days. The number of growing and mature follicles increased except in more repetitions and higher doses of l-arginine. The between groups' differences are based on Tukey's Post-hoc test (** P< 0.01).

3.4. Diameter of Ovary

Ovary diameter increased with more recurrences and highest dose of l-arginine (50 mg/kg) (Fig. 4).

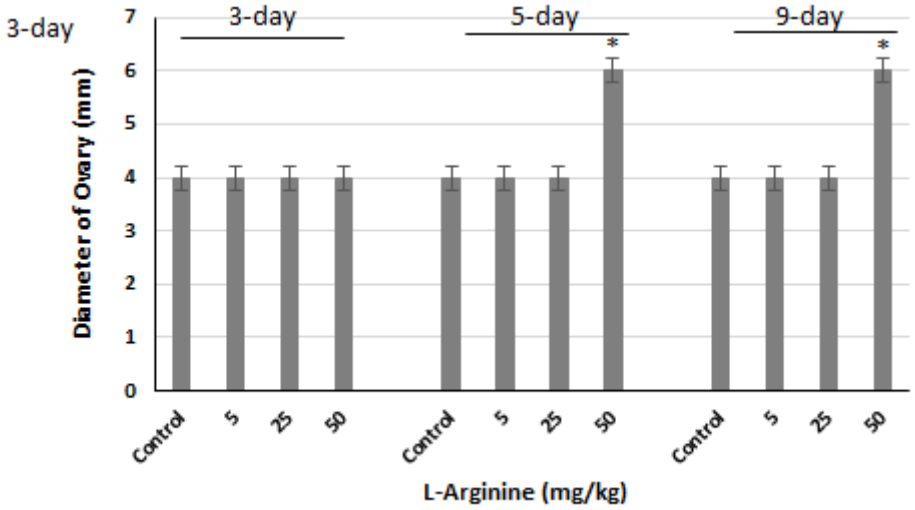

Figure 4. Saline or l-arginine (5, 25, and 50 mg/kg) were given to rats over a period of three to nine days. The diameter of ovary increased with more recurrences and highest dose of l-arginine (50 mg/kg). The between groups' differences are based on Tukey's Post hoc test (* P< 0.05).

4. Discussion

There is no cure for menopause and common treatments focus on reducing the effects of menopause. Menopausal-related physical symptoms include hot flashes, genitourinary disorders, skin changes, ischemic heart disease, obesity, high blood pressure, headaches, and more. Psychological symptoms of this period are agitation and stress, anxiety, distress, self-esteem problems, memory loss and sleep disorders, etc. (19). Decreased estrogen after menopause increases bone fragility, increases serum calcium levels, reduces calcitonin production, and decreases calcium absorption. There is a strong link between sex hormones such as estrogens and female mood disorders. The use of birth control pills in women who are able to reproduce is associated with a reduction in depression. Changes in estrogens during pregnancy and menopause increase the risk of these disorders in women (20).

Estrogen can cross the blood-brain barrier and mediate two cytoplasmic or nuclear receptors called estrogen receptors, which play an important role in regulating the behavior and function of the reproductive glands, and play an important role in the learning and memory

and especially affect the function of different parts of the brain (21).

One of the menopausal treatments is hormone therapy, during which estrogen is prescribed to compensate for the decrease in this hormone in the body. Reducing menopausal symptoms and increasing quality of life are major reasons for starting hormone therapy in women (22). Due to the significant negative effects of menopausal symptoms, most women tend to receive appropriate relief from these side effects. Hormone therapy with estrogen alone or in combination with progesterone is often recommended for the management of menopausal symptoms (23). In Iran, only 15% of postmenopausal women use hormone replacement therapy (HRT) (24). Due to the fact that hormone therapy has caused many and dangerous complications such as strokes, breast, ovarian and colorectal cancers, headaches, mood swings, nausea, vaginal bleeding and endometriosis (25), so the use of drugs or another substance that does not induce a risk of many of these dangerous side effects is very necessary. This study investigated the effect of l-arginine (a precursor of nitric oxide) on the ovaries and estrogen levels of postmenopausal rats.

In this study, estrogen levels in l-arginine doses (5, 25, and 50 mg/kg) showed a significant increase compared to control group. In review, according to previous studies, NO can increase estrogen levels (15, 17). In addition, in the three-day and five-day groups in two doses of l-arginine (5 and 25 mg/kg), a significant decrease in the number of ovarian cysts was seen compared to the control group. The same effect was seen only in the lowest dose of l-arginine (5 mg/kg) in longer-term treatment (9-day). Therefore, l-arginine was able to have a positive effect on menopausal difficulties and improve ovarian cysts. However, this effect is related to the dose and duration of treatment of the substance. Growing and matured follicles were also measured as evidence of normal ovarian activity, which appeared with lower doses of l-arginine. To interpret, according to previous studies, the presence of NO is essential for the development of follicles in the ovary (28).

According to the results obtained in this study, with increasing dose of l-arginine, we observed the side effects (increase in dimensions of the ovaries), probably due to the pro-inflammatory NO produced by i.p. injection of l-arginine, which previous studies confirm this feature (26). According to previous publications, excessive increase of NO in the body can lead to cytotoxicity as well as inflammation in the organs of the body (27). Some authors have also postulated that the increased concentrations of NO or its releasing agents prevent the production of steroids in luteal and granulosa cells in humans and rats but increase the androgen release in the ovaries, which in turn leads to cyst formation in the ovaries (31-39). In summary, using l-arginine only for a short period and in small doses can alleviate the problems of menopause to some extent. It may increase the serum level of estrogen by activating the ovaries, which can lead to positive results. However, as the present results show, the use of this substance during long periods and high doses due to inflammatory processes has adverse effects, and therefore until the mechanisms are clarified, we recommend a caution for long-term use of this substance.

Conclusion

Based on present finding, the l-arginine is able to eliminate menopausal (ovarian) complications in low doses and with less repetitions.

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