Effect of Letrozole during Pregnancy on Learning and Memory of Offspring Rats

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ABSTRACT

Background and Objectives: Hippocampus is the main structure involved in spatial learning and memory consolidation. Formation of spatial memory can be strongly influenced by medications, hormones and different substances. Due to importance of new pharmacotherapy on drug administration in pregnancy, the aim of this study was to investigate the effect of letrozole-therapy during pregnancy on memory and learning in offspring rats.

Methods: In this study, 24 pregnant rats were divided into a control and three experimental groups (N=6). The subjects received low dose (0.25 mg/Kg), average dose (0.5 mg/Kg) and high dose (1 mg/Kg) of letrozole orally during 16-19 days of gestation. After maturating, learning and memory of the offspring were assessed by passive avoidance learning apparatus. Data were analyzed by SPSS 20 using one-way analysis of variance and the Tukey's test. P-values less than 0.05 were considered statistically significant.

Results: Weights of the offspring who received letrozole decreased significantly compared with the control. There was no significant difference in the step-through latency between the experimental groups. However, the step-through latency and time spent in the dark compartment decreased significantly in the experimental groups compared with control group ($P<0.05$).

Conclusion: The results of this study show that letrozole can influence learning and memory of offspring rats.

Keywords: Letrozole, Gestation, Memory, Learning, Offspring, Rats.

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INTRODUCTION

Letrozole is an aromatase inhibitor that causes embryo toxicity in rats, increasing embryonic lethality and anomalies such as deformities of the axial skeleton (1-3). Beneficial effects of estrogen on brain functioning and morphology have been documented (4-6). It is well established that estrogens plays a pivotal role in synaptic plasticity, learning and memory, as well as in neuroendocrine and sexually dimorphic differentiation (7-9). Several brain regions such as the hippocampus, amygdala and cerebral cortex that are involved in learning and memory processes have a large number of estrogen receptors (10). Low level of estrogen has been linked to increased incidence of neurodegenerative diseases and deterioration of cognitive function in menopausal women (4,11). The biosynthesis of estrogens is catalyzed by the enzyme aromatase (AROM), which is distributed in specific brain regions such as the cerebral cortex, limbic system, hippocampus, hypothalamus, amygdala and midbrain (12,13). Since AROM is the limiting enzyme for estrogen synthesis, many elective AROM inhibitors have been used to explore the association of brain function and estrogen.

Letrozole is a successful drug for the treatment of estrogen-receptor-positive breast cancer with some adverse effects on the nervous system. However, the results of studies in this regard are limited and controversial. In addition, its underlying mechanism of action is not clear (14). Recently, a pilot study has shown that treatment of breast cancer patients with letrozole impairs processing speed and verbal memory (15). Other studies have reported that letrozole affects neuroendocrine function (16,17), inhibits cell proliferation and increases apoptosis. In the open field tests, letrozole has been shown to induce mild anxiety and increased latency (18).

Several investigation on hippocampal synaptic plasticity revealed that letrozole administration reduces spine synapse and axon outgrowth. However, some studies showed that letrozole could rescue spatial learning and memory deficiency induced by ovariectomy (4,18). Therefore, the exact role of this drug on brain function, and especially on the hippocampus structure and function needs to be clarified (7). While many studies have examined health-related outcomes of delayed motherhood for women, the potential consequences in their children remain to be elucidated. Majority of studies on rats show that pregnancy and motherhood are associated with enhanced cognition, particularly in tasks that assess spatial learning and memory (19,20).

The aim of this study was to clarify the effect of letrozole administration during pregnancy on learning and memory development in offspring rats.

MATERIAL AND METHODS

Adult male Wistar rats (Pasteur Institute, Tehran, Islamic Republic of Iran) weighing 200–250 g were maintained in an animal house (4 rats per cage) with 12-h light/dark cycle (beginning at 7 a.m.) and free access to food and water. All animals were allowed to adapt to the laboratory conditions, and were handled for 10 min/day during this adaptation period. The experiments were performed during the light cycle between 9:00 a.m. and13:00 p.m. Each animal was used only once. Two weeks after arrival, vaginal smears from the female rats were taken daily to determine the phase of the estrus cycle. On the proestrus day, female rat were placed along with male rats for 12 h. Presence of sperm in vaginal smears was checked the next morning. Pregnant females were randomly assigned to a control and three experimental groups, receiving low (0.25 mg/Kg), average (0.5 mg/Kg) and high (1 mg/Kg) dose of letrozole **(**21-23**)**. Oral letrozole was given during 16-19 days of gestation. Then, 6-8-month old male offspring were selected for the experiments. All procedures for the treatment of animals were approved by the Research and Ethics Committee of the School of Advanced Technologies in Medicine (Islamic Azad University of Hamadan), and were done in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Efforts were made to minimize the number of animals used and their suffering. A standard passive avoidance conditioning apparatus (a shuttle box) was used for training and testing. Training was performed in a conditioning chamber, which had two compartments (light and dark) with same dimensions (20 × 20 × 30 cm). A guillotine-type door that could be lifted or lowered by the observer separated the two compartments.

Stainless steel grids (2.5 mm in diameter) were placed at 1-cm intervals (distance between grids) on the floor of the dark compartment to deliver an electric shock. A grid floor shocker (50 Hz, 2 s, and 0.8 mA intensity) was connected to the steel rods of the dark compartment, and provided a scrambled foot shock.

All animals were allowed to habituate in the experimental room for an hour prior to testing. All training and testing were carried out between 10:00 and 14:00. All experimental groups were first habituated to the apparatus. Each animal was gently placed in the light compartment for 30s, after which the guillotine door was lifted and the latency with which the animal crossed to the dark (shock) compartment was recorded. Animals that waited for more than 120 s to cross to the other side were excluded from the experiment. Once the animal had crossed over with all four paws in the next compartment, the door was closed and the rat was taken from the dark compartment into the home cage. The habituation trial was repeated after 30 min and was followed after the same interval by the acquisition trial during which the guillotine door was closed and a shock (50 Hz, 2 s, 0.8mA intensity) was delivered immediately after the rat had entered the dark compartment. After 20 s, the rat was removed from the apparatus and placed temporarily in the home cage for 2 min. The rat was then retested in the same way as before. If the rat did not enter the dark compartment during the following 120 s, successful acquisition of a passive avoidance response was recorded (24).

A retention test was performed 48 h after the training to determine long-term memory. Each animal was placed in the light compartment and the door was opened after 5 s. Then, step-through latency (STL) was measured for entering the dark compartment. The test session ended when the animal either entered the dark compartment or remained in the light compartment for 300 s (criterion for retention). During these sessions, no electric shock was applied. On the retention test, the time spent in the dark compartment (TDC) was also recorded as a measure of retention performance.

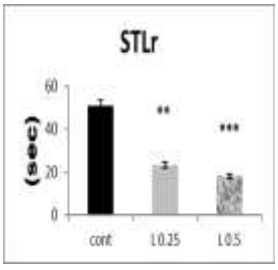
The number of trials required for acquisition in the training session, and the STL and TDC in the retention session were compared between experimental groups using a one-way analysis of variance followed by Tukey's test for multiple comparisons. All results were reported as mean ± standard error of mean (SEM). P-values less than 0.05 were considered statistically significant (25).

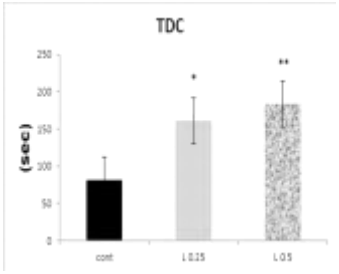
RESULTS

Weight of the animals receiving the low and medium doses of letrozole differed significantly with that of the control group ($P < 0.01$). However, there was no difference between the weights of animals in these two groups. Most of the animals that received 1 mg/Kg of letrozole died.

We studied the effects of letrozole (0.25, 0.5 mg/Kg) administration during pregnancy on memory using a behavioral test in the passive avoidance apparatus in adult offspring rats. Administration of letrozole in both groups decreased STL significantly compared with the control group (Figure 2). Chronic administration of letrozole during pregnancy increased the TDC significantly compared with the control group (Figure 2).

Figure 2a- Effect of different doses of letrozole administration during pregnancy on STL in adult offspring rats. Figure 2b- Effect of letrozole administration during pregnancy on TDC





DISCUSSION

We investigated the effect of letrozole administration during pregnancy on spatial memory of adult offspring rats. Chronic exposure to letrozole during pregnancy caused impairments in the acquisition of learning and memory retrieval in adult offspring rats. Moreover, we observed significant weight differences between the rats that received letrozole and those in the control group. Chronic exposure to letrozole decreased the STL and enhanced the number of trials to acquisition and TDC in a passive avoidance task indicating impairment in memory. These data support the results of previous experiments (7). Estrogen receptors are present in dopaminenergic neurons (26). Several brain regions that are involved in memory and cognition are rich in estrogen receptors (27,28). Although it is well-demonstrated that estradiol exposure can be deleterious to some neuronal populations, the potential clinical benefits of estrogen treatment for enhancing cognitive function may outweigh the associated central and peripheral risks (5). Fink et al. showed the effect of estrogen on central monoamine neurotransmission, mental state, cognition, emotion and behavior (29). Essential role of endogenous hippocampal estrogen synthesis in maintenance of hippocampal spine synapses has been demonstared previously (30,31). Studies have shown the presence of AROM in hippocampal neurons of rodents and primates on mRNA (32,33) and protein levels (34). In 2001, Azcoitia et al. demonstrated the functional activity of this enzyme (35). Yet, estrogen synthesis was shown only in hippocampal explants cultures obtained from newborn rats (12). The activity of AROM could be inhibited notably by the addition of the enzyme's inhibitor letrozole in culture models. Letrozole affects transcription of neural cell adhesion molecules (N-CAM) that might participate in synaptogenesis in neuronal plasticity (36). Several recent studies indicated a role for N-CAM in learning and establishment of long-term memory (37,38). In our study, spatial learning of offspring rats whose mothers had been treated with letrozole changed significantly compared with the control group. This may be partially in line with studies that showed that letrozole reduced the N-CAM expression in the hippocampus and cortex of adult rats (37). Since estradiol treatment could increase the release of catecholamine in hippocampus, reduction of noradrenaline and dopamine concentration in the brain by inhibiting estradiol synthesis is expected (39). A study has shown a positive correlation between the noradrenaline and dopamine reduction levels in the hippocampus and spatial memory function in rats (40).

In 2012, Vierk et al. showed that systemic inhibition of AROM activity significantly impairs long-term potentiation in the hippocampus of female and male mice (41). Another study indicated that systemic inhibition of AROM in mice affects structural synaptic plasticity in the hippocampus, which is in agreement with our study (42). However, a pilot study showed that women receiving AROM inhibitors for treatment of breast cancer had specific verbal memory deficits (15). According to our results, letrozole administration during pregnancy might decrease memory and learning in offspring rats. However, further clinical studies are necessary to investigate the molecular changes during letrozole exposure on cognitive deficits.

CONCLUSION

Administration of letrozole significantly affects learning and memory especially during embryogenesis, and probably the synaptogenesis in hippocampus.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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