The Effect of Treadmill Running on Passive Avoidance Learning in Animal Model of Alzheimer Disease

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Date of Submission: Apr 15, 2012

Date of Acceptance: Sep 13, 2012

How to cite this article: Hosseini N, Alaei H, Reisi P, Radahmadi M. The effect of treadmill running on passive avoidance learning in animal model of alzheimer disease. Int J Prev Med.2013;4:187-92.

ABSTRACT

Background: Alzheimer's disease was known as a progressive neurodegenerative disorder in the elderly and is characterized by dementia and severe neuronal loss in the some regions of brain such as nucleus basalis magnocellularis. It plays an important role in the brain functions such as learning and memory. Loss of cholinergic neurons of nucleus basalis magnocellularis by ibotenic acid can commonly be regarded as a suitable model of Alzheimer's disease. Previous studies reported that exercise training may slow down the onset and progression of memory deficit in neurodegenerative disorders. This research investigates the effects of treadmill running on acquisition and retention time of passive avoidance deficits induced by ibotenic acid nucleus basalis magnocellularis lesion.

Methods: Male Wistar rats were randomly selected and divided into five groups as follows: Control, sham, Alzheimer, exercise before Alzheimer, and exercise groups. Treadmill running had a 21 day period and Alzheimer was induced by 5 μg/μl bilateral injection of ibotenic acid in nucleus basalis magnocellularis.

Results: Our results showed that ibotenic acid lesions significantly impaired passive avoidance acquisition ($P < 0.01$) and retention ($P < 0.001$) performance, while treadmill running exercise significantly ($P < 0.001$) improved passive avoidance learning in NBM-lesion rats.

Conclusion: Treadmill running has a potential role in the prevention of learning and memory impairments in NBM-lesion rats.

Keywords: Alzheimer, nucleus basalis magnocellularis, passive avoidance learning, treadmill running

Original Article

INTRODUCTION

Basically, Alzheimer's disease (AD) described by Alois Alzheimer in 1907.[1] It is characterized by a complex of neuropathological, biochemical, and behavioral symptoms, that gradually impair memory and the ability to learn, carry out daily activities and behavior.[2] Nucleus basalis magnocellularis (NBM)

is one of the cerebral cortex function modulators.[3,4] Therefore, NBM plays a major role in all forms of learning and memory.[5] In particular, ibotenic acid-induced lesions of the NBM in rats produce impairment in acquisition and retention phase of passive avoidance tasks.[6,7] Studies have shown that NBM has been widely involved in the pathogenesis of AD and is accompanied with cognitive deficits.[5,8] A number of studies.[9] suggested that exercise in aged rats improves learning and neurogenesis. So it may slow the onset and progression of learning and memory deficit in AD. Other studies have demonstrated the beneficial effects of exercise on brain function and plasticity.[10,11] It is proved that regular exercise attenuates motor deficits, increases formation of new neurons, and ameliorates neurological impairment in several neurodegenerative diseases.[12-14]

Some studies on transgenic mouse models of AD have showed beneficial effects of exercise on general activity, spontaneous alternation, object recognition memory[15] and, spatial learning performance.[16,17] Some animal studies have revealed that treadmill running improves spatial and passive avoidance learning in NBM-lesion rats.[4,18] In previous studies, transgenic and NBM-lesion models, they investigated the effects of physical activity after onset of AD in these models and, there have been no basic researches performed to estimate the preventive effects of exercise. The aims of this study were to investigate preventive effects of treadmill running in NBM-lesion rats on learning and memory deficit by passive avoidance task in order to examine whether treadmill running can prevent learning impairment in this model of AD.

METHODS

Animals

Male Wistar rats (250-300 g; n = 54) were obtained from Jondishapour University, Ahwaz, Iran). The animals were kept in animal house and provided with food and water *ad libitum* and experienced a 12:12-h light-dark cycle (07:00-19:00) in a temperature controlled environment (22 ± 2°C). All behavioral experiments were carried out between 13:00 and 15:00. This study was approved by the Ethics Committee for Animal Experiments at Isfahan University approved the study, and all experiments were conducted in accordance with the international guiding principles for biomedical research involving animals, which was revised in 1985.

Rats were randomly allocated to the following groups:

- Control group (Co; n = 11): No injection, no exercise.
- Sham operation (Sh; n = 10): Saline (drug solvent) was injected in NBM.
- Alzheimer group (A; n = 11): Ibotenic acid was injected in NBM.
- Exercise before Alzheimer group (E-A; n = 12): Ibotenic acid was injected bilaterally in NBM, and then rats exercised 21 days.
- Exercise group (E; n = 10): Rats exercised 21 days.

Rats were anesthetized with chloral hydrate (450 mg/kg, i.p) and then placed in a Stoelting stereotaxic apparatus (incisor bar ± 3.3 mm, ear bars positioned symmetrically). The scalp was cleaned and incised on the midline and a burr hole was drilled through the skull and ibotenic acid (cat number 12765, Sigma) was injected at coordinates of: AP = 1.2, ML = ±3.2, DV = 7.5 mm from surface skull.[19] 10 μg/μl of ibotenic acid was injected (5 μg/μl each sides) with microinjection pump at the speed of 120 μ lit/h. Instead of ibotenic acid solution, Sham-operated rats received 0.9% normal saline (5 μl bilaterally). Post-operatively, the rats were given special care until spontaneous feeding was restored. Behavioral tests were conducted four weeks after the surgery and were evaluated blind to the treatments by the observer.

Exercise protocol

Rats in the exercise group run on a treadmill at a speed of 20-21 m/min for 60 min daily (6 days a week), for 3 weeks at 0° inclination. To familiarize animals with the experimental set up, the treadmill was switched on and the speed was increased from 5 to 21 m/min and over the course of 6 days, the duration was increased from 10 to 60 min. When exercising rats moved back on the treadmill, electric shocks were sparingly used to impel the animal to run. From week 2 onwards, speed and duration were kept constant at 20-21 m/min, 60 min per run after warm up. The non-runners groups were not put on the treadmill for the same duration of running as runners did.[4] After 21 days, rats in all exercise groups, were subjected to passive avoidance learning (PAL) test.

Passive avoidance learning test

The training apparatus had two compartments comprising a small chamber (25 × 25 × 20 cm) and a large dark compartment (50 × 25 × 20 cm). These compartments were separated by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator.

At the beginning of the test, each rat was placed in the apparatus for 5 min to become habituated. On the second day, an acquisition trial was performed; rats were placed individually in the illuminated chamber. After a habituation period (1 min), the guillotine door was lifted. Then after the rat entered the dark chamber, the door was lowered and an inescapable scrambled single electric shock (75V, 0.2 mA, 50HZ) was delivered for 3 second. Latency to cross the dark compartment (i.e., pre-shock latency) was recorded. After exposure to the foot shock, the rat was removed from the passive avoidance apparatus to its home cage. Retention of passive avoidance performance was tested 24 h afterwards. The rat was placed in the lighted (safe) compartment again with access to the dark compartment without any shock. The latency to enter the dark compartment was measured (i.e., testing latency) up to a maximum of 300 seconds.

Statistical analysis

In the passive avoidance test, all results were compared using a Kruskal-Wallis nonparametric one-way analysis of variance corrected for ties, followed by a two-tailed Mann-Whitney *U* test. The comparisons of retention time 24 h (within groups) were analyzed by Friedman test, followed by a Wilcoxon signed ranks test. Results are expressed as the mean ± SEM.

Histology

After the completion of behavioral tests, the rats were sacrificed and brains were removed and fixed in formalin, and then were sectioned to verify ibotenic acid injection site. The lesions were reconstructed on standardized sections of the rat brain[19] [Figure 1].

RESULTS

Passive avoidance test in rats

The latency was measured in pre foot shock (Acquisition time) and 24 h post foot shocks (Retention time). Results indicated that the pre-shock latency was the same among all groups, but compared to group Alzheimer (A) as well as between group A and group Exercise-Alzheimer (E-A), acquisition time was longer in control (Co) group ($P < 0.01$, $P < 0.05$ respectively). There was a significant difference between group Exercise (E) and A ($P < 0.01$), as well as group Sham (Sh) and A [$P < 0.05$, Figure 2].

However, the retention time during testing (i.e., testing latency carried out 24 h after receiving

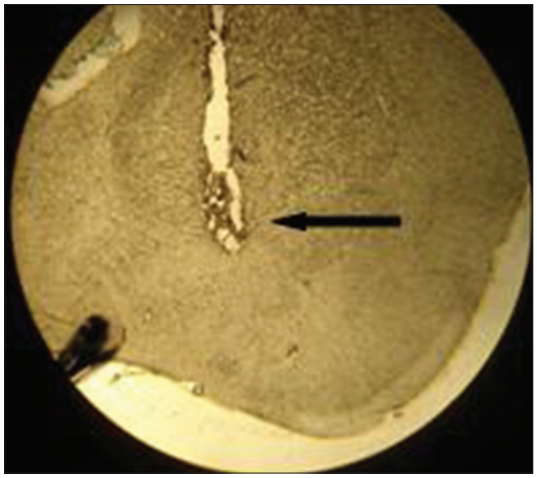

Figure 1: Histological representation of NBM lesion area marked. The arrows indicated the stained place of injection in NBM

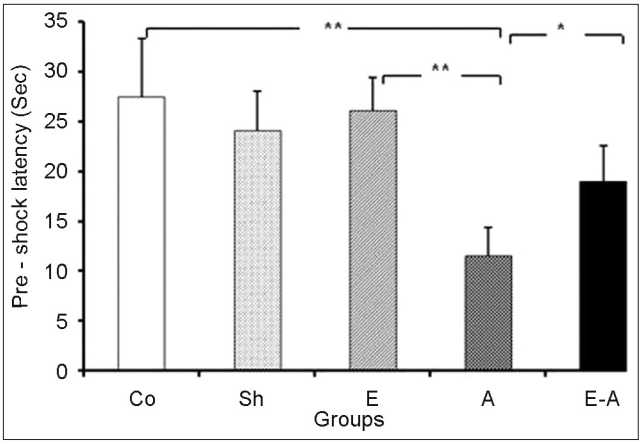

Figure 2: Comparison of latency to enter the dark chamber before receiving foot shock (Acquisition time). Each bar represents the mean ± S.E.M. There were significant differences between control ($n = 11$) and A ($n = 11$) group (**$P < 0.01$), as well as A and E ($n = 12$) group (**$P < 0.01$). There were significant differences between A and others (Sh and E-A groups, *$P < 0.05$). All results were analyzed by Kruskal-Wallis test followed by Mann-Whitney U test

foot shock) was significantly decreased in group A when compared to other groups (Co, Sh, E and groups E-A; $P < 0.001$, $P < 0.01$, $P < 0.001$, $P < 0.001$ respectively). The retention time changes were not significant between group E and group Sh. Also, there were not significant differences in retention time between control group, as well as Sh and E-A groups [Figure 3].

The results of pre foot shock and post foot shock latency were analyzed by a paired sample to evaluate changes within groups. In this part, our data showed that there were significant differences in pre and post foot shock latency among all groups [$P < 0.01$, Figure 4].

DISCUSSION

In the present study, the passive avoidance retention and retrieval function was impaired by ibotenic acid NBM-lesions. Our results showed that treadmill exercise training significantly improves passive avoidance performance in normal and NBM-lesion rats [Figures 2 and 3].

These results were in agreement with those of previous studies.[12,18] Chopin *et al.*[20] demonstrated significant performance deficits in both passive avoidance and Morris water maze tests in bilateral NBM-lesion rats. They showed that regular exercise significantly attenuated lesion-associated decrease in brain functions. In these studies, animals exercised after onset AD in this animal model.

In this research, relationship between treadmill running and preventive effects on learning and memory deficits observed in NBM-lesion rats were investigated. Our results indicated that in comparison to NBM-lesion rats, learning and memory in groups E-A were improved that is, exercise has preventive effects on acquisition and retention time impairment in passive avoidance test in NBM-lesion [Figures 2 and 3]. Furthermore, it will postpone memory impairments that may result from some exercise mechanisms such as increase in dopamine[21] and muscarinic receptor density, Acetylcholine level,[22] neurotransmitter release in the hippocampus,[23] brain derived neurotrophic factor, BDNF gene expression,[24] neuron proliferation and survival in the animal's brain.[25] Similarly, Van Praag *et al.*[26] suggested that exercise in animals can enhance both cognition and neuron proliferation. Conversely, Yuede, *et al.*[15] reported forced exercise did not have a successful effect on preventing memory deficits. Also, other researchers suggested that exercise training (swimming) increased memory of rats in passive avoidance test, but such increase was temporary after stopping exercise[27] because swimming requires physical effort and compared to treadmill running in rats, was known as an effective stressor.[28] According to our results, treadmill running had beneficial effects on encountering learning and memory deficits with NBM-lesion as animal model of AD and probably can reduce dementia in patients.

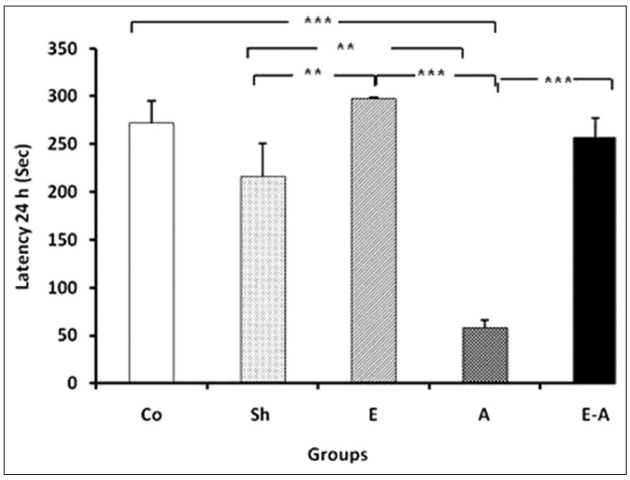

Figure 3: Comparison of latency to enter the dark chamber 24 h after receiving foot shock (the retention time). Each bar represents the mean ± S.E.M. The retention time was significantly decreased in the A group compared to others (control, Sh and E-A group, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{***}P < 0.001$ respectively). All results were analyzed by Kruskal-Wallis test followed by Mann-Whitney U test

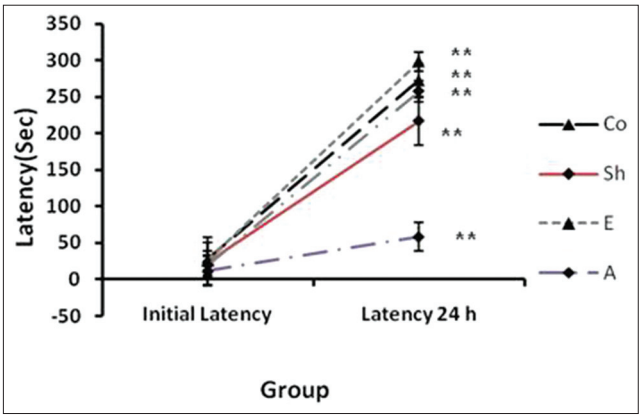

Figure 4: Comparison of latency to enter the dark chamber pre and post foot shock receiving. Each bar represents the mean ± S.E.M. There were significant differences in pre and post foot shock latency in all groups ($^{**}P < 0.01$)

Exercise effects might be the result of structural and biological changes in brain,[29] which enhance neuron numbers.[30] A cell proliferation increase or cell death decrease[31] increases the length and number of dendrites connection between neurons, as well as synaptic plasticity in hippocampus, which is involved in learning and memory.[25,32-34] It has been suggested that mechanisms for some mentioned effects of exercise including gene expression, increment of neurotrophic factors such as brain derived neurotrophic factor and insulin-like growth factor I, which are important for neuronal survival and differentiation, as well as synaptic plasticity.[35-37] Hence, all of the aforesaid factors may have a pivotal role in learning and memory enhancement by exercise.

CONDCLUSION

Our results at the behavioral level (passive avoidance test) emphasize the role of treadmill running in the prevention of learning and memory impairments in NBM-lesion rats. Memory deficit caused by NBM lesion was also reversed by treadmill running, suggesting enhancement of learning and memory functions through physical activity. Identifying such mechanisms in animal models and in human requires conducting further research.

ACKNOWLEDGMENTS

This research was supported by Isfahan University of Medical Sciences, Isfahan, Iran.

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Source of Support: This study was conducted as a thesis funded by Isfahan University of Medical Sciences, **Conflict of Interest:** None declared.