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Aerobic exercise is a feasible intervention for delaying disease progression in Alzheimer's disease

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Abstract

Introduction: Alzheimer's disease (AD) is a chronic neurodegenerative disease that is characterized by a gradual loss of memory and cognitive function. Tau hyperphosphorylation and aggregation is major proximal causes of neuron loss in AD pathogenesis. Physical exercise may be an important adjunct to pharmacological treatment of AD, but the effects of aerobic exercise on tau gene expression are not well known. Thus, the purpose of present study was to determine the effects of aerobic exercise on tau

gene expression in rats with trimethyltin (TMT) model of AD.

Material & Methods: In this experiment, thirty tow mature Sprague-dawley male rats were subjected to Alzheimer's disease through intraperitoneally injection of 8 mg/kg TMT and then were divided into (1) control, (2) Alzheimer-infected control group, (3) endurance training, and (4) sham to study the impact of the disease on the variables. The rats in the endurance training group ran on a rat treadmill with the speed of 15 to 20 meters per minute for 15 to 30 minutes in each session, 3 times a week for 8 weeks. To analyze the results of the tests, one-way ANOVA and Tukey post hoc test were run using SPSS.

Results: The results indicated that TMT injection increases the tau gene expression in the Alzheimer-infected control group. No significant differences were observed between sham and control groups. TMT rats had increased levels of tau gene expression that were significantly ameliorated by exercise ($P<0.05$).

Conclusions: Our results suggest that aerobic exercise is a feasible intervention for delaying disease progression in AD.

Keywords: Aerobic exercise, Alzheimer's disease, Tau, Cognitive function, Hippocampus

1. Introduction

Alzheimer disease (AD), the most common neurodegenerative disorder in which the nervous system progressively and irreversibly deteriorates, affects millions of people worldwide (1). AD clearly is associated with systemic manifestations that extend beyond the central nervous system. In fact, triggered by environmental and endogenous factors, the risk for brain dysfunction and AD is augmented by obesity, diabetes, hypertension, hypercholesterolemia and chronic inflammation (2).

The symptoms of AD appear several years after the disease initiation and are characterized by a progressive cognitive decline, mostly related with memory and thinking language impairment, confusion and disorientation (3). Additionally, AD is related with neurobehavioral disarrangements, including apathy, depression, agitation and anxiety (3). The AD brain is further characterized by decreased neuronal cell proliferation (4), survival (5) and differentiation (6), and a progressive loss of neurons and synapses number in specific brain regions, particularly in the hippocampus, followed by changes in the cortical and subcortical structures and complexity (5). Many factors might cause AD, including genetic defects, appearance of neurofibrillary tangles, altered amyloid precursor processing, mitochondrial defects, deficiency of neurotropic factors, trace element neurotoxicity, energy metabolism deficit, and oxidative stress (7). Researchers have shown that microtubule dysfunction is associated with AD and cognitive dysfunction (8). The hippocampus is a key region in neurological diseases, especially dementia and AD. Studies have shown that damage to the hippocampus region causes to cognitive dysfunction that leads to AD (9).

Central nervous system (CNS) accumulation of hyperphosphorylated tau and amyloid-beta (Aβ) proteins are pathological hallmarks of AD, whereas tau accumulation also occurs in other tauopathies such as frontotemporal dementia, Pick's disease, progressive supranuclear palsy and corticobasal degeneration (10). These diseases are all characterized by the intraneuronal or glial accumulation of neurofibrillary tangles (NFTs), which are comprised of hyperphosphorylated and aggregated tau protein (11). There are no approved treatments for diseases with only tau inclusions (12), whereas the currently approved drugs for AD temporarily relieve symptoms without altering disease progression (13). While much research has been devoted to understanding the mechanisms by which physical activity can reduce or prevent the pathological consequences of toxic Aβ accumulation in AD (14,15), the impact of exercise on the neurodegenerative process in tauopathy is not as well understood. Tau hyperphosphorylation was reported to decrease after exercise in rats and mice (10,16,17); on the other hand, Omidi et al. (2018) reported that they failed to find such an effect in diabetic rats (18). Thus, the purpose of present study was to determine the effects of

aerobic exercise on tau gene expression in rats with trimethyltin (TMT) model of AD.

2. Material & Methods

Animals

The present study was approved by the Ethics Committee on Animal Use of Larestan branch, Islamic Azad University, Larestan, Iran. Adult Sprague-dawley male rats weighing 220 ± 30.6 g (at the beginning of the study) were used in this experiment. The animals were kept in accordance with the Guide to the Care and Use of Experimental Animals (1993).

Rats were submitted to seven days of acclimatization in polypropylene boxes (dimensions 41 cm × 34 cm × 17.5 cm), containing wood shavings (for absorbing urine and water). Five animals were placed in each box. Throughout the experimental period, rats were housed under controlled temperature (20–24°C), humidity (60% ± 5%) and lighting conditions (7:00 a.m. to 19:00 p.m.) with food and water made available *ad libitum*. Animals were randomly divided into four groups (n=8 in each group): (1) control, (2) Alzheimer-infected control group, (3) endurance training, and (4) sham to study the impact of the disease on the variables. The sham surgery group received normal saline using the same route and condition. All efforts were made to minimize animal numbers and suffering.

Induction of Alzheimer disease

At the end of the acclimatization period, all animals were submitted to AD induction protocol as described by Ishikawa et al. (1997) and Malekzadeh et al. (2017) (19,20). To induce AD, a single intraperitioneal injection of Trimethyltin (TMT) (8 mg/kg, body weight; dissolved in 0.9% saline) was given to each animal.

Treadmill exercise protocol

In the first week of the preliminary experiments, the rats were adapted to treadmill (Danesh Salar, Tehran, Iran). The adaptation consisted of 10 min of exercise at a speed of 8 $m.min^{-1}$ on a 0° incline. The rats in the

endurance training group ran on a rat treadmill with the speed of 15 to 20 meters per minute for 15 to 30 minutes in each session, 3 times a week for 8 weeks. Electric shocks were used sparingly to motivate the animals to run. Electrical shocks were applied to the metal grid behind the lane to stimulate rats that failed to run spontaneously. The non-exercise groups remained in their cages.

RNA extraction and real time PCR

The rats were sacrificed by CO_2 asphyxiation and were perfused via a transcardial approach with 15 ml of cold 0.9% saline to rinse away residual blood. The brains were removed and immediately immersed in precooled 4% paraformaldehyde in phosphate buffer and kept at 4°C for further studies. The frontal cortices and hippocampi were dissected from the right hemispheres. The mRNA of the brains was isolated by RNAiso plus (Takara, Japan) as previously described (21). Isolated mRNA was dissolved in DEPC water and 1μg of mRNA was converted to complementary DNA sequence by reversed transcription using cDNA synthesis kit according to manufactures' protocol (Takara, Japan). cDNA expression of tau was assessed by real-time PCR with the following primers respectively: (1) tau, forward:

TGCCCATGCCAGACCTAAAG, reverse:
CCACACTTGGACTGGACGTT; (2) GAPDH, forward:
AGTGCCAGCCTCGTCTCATA, reverse:
GAGAAGGCAGCCCTGGTAAC.

Statistical Analysis

Results were expressed as the mean ± SD and distributions of all variables were assessed for normality. To analyze the results of the tests, one-way ANOVA and Tukey post hoc test were run using SPSS software for windows (version 17, SPSS, Inc., Chicago, IL). The level of significance in all statistical analyses was set at $P \leq 0.05$.

3. Results

Control and sham surgery groups showed no significant difference in the tau gene expression. The results indicated that TMT injection increases the tau gene expression in the Alzheimer-infected control group. TMT

rats had increased levels of tau gene expression that were significantly ameliorated by exercise (P<0.05).

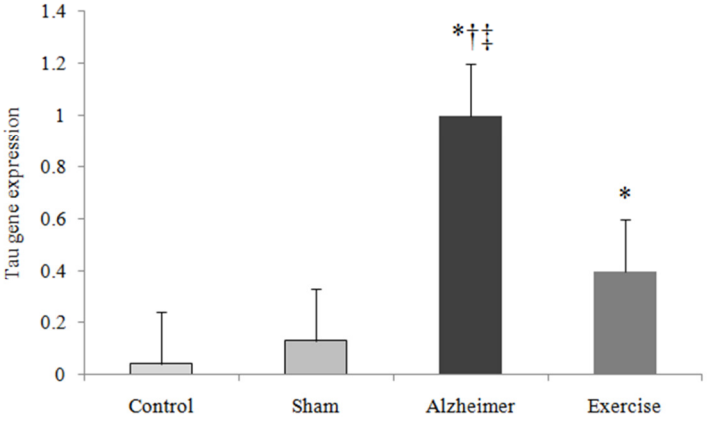

Figure 1. Comparison of the tau gene expression within the groups
* Significant differences with the control group (P <0.05)
† Significant differences with the sham group (P <0.05)
‡ Significant differences with the exercise group (P <0.05)

4. Discussion

Currently, there are no disease-modifying drugs available for AD and available pharmacological and supportive therapy are aimed at slowing disease progression. Clinical studies suggest physical exercise improves cognitive function and reduces the risk of developing AD (22,23). The purpose of present study was to determine the effects of aerobic exercise on tau gene expression in rats with TMT model of AD. Our results revealed that TMT injection increases the tau gene expression in the Alzheimer-infected control group. TMT chloride (C3H9ClSn) has neurotoxicant effects when used in neuronal degeneration research. Studies have shown that TMT treatment caused loss of pyramidal neurons in the hippocampus of rats (24). After TMT drug injection, animals showed some behavioral changes such as seizures, aggressive behavior, self-biting, impairment of working memory, and hyperactivity. Studies showed that TMT intoxication caused cognitive and behavioral dysfunction in experimental animals and humans (25). Studies have shown that TMT is a drug used in research of Alzheimer-like diseases in experimental model (20,26). The first target of TMT is the hippocampus,

where it has toxic effects on pyramidal neurons. Structural damage begins 2–3 days after TMT injection (that appeared within 21 days), and continues during several weeks (27), although the time of onset and prolonged duration is a consequence of the relationship with hemoglobin of rat and TMT. The hemoglobin is associated with slowly and continuously releasing TMT into the plasma, and subsequently into the brain (28). The TMT caused a cytotoxic effect on glial cells; studies have shown that following TMT administration, increased glial fibrillary acidic protein levels (29) and Na^+ – K^+ – ATPase activity changes due to swelling of primary cultures of astrocytes (30).

The results indicated that TMT rats had increased levels of tau gene expression that were significantly ameliorated by exercise. Along with Aβ, tau hyperphosphorylation and aggregation are major proximal causes of neuron loss in AD pathogenesis (31). Tau is an important microtubule-associated protein abundant in the central nervous system (32). Under physiological conditions, tau is a soluble protein that promotes microtubule assembly and stabilization, and affects the dynamics of microtubules in neurons (33). The phosphorylation of tau regulates microtubule binding and assembly (34); however, under pathologic conditions, tau overexpression and hyperphosphorylation at certain residues appears to impair axonal transport of organelles through microtubules, including mitochondria, causing synapse "starvation" and depletion of ATP (35). Under this pathological condition, tau undergoes a series of posttranslational changes including abnormal phosphorylation, glycosylation, glycation and truncation, which may render tau more prone to form NFT, a major hallmark of AD (23,36). Following aggregation, microtubules disintegrate, impairing the neuronal transport system and eventually leading to cell death. Hyperphosphorylation is believed to be an early event in the pathway that leads from soluble to insoluble and filamentous tau protein (37), resulting in the formation of the potently cytotoxic filamentous structures (38). Factors affecting tau hyperphosphorylation are not fully understood and in fact, it has been suggested that tau pathology can be triggered by different mechanisms, dependent and/or independent of Aβ (23). In line with the present study, previous studies had been reported that exercise training decreases tau hyperphosphorylation in samples with AD (10,16,17,39).

Stranahan et al. (2012) were studied the effects of physical activity on tau protein in humans and mouse models of Alzheimer's disease. They reported that treadmill exercise has been shown to ameliorate the accumulation of phosphorylated tau, an essential component of neurofibrillary tangles in Alzheimer's models (40). Tg-NSE/htau23, express the human tau23 isoform protein under the control of the neuron-specific enolase (NSE) promoter in the genetic background of C57BL/6 mice (41). These mice express excessive tau hyperphosphorylation in a manner characteristic of the mature human AD condition. Previous studies indicated that involuntary treadmill training attenuated this tau pathology in male and female TgNSE-htau23 mice (40,42). Stranahan et al. (2012) indicated that treadmill training restored normal levels of copper-zinc superoxide dismutase, catalase, and manganese superoxide dismutase in both male and female TgNSE-htau23 mice (40). They also found that running restored normal catalase and superoxide dismutase activity. Running increased the phospho-PKCalpha/PKCalpha ratio, reduced the phospho-PKA/PKA ratio, and reversed the increased ratio of active phospho-p38 mitogen-activated protein kinase (MAPK) to total p38-MAPK. Running also reduced the active phospho-extracellular signal-regulated (ERK)/ERK ratio, and attenuated the increase in the active phospho-c Jun N-terminal kinsae (JNK) to JNK ratio. In contrast, running increased the active phospho-Akt to total Akt ratio, which was reduced in both male and female TgNSE-htau23 mice (40). Overall, Stranahan et al. (2012) indicated that running influences the activity status of serine-threonine kinases that may regulate the degree of tau phosphorylation as running effectively reduced tau phosphorylation at multiple sites (40).

5. Conclusion

In conclusion, the present study suggests that aerobic exercise is a feasible intervention for delaying disease progression in AD by ameliorating tau gene expression. Thus not only healthy active lifestyle is a preventative strategy to reduce the likelihood of developing neurodegenerative disease, exercise has also been shown to be effective as an intervention in aged populations at risk for or suffering from AD.

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Conflict of interests: The authors have no conflicts of interest to declare.

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