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Research paper

Short-term treadmill exercise increased tau insolubility and neuroinflammation in tauopathy model mice

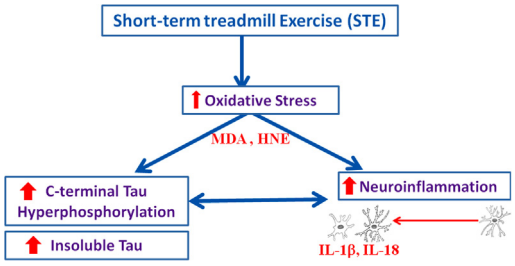
Montasir Elahi[a,b], Yumiko Motoi[a,b,c,*], Shin-Ei Matsumoto[b,c], Zafrul Hasan[a], Koichi Ishiguro[c], Nobutaka Hattori[b,c]

[a] Sportology Center, Juntendo University Graduate of Medicine, Japan
[b] Department of Diagnosis, Prevention and Treatment of Dementia, Juntendo University Graduate School of Medicine, Japan
[c] Department of Neurology, Juntendo University Graduate School of Medicine, Japan

HIGHLIGHTS

- 3 weeks of treadmill exercise markedly increased insoluble fraction of tau in tauopathy model mice.
- Increase in tau insolubility was related to C-terminal tau phosphorylation.
- Short-term treadmill exercise (STE) increases the Iba-1-positive microglia in the hippocampus.
- STE increases the release of proinflammatory cytokines and oxidative stress.
- STE would not be beneficial to the Alzheimer's disease pathology.

GRAPHICAL ABSTRACT



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ABSTRACT

Physical exercise has been identified as a preventive measure for Alzheimer's disease (AD), one of the neuropathological hallmarks of which, neurofibrillary tangles, consist of hyperphosphorylated insoluble tau. Previous studies demonstrated that long-term treadmill exercise reduced tau hyperphosphorylation and insolubility; however, whether short-term treadmill exercise (STE) alters tau modifications currently remains unknown. In the present study, we attempted to characterize the effects of STE on tau solubility and determine its relationship with neuroinflammation using tauopathy model mice (Tg601), which express wild-type human tau. The results obtained showed that 3 weeks of non-shock treadmill exercise in Tg601 and non-transgenic female mice markedly increased insoluble tau. An analysis of phosphorylation patterns indicated that changes in tau solubility were related to an increase in phosphorylation at the tau C-terminal end. The results of immunohistochemical analyses revealed that STE increased the number

Abbreviations: AD, alzheimer's disease; BDNF, brain-derived neurotropic factor; CXCL, chemokine (C-X-C motif) ligand; EXE, exercised; IL, interleukin; HNE, 4-hydroxy-*trans*-2-noneal; MDA, malondialdehyde; NFT, neurofibrillary tangles; NTg, non-transgenic; SED, sedentary; STE, short-term treadmill exercise; TNF-α, tumor necrosis factor alpha; Tg, transgenic.

* Corresponding author at: Department of Prevention, Diagnosis and Treatment of Dementia, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan.
E-mail address: motoi@juntendo.ac.jp (Y. Motoi).

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of Iba-1-positive microglial cells in the hippocampus. Elevations in the levels of the lipid peroxidation markers, 4-hydroxy-*trans*-2-noneal and malondialdehyde, indicated the presence of oxidative stress. Moreover, higher levels of cytokines, IL-1β and IL-18, and chemokines, CXCL-1 and CXCL-12, supported neuroinflammation.

1. Introduction

Alzheimer's disease (AD) is a leading cause of dementia among elderly adults, and affects approximately 36 million people worldwide [1]. Two neuropathological hallmarks of AD are senile plaques and neurofibrillary tangles (NFTs) [2]. Senile plaques are formed by amyloid-β (Aβ), whereas the major constituent of NFTs is the abnormally hyperphosphorylated microtubule-associated tau protein [3]. Previous studies demonstrated that N-terminal hyperphosphorylation was related to the inhibition of microtubule assembly, whereas C-terminal phosphorylation was mostly involved in tau self-aggregation [4,5]. Abnormally hyperphosphorylated tau and Aβ deposits have been shown to stimulate a local immune response by activating microglial cells, and the products of oxidative stress have been associated with NFTs [6,7].

The curation process from AD remains elusive due to the lack of specific drugs or medication. Lifestyle treatments such as physiological exercise have been examined as preventive measures for AD in human and animal models [8,9]. In animal studies, exercise was found to be beneficial because it improved cognition and memory functions [10]. In young transgenic mice (3 months old) expressing the amyloid precursor protein and Presenilin1 (APP/PS1), long-term treadmill exercise inhibited the progression of AD pathology by decreasing the load of Aβ plaques [11]. Most studies on treadmill running have employed Aβ model mice, while a few have focused on tauopathy model mice subjected to long-term treadmill treatments [12]. Long-term treadmill exercise (≥12 weeks) was previously shown to decrease total and hyperphosphorylated tau in the brain and spinal cord of AD model mice [11,13–15]. In P301S tau transgenic mice expressing 1N4R human tau containing the Pro301Ser mutation, a long-term treadmill exercise protocol (12 weeks) reduced N-terminal phosphorylation (Ser202 and Thr205) and insoluble tau in the cortex [15]. However, the effects of STE on tau modifications in tau transgenic mice have not yet been examined.

Therefore, the aim of the present study was to determine the effects of non-shock STE on tau modifications and neuroinflammation in tauopathy model mice. We used Tg601 mice, which overexpress the wild-type human tau sequence (2N4R) [16]. We examined tau solubility and phosphorylation patterns using various phosphorylation-dependent tau antibodies, and then assessed brain inflammatory cytokines, oxidative stress, and microglial proliferation in Tg601 mice and their non-transgenic (NTg) littermates in order to identify the effects of STE on tauopathy.

2. Materials and methods

2.1. Animals

Tau transgenic (Tg601) mice overexpressing wild-type human tau (2N4R) under the control of the calcium/calmodulin-dependent protein kinase IIα (CAMKIIα) promoter, which was developed in our laboratory, were employed in the present study [16]. Tg601 and their non-transgenic littermates (NTg/WT) had the same genetic background. All experiments were performed according to the animal experiment guidelines of Juntendo University, which were prepared by following the international guidelines of "Principles of Laboratory Animal Care" (NIH publication no. 85-23, 1985). The protocol used was approved by the Committee on the Ethics of Animal Experiments of Juntendo University (Permit Number: 260199). All efforts were made to minimize suffering. All mice were confirmed to be healthy before starting the experiment.

2.2. Experiment design, exercise paradigm, and sample collection

Nineteen-month-old Tg601 and non-transgenic (NTg) female mice were separated into sedentary and exercised groups ($N = 6$). In the exercised group, continuous non-shock exercise with gentle tail touching was performed using a motor-driven mouse treadmill (Moromachi Kikai, Japan). Pre-exercise (5 m/min, 10 min/day) was performed for 5 days for acclimatization. The actual treadmill training protocol was conducted at 10 m/min, 30 min/day, 5 days/week on a 0% gradient for 3 weeks [17]. During this period, sedentary mice were kept in their home cage.

Mice were sacrificed by cervical dislocation after 3 weeks of exercise training and the brains were removed. The left side of the brain was maintained in 4% paraformaldehyde at 4 °C for 24 h, and transferred to 20% sucrose solution until further use, while the right side was stored at −80 °C as a frozen sample.

2.3. Preparation of soluble and insoluble tau fractions

Frozen brain tissues, excluding the olfactory bulbs and cerebellum (~0.2 g), were homogenized (polytron PT 3100) in 10X volume of homogenization buffer (HB) containing 10 mM Tris–HCl, pH7.5, 0.8 M NaCl, 1 mM ethylene glycol tetraacetic acid (EGTA), 1 mM dithiothreitol, and 0.1 μM phenylmethanesulfonylfluoride (PMSF). After sonication, homogenized samples were centrifuged at 100,000 × *g* at 4 °C for 20 min. The supernatants (TS: Tris soluble) were collected while the pellets were resuspended in 2 ml of HB containing 1% (V/V) Triton X-100 by sonication and incubated at 37 °C for 30 min. After centrifugation at 100,000 × *g* at 4 °C for 20 min, the Triton X-soluble fractions (TX) were harvested and the pellets were further suspended in 1 ml of HB containing 1% Sarkosyl. Samples were centrifuged and the supernatants were collected as Sarkosyl-soluble fractions (SS). The resulting pellets were used as the Sarkosyl-insoluble fraction (Ppt), which was suspended in 0.1 ml of sodium dodecyl sulfate (SDS) loading buffer (0.25% bromophenol blue, 0.5 M dithiothreitol, 50% glycerol, and 10% SDS) by sonication for western blotting.

2.4. Western blotting

TS, TX, SS, and Ppt samples were separated by SDS-PAGE SuperSep 5–20% (Wako, Japan), and were blotted onto polyvinylidene membranes (Immobilon-P, Millipore, USA). The blots were incubated at room temperature overnight with a primary antibody, followed by interactions with an appropriate biotinylated secondary antibody. The following primary antibodies were used for analysis: TauC (1:12000, Rabbit polyclonal [18]), mAb AT100 (1:200,Thermo), (mAb AT270 1:1000,Thermo), PS396 (1:3000, Rabbit, Invitrogen), and PTau400 (1:1500, Rabbit, Cell Signaling). The VECTASTAIN ABC Kit (VECTOR Laborato-