Voluntary Physical, Not Forced Exercise, Ameliorates Tau Pathology and Cognition in Tauopathy Model Mice

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Background: Physical exercise has been considered as an effective non-pharmaceutical measure for the treatment of Alzheimer's disease (AD). In this report, we described the effect of long-term forced and voluntary exercise on tau protein modification using tauopathy model mice, Tg601.

Methods: 12-month-old non-transgenic and Tg601 mice were subjected to forced and voluntary exercise by means of treadmill (TD) and wheel running (WH), respectively. After 5 months, behavioral analysis was performed by Morris water Maze (MWM) and Y-Maze (YM) test. Tau modification was analyzed by using detergent soluble and insoluble tau fractions from brain homogenate. Neuroinflammation was analyzed by immunohistochemistry (IHC) and the cytokine profile by total cytokine microarray.

Results: The MWM and YM results indicated improvement of cognition only in WH. However, TD-treated mice were unaffected. A marked decrease in sarkosyl insoluble tau, and hyper phosphorylated tau was observed in the WH group. IHC data indicated Iba-1 positive microglia were reduced in WH mice. Cytokine microarray illustrated that IL-1 α , IL-1 r , IL-9, IL-15 r , IFN γ , CRP, ICAM-1, CXCL-12, and TNF α were elevated in TD group, however WH was not altered.

Conclusion: Our findings indicated that voluntary exercise would be beneficial for AD in mice.

Key words: physical exercise, dementia, tau protein

Introduction

Alzheimer's disease (AD) is a leading cause of dementia, affected approximately 46.8 million people worldwide¹⁾. The most remarkable neuropathological hallmarks of AD, is neurofibrillary tangles (NFTs) are composed of abnormally hyperphosphorylated microtubule-associated protein tau²⁾. Due to the lack of effective drugs or medication, lifestyle treatments such as physiological exercise have been examined as one of the effective preventive measures for AD both in human and animal models^{3) 4)}.

Epidemiological findings have suggested that a

sedentary to active transition in lifestyle delayed the onset of dementia and improved cognitive function in older adults³⁾. However, some epidemiological studies on physical exercise in patients with AD was shown as inconsistent. An inverse association or no beneficial effect between physical activity and cognitive decline were also reported⁵⁾⁻⁷⁾. Therefore, the method in which exercise employed may determine its effects in the brain. In animal model of AD, exercise has been practiced by means of voluntary wheel running and continuous treadmill running. Most of the report described the beneficial effect of both phenomena for model mice but some studies describe the adverse effect of only

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treadmill running. Till to date, we observe only a very few reports described a comparative view on exercise methodology.

The aim of the present study was to differentiate between the effects of voluntary and forced exercise on immune profile and on tau modifications in tauopathy model mice. We used Tg601 mice, which over-express the wild-type human tau sequence (2N4R)⁸⁾. We assessed brain cytokines profile, and microglial proliferation and examined tau solubility and phosphorylation patterns in Tg601 mice and their non-transgenic (NTg) littermates to give a comprehensive view on the effects of exercise on tauopathy.

Materials and Methods

1. Animals and exercise training

Tau transgenic model (Tg601) mice overexpressing wild-type human tau (2N4R) under the control of the calcium/calmodulin-dependent protein kinase II α (CAMKII α) promoter was developed in our laboratory⁸⁾. Twelve-month-old female mice were separated into treadmill runner, wheel runner and sedentary (N = 8) groups. Treadmill group were subjected to continuous non-shock exercise with gentle tail touching using a motor-driven mouse treadmill (Muromachi Kikai, Japan). After pre-exercise the actual treadmill training protocol was conducted at 10 m/min, 60 min/day, 5 days/week on a 0% gradient for one hour⁹⁾. Mouse of the wheel running group were kept to a cage (equipped with a running wheel and a counter) for 60 min/day, 5 days/week (Tokiwa, Japan). Single mouse was allowed in a cage for this period. During this time, sedentary mice were kept in their home cage.

2. Behavioral analysis

1) Y-maze

As a measure of general activity and short term working memory function, mice were tested in a Y-maze with three arms (Identified as A, B, and C). Each mouse was placed into one of the three arms facing the middle area, and allowed to explore the maze for 8 min. The total number of arm entries and sequence of arm choices were observed and recorded. Alternation was defined as the ratio of

arm choices differing from the previous two choices divided by the total number of entries. The score of alternation was expressed as percentage.

2) Morris water maze

To measure reference learning (acquisition) and memory (retention), mice were placed into a 122-cm water-filled circular pool. The pool was divided into four equal-sized quadrants and was surrounded by various visual cues, including beach ball, colored wall poster with specific sign, which helped the mice to orient their location in the pool. A clear, 9-cm platform was placed in the middle of quadrant II. The surface of the platform was exactly 1.0 cm below the water surface and visually indiscernible to the mice. Reference learning (acquisition) was evaluated over a 5-day period, with three successive sessions per day. The swimming path, swimming time, latency to find the platform (up to 60 s) for each trial was recorded by using an automated video tracking system (Muromachi Kikai, Japan).

3. Immunohistochemistry (IHC)

For floating section IHC thirty-micrometer-thick sections was performed as previously described¹⁰⁾. The number of Iba-1-positive cells was counted for the whole hippocampus including the CA1, CA3, CA4, and dentate gyrus regions in three sections/slices from each group of mice (N = 3) using AxioVision Rel. 4.8.2 (Carl Zeiss AB, Sweden) software. Data were represented as the number of Iba-1-positive cells per square millimeter.

4. Cytokine micro array

Cytokine micro array was performed using Raybio Label-Based (L-Series) mouse antibody array 308 to analyze the total cytokine profile in the mouse brain (Ray biotech Inc., USA). Frozen half right of the brain were homogenized in buffer provided by the kit. Micro array slides were prepared following the protocol provided by the kit and scanning of the slides were performed using GenePix 4400 microarray laser scanner (Molecular device). Data acquisition and intensity scaling were performed by GenePix Pro7.2 software. Data normalization and analysis were performed using Ray bio analysis tool.

5. Preparation of soluble and insoluble tau fractions and western blotting

Detergent soluble and insoluble Tau fractions were prepared from frozen half right of the brain following the protocol as previously described¹⁰. TS, TX, SS, and Ppt samples were separated by SDS-PAGE SuperSep 5–20% (Wako, Japan), and were blotted onto polyvinylidene membranes (Immobilon-P). 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 poly¹¹), mAb AT270 (1:1500, Thermo), PS202, 1:2000, Rabbit poly), PS396 (1:3000, Rabbit, Invitrogen), PHF1 (1:500, mouse). The VECTASTAIN ABC Kit (VECTOR Laboratories, USA) and 3,3'-diaminobenzidine (DAB)

containing Nickel (II) chloride (NiCl₂) substrate were used to visualize bands. Band intensities were analyzed using Image J software (National Institutes of Health, USA).

Results and Discussion

1. Exercise alter neuroinflammation and cytokines profile

Microglial cells play a major role in inflammatory responses in the brain by secreting proinflammatory (e.g., IL-1 β , IL-6, and TNF- α) and anti-inflammatory cytokines and neuroprotective factors in neurodegenerative diseases^{12) 13)}. To understand the exercise induced neuroinflammation or microglial change in the brain, we performed immunohistochemical analysis using an Iba-1 antibody. A one-way ANOVA with posthoc Tukey's HSD for

Figure-1 Iba-1 IHC showed Microglial activation after treadmill exercise

Figure-2 Change in brain cytokine profile after exercise

Iba-1-positive microglia revealed the effect of exercise in both Tg601 and NTg mice. We observed that only treadmill running mice showed elevated number of microglia and activated form of microglia in their brain, however, wheel running was unaffected (Figure-1).

The total cytokine profile in the brain were determined by protein microarray, which allowed us to measure the level of cytokine semi-quantitatively. Statistical analysis with one-way ANOVA followed by posthoc Tukey's HSD of the normalized intensity data revealed that due to treadmill exercise altered IL-1 α , IL-1 r , IL-9, IL-15 r , IFN γ , CRP, ICAM-1, IFN γ R1, CXCL-12 and TNF α were elevated in TD group (Figure-2).

2. Exercise modified Tau solubility

Sarkosyl insoluble, aggregated Tau formed by dephosphorylated and abnormally phosphorylated tau species has considered as the toxic form of tau in AD¹⁴⁾. In order to determine the effects of exercise, on tau solubility, we pre-pared different detergent soluble and insoluble fractions from brain homogenates and then performed a western blot analysis using site-specific anti-tau anti-body TauC. The TauC antibody, which recognizes total

mouse and human tau, revealed a sharp decrease in transgenic human Tau (~ 75 KD) in the sarkosyl-insoluble fractions (Ppt) of wheel running Tg601 mice ($p < 0.005$) (Figure-3). A one-way factorial ANOVA followed by Tukey's HSD analysis indicated that wheel running also decreased the amount of endogenous mouse tau (~ 50 KD) in the sarkosyl-insoluble fraction of Tg601 mice. A similar phenomenon (decrease in hTau and endogenous mTau) in the Tris soluble Tau fractions (TS) ($p < 0.05$) but in TX and SS we did not observed any significant change between group.

3. Exercise modified tau phosphorylation

Phosphorylated Tau has been reported as the primary toxic Tau as these tau moieties ultimately form the neurofibrillary tangles (NFTs). The microtubule-associated protein tau consists of more than 30 serine/threonine residues that become hyperphosphorylated under pathological conditions¹⁵⁾. Antibodies recognize N-terminal proline rich domain (PRD) phosphorylation AT 270 (pThr181) PS202 (pSer202) and showed a decreased amount of phosphorylation in the brain homogenate of the WH group than in that of the SD and TD group (Figure-4). The effect was higher in

Figure-3 WH decreased insoluble Tau fractions activation after treadmill exercise

Figure-4 Wheel running exercise decrease tau phosphorylation

Figure-5 Wheel running increased memory function in tauopathy model mice

C-terminal phosphorylation (PS 396, PHF1). The result of western blotting was perfectly evaluated in AT8 immunofluorescence.

4. Exercise changed the cognition

Tau pathology like AD impair the cognition and exercise has shown to restore the tau pathology and memory function in animal model¹⁶. After five months of exercise, the general activity and short term working memory function of mice were tested in a Y-maze test. In Y-maze the alteration score is a measure of memory function. For running wheel, treadmill and sedentary groups, the scores were 59.98, 39.82 and 43.58, respectively. Statistical analysis revealed that WH mice showed signifi-

cantly higher alteration score than TD and SD mice ($p < 0.0001$). However, TD mice did not show any change compare to SD mice. Spatial learning and memory was evaluated using the Morris water maze (MWM). During the 5-day training, mice were given three 60-sec session training per day to find out the hidden platform in a pool. Compared to the adult NTg mice, the Tg601 mice showed much longer escape latencies on training day 3 ($p < 0.0001$), day 4 ($p < 0.001$, and day 5 ($p < 0.0001$) (data not shown). In addition, WH exercise significantly reduced the escape latencies in transgenic mice compared to SD and TD mice on day 2 ($p < 0.005$, $p < 0.05$), day 3 ($p < 0.005$, $p < 0.05$), day 4 ($p < 0.005$, $p < 0.05$) and day 5 ($p < 0.005$, $p < 0.05$).

0.05) (Figure-5). However, we did not observe any difference between TD and SD group. Therefore, these results suggest that the acquisition or retention of memory and cognition was enhanced in WH mice by a running wheel exercise regime and treadmill exercise has no effect of memory and cognition.

Conclusion

Previously we reported that, short term treadmill exercise (STE) increased tau pathology by increasing tau phosphorylation, tau aggregation, and neuroinflammation¹⁰⁾. Current study showed that Neuroinflammation persist in tauopathy model mice after long term treadmill exercise (TD). On the other hand, voluntary wheel running exercise (WH) decreased tau phosphorylation, tau aggregation, and has no effect on neuroinflammation. In addition, WH improved the cognition function in model mice. Therefore, the neuroinflammation might hinder the beneficial effect of treadmill exercise in Tauopathy model mice. Further analysis might require identifying the specified molecule behind the beneficial effect for voluntary exercise.

References

- 1) Prince M, Comas-Herrera A, Knapp M, Guerchet M, Karagiannidou M: World Alzheimer Report 2016, Improving healthcare for people living with dementia. (<https://www.alz.co.uk/research/WorldAlzheimerReport2016.pdf>)
- 2) Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI: Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci U S A*, 1986; 83: 4913-4917.
- 3) Larson EB, Wang L, Bowen JD, *et al*: Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. *Ann Intern Med*, 2006; 144: 73-81.
- 4) Pasinetti GM, Eberstein JA: Metabolic syndrome and the role of dietary lifestyles in Alzheimer's disease. *J Neurochem*, 2008; 106: 1503-1514.
- 5) Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K: Physical activity and risk of cognitive impairment and dementia in elderly persons. *Arch Neurol*, 2001; 58: 498-504.
- 6) Rovio S, Kareholt I, Helkala EL, *et al*: Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. *Lancet Neurol*, 2005; 4: 705-711.
- 7) Verghese J, Lipton RB, Katz MJ, *et al*: Leisure activities and the risk of dementia in the elderly. *N Engl J Med*, 2003; 348: 2508-2516.
- 8) Kambe T, Motoi Y, Inoue R, *et al*: Differential regional distribution of phosphorylated tau and synapse loss in the nucleus accumbens in tauopathy model mice. *Neurobiol Dis*, 2011; 42: 404-414.
- 9) Ke HC, Huang HJ, Liang KC, Hsieh-Li HM: Selective improvement of cognitive function in adult and aged APP/PS1 transgenic mice by continuous non-shock treadmill exercise. *Brain Res*, 2011; 1403: 1-11.
- 10) Elahi M, Motoi Y, Matsumoto SE, Hasan Z, Ishiguro K, Hattori N: Short-term treadmill exercise increased tau insolubility and neuroinflammation in tauopathy model mice. *Neurosci Lett*, 2010; 478: 207-212.
- 11) Ishiguro K, Sato K, Takamatsu M, Park J, Uchida T, Imahori K: Analysis of phosphorylation of tau with antibodies specific for phosphorylation sites. *Neurosci Lett*, 1995; 202: 81-84.
- 12) Akiyama H, Barger S, Barnum S, *et al*: Inflammation and Alzheimer's disease. *Neurobiol Aging*, 2000; 21: 383-421.
- 13) Saijo K, Winner B, Carson CT, *et al*: A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. *Cell*, 2009; 137: 47-59.
- 14) Hanger DP, Gibb GM, de Silva R, *et al*: The complex relationship between soluble and insoluble tau in tauopathies revealed by efficient dephosphorylation and specific antibodies. *FEBS Lett*, 2002; 531: 538-542.
- 15) Hanger DP, Anderton BH, Noble W: Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. *Trends Mol Med*, 2009; 15: 112-119.
- 16) Belarbi K, Burnouf S, Fernandez-Gomez FJ, *et al*: Beneficial effects of exercise in a transgenic mouse model of Alzheimer's disease-like Tau pathology. *Neurobiol Dis*, 2011; 43: 486-494.