Neurochemistry International xxx (2017) 1–9

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Effects of task-specific rehabilitation training on tau modification in rat with photothrombotic cortical ischemic damage

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ARTICLE INFO

Article history:
Received 3 February 2017
Received in revised form
2 May 2017
Accepted 8 May 2017
Available online xxx

Keywords:
Stroke
Tau
Rehabilitation
Phosphorylated tau
Dementia
Cerebral cortex

ABSTRACT

Although stroke elicits progressive cognitive decline and is a leading cause of dementia, molecular interplay between stroke and Alzheimer's disease (AD) pathology has not been fully elucidated. Furthermore, studies on the effects of post-stroke rehabilitation on AD pathology are limited. We evaluated the acute effect of stroke on tau modification, and the molecular effects of task-specific training (TST) on tau modification using a model of photochemically-induced thrombosis (PIT)-induced cortical infarction. Following PIT in the dominant side of sensorimotor cortex, the rehabilitation group received 4-weeks of TST rehabilitation once daily by single pellet reaching training, whereas the sedentary control group did not received any type of training. Cortical expression levels of proteins related to tau modification were evaluated on post-stroke day 1 (PSD1) and 28; functional tests were also evaluated performed every week. The expression levels of acetyl-tau, phosphorylated-tau (p-tau), cyclooxygenase-2 and Akt-mTORC1-p70S6K pathway in infarcted cortices on PSD1 were significantly greater, whereas the expression levels of *p*-AMPK were significantly lower than in the paired contralateral sides. TST rehabilitation for 4 weeks greatly improved functional motor performance but not memory, which concurred with the down-regulations of ipsilateral *p*-AMPK, cyclooxygenase-2, Akt-mTORC1-p70S6K pathway, and p-tau in rehabilitation group. PIT-induced cortical infarction was found to induce cortical tau modification through the Akt-mTORC1-p70S6K activation, and to suppress the expression of AMPK-related proteins. TST rehabilitation greatly improved motor function, but not memory, and suppressed p-tau expression and neuroinflammation. Nevertheless, the role of TST-mediated regulation of tau hyperphosphorylation required further clarification.

1. Introduction

Stroke is a leading cause of severe physical disability and elicits progressive cognitive decline in adults. The prevalence of dementia in those that have experienced stroke is 3.5–5.8 times that of normal elderly people (Prencipe et al., 1997). Furthermore, Alzheimer's disease (AD) pathology is frequently observed in the brains of patients with post-stroke dementia, and has a prevalence of 19–41% in new-onset post-stroke dementia (Leys et al., 2005). In addition, those that develop dementia after stroke have a 2- to 6-

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http://dx.doi.org/10.1016/j.neuint.2017.05.004
0197-0186/

fold higher long-term mortality rate than stroke patients that do not develop dementia (Baldereschi et al., 1999; Desmond et al., 2002; Barba et al., 2002). Therefore, the role of AD pathology in post-stroke dementia development is critical, and in particular, the molecular mechanisms underlying AD-related post-stroke dementia should be elucidated.

Tau is a microtubule-associated protein that contributes to microtubule stability, and tau hyperphosphorylation has been reported in the cortices of animal after stroke (Han et al., 2008; Wen et al., 2004). Furthermore, this hyperphosphorylation causes tau detachment from microtubules and subsequent microtubule instability, self-aggregation, and tangle formation. Many tau kinases and phosphatases regulate the phosphorylation status of tau in phosphorylation site-dependent manners (Hanger et al., 2009). In addition, acetylation of tau (Ac-tau) promotes tau aggregation, which suggests Ac-tau play a role in the pathologic transformation of tau (Min et al., 2010). Previous studies on the molecular link between tauopathy and stroke have focused the triggering of tau hyperphosphorylation by ischemia-reperfusion (Wen et al., 2004; Gordon-Krajcer et al., 2007; Dong et al., 2014), and as a result, the detailed mechanisms responsible for the progression of tau modification have not been elucidated. In particular, acute stroke causes cellular hypoxia and energy depletion, which induce energy restoring mechanisms, such as, 5′-AMP-activated protein kinase (AMPK) activation. In addition to the known involvements of glycogen synthase kinase 3β (GSK3β) and cyclin-dependent kinase 5 (cdk5)-mediated pathways, we previously provided evidence that AMPK signaling pathways were associated with tau phosphorylation in an animal model of AD (Kim et al., 2015). However, whether tau phosphorylation in acute stroke is regulated by an AMPK-related pathway and whether rehabilitation is affected tau modification remain to be determined.

Physical activity programs involving moderate to maximal aerobic exercise with resistance exercise components have been suggested to slow down progressive cognitive decline effectively in the elderly (Zheng et al., 2016; Ikudome et al., 2016; Etgen et al., 2010). Previous studies tended the focus on the efficacy of physical activity programs in elderly without stroke and/or motor disability (Zheng et al., 2016; Ikudome et al., 2016; Etgen et al., 2010), but motor disabilities caused by stroke usually restrict physical exercise ability, and hence, post-stroke rehabilitation programs based on task-specific training (TST) are used in practice. Several *in vivo* animal studies suggest aerobic exercise inhibits amyloid beta accumulation and tau hyperphosphorylation in AD (Ohia-Nwoko et al., 2014; Cho et al., 2015; Walker et al., 2015). However, little is known of the effects of TST after stroke on tau modification and related mechanisms. Accordingly, the present study was undertaken to characterize tau modifications after acute ischemic damage, and to evaluate the effect of rehabilitative TST on tau and on the mechanisms regulating tau modifications in a photochemically-induced thrombosis (PIT) model of cortical infarction.

2. Materials and methods

2.1. Experimental animals

Male Sprague–Dawley rats (8 weeks old; Orient Bio, Seongnam, Korea) weighing 250–300 g were used in the study. Animals were housed in a temperature- (22 ± 2 °C) and humidity-controlled (45–55%) room under a 12 h light/dark cycle (07:00–19:00) with free access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee of Inha University. All rats underwent photothrombotic ischemic surgery, and were sacrificed one day after surgery (PSD1, n = 12) for molecular works, or randomly allocated to a rehabilitation group (RG, n = 23) or a sedentary control group (SC, n = 23) for evaluating the effects of rehabilitation on behavioral performance for 4 weeks. 28 days (PSD28) after PIT-induced stroke, rats (n = 16) of RG and SC were sacrificed after behavioral test for immunoblot, immunohistochemistry, and Nissl staining. We evaluated the tau modification and related molecular pathways in ischemic cortices at PSD1, and effects of 4-weeks of rehabilitation on these molecular changes at PSD28.

2.2. Induction of photothrombotic infarction

Focal, permanent cortical infarcts were produced by PIT in the motor and sensorimotor cortex by focusing light on targeted regions in intraperitoneally Rose Bengal-treated rats (Watson et al., 1985). In brief, 5 min after Rose Bengal dye (10 mg/kg) injection followed by anesthesia (tiletamine/rompun mixture, 30/10 mg/kg by intramuscular injection), rats were placed on a stereotaxic apparatus (David Kobf, Tujunga, CA). The skull opposite the dominant side of forelimb was exposed and the fiber optic bundle of a KL1500 LCD cold light source (Carl Zeiss, Jena, Germany) with an 8-mm in diameter (6 mm aperture) was positioned as close as possible on the skull at bregma and 4.0 mm lateral to midline over the sensorimotor and motor cortex. Focal ischemic lesions were produced by irradiating the exposed skull for 20 min with the fiber optic bundle. The scalp was sutured and rats were allowed to awake. At the time of sacrifice, rats were anesthesized with intramuscular injection of tiletamine/rompun (30/10 mg/kg) and decapitated. Brain was quickly removed and sectioned coronally into 2-mm slices using a brain marix. Slices were immediately stained with 2% (w/v) 2,3,5-triphenyltetrazolium chloride (TTC) in pre-warmed phosphate-buffered saline and stained for 20 min. As expected, the infarction areas were 6 mm in diameter and 3 mm in depth by TTC staining, as shown in Supplementary Figure S1.

2.3. Immunohistochemistry

Anesthetized rats were perfused transcardially with 0.9% saline at 4 °C, followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH7.4) for 10 min. Brains were isolated, and post fixed in the same fixative for 4 h at 4 °C. Coronal sections (15 μm thick) at the level of the frontoparietal level (infarct area) were cut on a cryostat (CM1950, Leica, Heidelberger, Germany). Nissl staining was performed with 0.1% cresyl violet (Sigma, StLouis, MO) to evaluate neuronal damage. For immunohistochemical analysis, 3 μm sections of paraffin-embedded blocks were deparaffinized, hydrated, treated with 3% hydrogen peroxide in 0.05 M Tris-HCl (0.25% Triton X-100) for 15 min at room temperature (RT), and then washed three times in PBS. After blocking with a 5% diluent solution (Invitrogen, Carlsbad, CA) in 0.05 M Tris-HCl containing 1 mg/mL bovine serum album, 1 mM NaF and 0.05% Triton X-100 for 1 h at RT, sections were incubated with primary antibodies overnight at 4 °C, and then rinsed three times in PBS. The signal was detected with EnVision system-HRP labelled Polymer anti-rabbit IgG (Dako, K4002) for 30 min at RT and 3,3'-diaminobenzidine peroxidase substrate. The intensity of *p*-AMPK immunoreactivity was quantified using Image J by multiplying the mean intensity of 5 randomly selected regions in each animal (n = 5). The intensity of *p*-AMPK in selected region was normalized by intensity of background counterstaining.

2.4. Western blot

Isolated cerebral cortices were homogenized on ice in the radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 7.5, 1% Triton X-100, 150 mM NaCl, 1% sodium deoxycholate, 2 mM EDTA

Please cite this article in press as: Joa, K.-L., et al., Effects of task-specific rehabilitation training on tau modification in rat with photothrombotic cortical ischemic damage, Neurochemistry International (2017), http://dx.doi.org/10.1016/j.neuint.2017.05.004