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Cognitive improvement of mice induced by exercise prior to traumatic brain injury is associated with cytochrome c oxidase

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HIGHLIGHTS

- Running wheel (RW) counteracted the cognitive deficits induced by TBI.
- RW restored the neuron and synaptic density loss associated with TBI.
- RW increased the levels of COX I, II, III, BDNF, synapsin I and SYP.
- RW switched the mitochondrial CcO activity and ATP amounts.
- COX might play an important role in exercise's cognitive effects in TBI.

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ABSTRACT

Though the evidence demonstrated that voluntary exercise programs could be implemented to enhance recovery of cognitive function induced by traumatic brain injury (TBI), the exact mechanisms were still not known. We proposed that the cognitive improvement induced by exercise in TBI mice is associated with cytochrome c oxidase (COX). To demonstrate this hypothesis, adult mice were housed with or without access to a running wheel (RW) for three weeks followed by TBI operation. Acquisition of spatial learning and memory retention was assessed by using the Morris Water Maze (MWM) on days 15 post TBI. The synaptic density was measured by Golji staining. Immunohistochemistry (IHC) for NeuN, GFAP and growth associated protein 43 (GAP43) were also performed. Using Western blot, the expressions of COX I, II, III, BDNF, synapsin I, synaptophysin (SYP) and GAP43 in hippocampus of TBI mice were determinated. Lastly, CcO activity and ATP amount were also detected. Results showed that voluntary exercise prior TBI: (i) counteracted the cognitive deficits and neuron and synaptic density loss associated with the injury; (ii) increased the levels of COX I, II, III, BDNF, synapsin I, SYP and GAP43; (iii) switched the mitochondrial CcO activity and ATP amounts. These studies demonstrated that the COX plays an important role in exercise's cognitive effects in TBI model and also provide evidence that RW training is a promise exercise for traumatically injured mice.

1. Introduction

It is estimated that nearly 10 million people sustain severe brain injuries leading to hospitalization and/or death every year in the world. Amongst survivors, traumatic brain injury (TBI) results in a wide variety of physical, emotional and cognitive deficits. TBI is followed by an energy crisis that compromises the capacity of the brain to cope with challenges, and often reduces cognitive ability, which is one of the prevalent features of TBI [1,4]. Cognitive deficits are frequently related to impaired hippocampal function [23], and have been reproduced in animal models of TBI [7,12]. Unfortunately, there are no scientifically established effective treatments [2,5].

TBI is documented to have detrimental effects on mitochondria, such as alterations in glucose utilization and the depression of mitochondrial oxidative phosphorylation. Cytochrome c oxidase (COX, complex IV) is a key enzyme of the mitochondrial oxidative phosphorylation machinery, which creates cellular energy in the form of ATP [18]. COX consists of thirteen subunits. Of which, the largest subunits, COX I-III, are encoded by the mtDNA and activated in transcriptional level by mitochondrial transcription factors. Studies on mitochondrial metabolism have also provided evidences for

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reduced activity of the COX after TBI, which indicate that COX is vital for mitochondrial oxidative phosphorylation [9,15]. However, the exact role of COX involved in cognitive impairment after TBI is still unknown.

Both human and animal studies have demonstrated the effects of exercise supporting cognitive function [9]. Based on evidence that voluntary exercise activates neuroplasticity mechanisms within the hippocampus and counteracts cognitive deficits that are typically exhibited after experimental TBI [10], we hypothesize that voluntary exercise could be implemented to enhance recovery of cognitive functions through restores of mitochondrial COX induced by voluntary exercise prior to TBI.

In the present study, we explored the neuroprotective effects of voluntary exercise programs in recovery of cognitive functions induced by TBI. Mice were housed with voluntarily access to running wheel (RW) or immobilized RW for three weeks before injury. Then all mice were subjected to TBI or sham-operation and processed for experimental analysis. Acquisition of spatial learning and memory retention was assessed by using the MWM test 15 days post TBI. Golji staining was performed to analyze the alterations of synaptic density. We also employed IHC to evaluate the neuron and glia loss (NeuN and GFAP) as well as regeneration (GAP43 expression). The expressions of COX I, II, III, cognitive associated proteins (BDNF, synapsin I and SYP) and GAP43 were detected by Western blot in hippocampus of TBI mice. Additionally, hippocampal mitochondria CcO activity and ATP amount were measured.

2. Materials and methods

2.1. Animal grouping

Animal use and care were in accordance with the animal care guidelines, which conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Adult male C57BL/6J mice (4–4.5 months) were purchased from Harbin Medical University.

Runner groups were individually housed in cages equipped with a RW (diameter = 12 cm, width = 5 cm; Nalge Nunc International, Rochester, NY, USA) each. The equipped RW was attached to a receiver that monitored the number of revolutions (Vital Viewer Data Acquisition System software, Mini Mitter, Sunriver, OR, USA). The Non-runner groups were exposed to immobilized RW to control for the possibility of the RW serving as sources of environmental enrichment. After completing a 3-week period, animals were divided into four groups: animals received sham operation (Non-Injured runners), animals received a brain injury described as following (Injured runners), animals received sham operation (Non-Injured, Non-runners), and animals received a brain injury (Injured Non-runners). All of the mice were then processed for MWM test and sacrificed for other analysis. There were thirteen mice in each group ($N = 52$, $n = 13$), in which seven of them were employed for protein detections (Western blot), another three ones were prepared for IHC and Golgi staining, while the speared were prepared for mitochondria analysis (evaluations of the CcO activity assay and ATP amounts).

2.2. TBI

Animals underwent anesthesia with 3.6% chloral hydrate (100 mg/kg, Fluka, Germany) by intraperitoneal injection and fixed on a stereotactic platform. The TBI model of mice was performed according to described as before [19]. Briefly, a 3 mm diameter convex impact tip was used, and the impact was set to a 1.0 mm deformation delivered at a velocity of 4.5 m/s. In sham operated rats, the brain was spared. After the procedure, the scalp was sutured and each animal received a subcutaneous injection of warm physiologic saline (1 ml) to prevent dehydration. During surgery and subsequent recovery, body temperature was maintained with a circulating water heating pad.

2.3. MWM test

A Morris Water Maze paradigm was employed to assess spatial learning by training mice to locate a hidden, submerged platform using examination visual information was conducted exactly as described previously [17]. The test was conducted at 15–20 days after injury, and each mouse was tested for three trials per day for six consecutive days. The time required (escape latency) to find the hidden platform with a 90 s limit was recorded by a blinded observer and tracked using TOPSCAN (Clever Sys Inc.). A probe trial of 90 s was given 1 day after the final learning trial. The percentage of time spent in the quadrant where the platform was previously located was recorded. After the last probe trial, mice were sacrificed immediately for further analysis.

2.4. Golgi stain

After behavioral testing was completed, mice were deeply anesthetized with 3.6% chloral hydrate and decapitated. The brains were then rapidly removed. FDRapid Golgistain kits (FD NeuroTechnologies, Baltimore, MD, USA) were used for Golgi staining. Neurons were chosen on the basis of the following criteria as described before [13]. Diameter of somata, dendritic length and basal/apical branch points were also quantified. Somata diameter and spine density were analyzed using a two-way ANOVA, followed by Mann–Whitney U test. P values less than 0.05 were considered being significant.

2.5. IHC

After anesthesia with 3.6% chloral hydrate (1 ml/100 g), mice were perfused with 150 ml of cold phosphate-buffered saline (PBS) for 5 min followed by 150 ml of 4% paraformaldehyde solution for 30 min. The cerebral cortex from mice in each group was harvested for IHC detections. The sections were incubated in primary antibodies for NeuN (1:500, Santa Cruz), GFAP (1:200, Santa Cruz) and GAP43 (1:100, Santa Cruz), respectively. Immunoreactive products were observed and photographed with a light microscope (Leica. DMIRB, Germany) coupled with a computer assisted video camera. The immunopositive staining of NeuN, GFAP and GAP43 were quantified according to others' reports [25].

2.6. Western blot

After the last probe trial, mice were killed by cervical dislocation and decapitated. Hippocampus were removed quickly (within 60 s) and frozen in −70 °C isopentane until processed for further analysis. The protein preparation and Western blotting was performed as previously described [24]. The optical densities (ODs) of the protein bands for COX-I (1:1000, Santa Cruz), COX-II (1:800, Santa Cruz), COX-III (1:500, Santa Cruz), synapsin-I (1:400, Santa Cruz), SYP (1:400, Santa Cruz), BDNF (1:500, Santa Cruz) and GAP43 (1:1000, Santa Cruz) were analyzed for each group in order to semi-quantitatively assess these protein levels. GAPDH (1:800, Santa Cruz) was used as internal control.

2.7. Mitochondria preparation

Mitochondria preparation has been described according to the instructions of manufacturer (Sigma, Saint Louis, MO, USA).