Experimental Neurology 300 (2018) 222–231

Contents lists available at ScienceDirect

Experimental Neurology

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

Research Paper

Combined therapy involving electroacupuncture and treadmill exercise attenuates demyelination in the corpus callosum by stimulating oligodendrogenesis in a rat model of neonatal hypoxia-ischemia

Malk Eun Pak[a,b], Da Hee Jung[a,b], Hong Ju Lee[a,b], Myung Jun Shin[c,d], Soo-Yeon Kim[e,f], Yong Beom Shin[c,d], Young Ju Yun[g], Hwa Kyoung Shin[a,b,h], Byung Tae Choi[a,b,h,*]

[a] Department of Korean Medical Science, School of Korean Medicine, Pusan National University, Yangsan 50612, Republic of Korea
[b] Graduate Training Program of Korean Medicine for Healthy-aging, School of Korean Medicine, Pusan National University, Yangsan 50612, Republic of Korea
[c] Department of Rehabilitation Medicine, School of Medicine, Pusan National University, Busan 49241, Republic of Korea
[d] Biomedical Research Institute, Pusan National University Hospital, Busan 49241, Republic of Korea
[e] Department of Rehabilitation Medicine, Pusan National University Yangsan Hospital, Yangsan 50612, Republic of Korea
[f] Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan 50612, Republic of Korea
[g] Department of Integrative Medicine, School of Korean Medicine, Pusan National University, Yangsan 50612, Republic of Korea
[h] Korean Medical Science Research Center for Healthy-Aging, Pusan National University, Yangsan 50612, Republic of Korea

ARTICLE INFO

Keywords:
Neonatal hypoxia-ischemia
Electroacupuncture
Exercise
Oligodendrogenesis
Brain-derived neurotrophic factor

ABSTRACT

We investigated whether electroacupuncture (EA) and treadmill (TM) exercise improve behaviors related to motor and memory dysfunction in a cerebral palsy-like rat model via activation of oligodendrogenesis. A neonatal hypoxia-ischemia model was created using Sprague-Dawley rats (P7), and these underwent EA stimulation and treadmill training from 3 to 5 weeks after hypoxia-ischemia induction. EA treatment was delivered via electrical stimulation (2 Hz, 1 mA) at two acupoints, Baihui (GV20) and Zusanli (ST36). Behavioral tests showed that EA alleviated motor dysfunction caused by hypoxia-ischemia on a rotarod test, and TM exercise alleviated motor and memory dysfunction seen on cylinder and passive avoidance tests. Combined therapy with EA and TM exercise showed synergistic effects on the cylinder, rotarod, and catwalk tests. TM exercise significantly restored corpus callosum thickness, and combined therapy with EA and TM restored myelin basic protein (MBP) levels in this region. While EA stimulation only increased activation of cAMP-response element binging protein (CREB) in oligodendrocytes of the corpus callosum, TM exercise increased newly generated oligodendrocyte progenitor cells or oligodendrocytes via activation of CREB. Synergistic effects on oligodendrogenesis were also observed by the combined therapy. Furthermore, the combined therapy induced mature brain-derived neurotrophic factor (BDNF) expression in the cerebral cortex. These results demonstrate that combined therapy with EA and TM exercise may restore myelin components following neonatal hypoxia-ischemia via upregulation of oligodendrogenesis involving CREB/BDNF signaling, which subsequently improves motor and memory function. Therefore, combined therapy with EA and TM exercise offers another treatment option for functional recovery from injuries caused by neonatal hypoxia-ischemia, such as cerebral palsy.

1. Introduction

Cerebral hypoxia-ischemia in newborn babies is a major cause of cerebral palsy (Bennet et al., 2012; Levison et al., 2001; Nelson and Lynch, 2004). Further, neonatal encephalopathy due to early-life hypoxia-ischemia can cause profound and lifelong chronic nervous system and behavioral disabilities, including sensorimotor impairments and cognitive deficits (Aarnoudse-Moens et al., 2009; van Handel et al., 2007; Yager, 2004). Perinatal hypoxia is caused by injury to vulnerable neurons and oligodendrocytes, which contributes considerably to cerebral white matter injury (Kaindl et al., 2009; Salmaso et al., 2014). Thus, cerebral palsy is considered a demyelinating disorder where white matter is injured as a result of the loss of oligodendrocytes, causing damage to myelin and disruption of nerve conduction (Sharma et al., 2015).

White matter injuries such as periventricular leukomalacia are the leading known causes of cerebral palsy and other neurocognitive deficits in premature infants (Deng et al., 2008; Porambo et al., 2015). The

* Corresponding author at: School of Korean Medicine, Pusan National University, Yangsan 50612, Republic of Korea.
E-mail address: choibt@pusan.ac.kr (B.T. Choi).

https://doi.org/10.1016/j.expneurol.2017.11.014
Received 14 September 2017; Received in revised form 9 November 2017; Accepted 29 November 2017
Available online 02 December 2017

corpus callosum, a large white matter structure that connects the two cerebral hemispheres, contains fibers projecting into prefrontal and other cortical areas as well as motor and sensory areas (Hofer and Frahm, 2006; Wahl et al., 2007). Significant atrophy of these connecting tracts explains some clinical conditions, such as motor dysfunction and cognitive impairment (Huang et al., 2005; Laporta-Hoyos et al., 2017).

There are no restorative therapies for white matter injury, but oligodendrocyte precursor cells (OPCs) can generate myelin-forming mature oligodendrocytes as an endogenous repair mechanism even after adolescence (Kaindl et al., 2009; Miyamoto et al., 2013). Therefore, oligodendrocytes are cellular targets in the treatment of neonatal white matter injury leading to cerebral palsy, and oligodendrogenesis can improve myelination in the brain through proliferation and differentiation of myelinating oligodendrocytes (Verney et al., 2012; Zhang and Chopp, 2009).

Electroacupuncture (EA) has been clinically used to treat many neurological disorders and is often used as a common complementary treatment for cerebral palsy (Duncan et al., 2012; Liu et al., 2013). EA treatment exerts protective effects on the neural myelin sheath via enhancement of oligodendrocyte proliferation and regeneration in spinal cord injury or cerebral hypoperfusion (S.M. Ahn et al., 2016; Huang et al., 2015). Treadmill (TM) exercise, both full and partial weight-bearing, is a dynamic exercise approach that is commonly used in the rehabilitation of children with cerebral palsy (Wessels et al., 2010). Activity-based therapy such as TM exercise promotes oligodendrogenesis and, thus, myelination. This activity-dependent myelination in adulthood is a significant contributor to brain repair following pathological demyelination (Alvarez-Saavedra et al., 2016; Jensen and Yong, 2016).

Brain-derived neurotrophic factor (BDNF) administration or secretion by astrocytes supports oligodendrogenesis and remyelination through the response of residual OPCs after white matter damage (Miyamoto et al., 2015; Ramos-Cejudo et al., 2015). EA stimulation upregulates neurotrophins, in particular BDNF. Neurotrophins are the main mediators of neural plasticity and neuronal stem cell proliferation and differentiation (Kim et al., 2014; Manni et al., 2010). Physical activity such as TM exercise increases BDNF expression in the brain and spinal cord and, thus, ameliorates ischemia-induced damage to myelin (J.H. Ahn et al., 2016; Skup et al., 2002).

If we have a deeper understanding of the underlying mechanisms regulating OPCs, it may lead to new therapeutic approaches for ameliorating white matter dysfunction in brain disorders (Maki et al., 2013). However, whether EA and TM exercise treatment stimulates neural repair responses, including oligodendrogenesis with recovery of white matter, in experimental models of white matter disorders is not clear. Thus, we hypothesized that combined therapy with EA and TM exercise may restore the components of myelin in a cerebral palsy-like rat model via activation of oligodendrogenesis and, subsequently, ameliorate behavioral deficits; and that it may provide an additional treatment for motor and memory deficits via targeting oligodendrogenesis involving BDNF signaling in cerebral palsy. To test our hypotheses, we examined the effects of EA and TM exercise on behavioral dysfunction and the expression of oligodendrocyte lineage markers in a rat neonatal hypoxia-ischemia model. We also explored the effects on BDNF and its related transcription factors.

2. Materials and methods

2.1. Animals

Pregnant Sprague-Dawley rats at embryonic day 17 (E17, n = 12) were obtained from Dooyeol Biotech (Seoul, Korea) and housed at a controlled temperature (20 ± 2 °C) with a 12-h/12-h light-dark cycle (lights on from 9:00 to 21:00). Food and water were provided ad libitum for the duration of the study. The Pusan National University Animal Care and Use Committee approved all experimental procedures in accordance with the National Institutes of Health Guidelines (approval number, PNU-2016-1099).

2.2. Neonatal hypoxia-ischemia model

To induce an animal model of hypoxia-ischemia, rat pups were anesthetized with isoflurane on postnatal day 7 (P7), and their left common carotid artery was exposed. The artery, isolated from neighboring nerves and veins, was ligated using 5-0 surgical silk to ensure cessation of blood flow. The total duration of the surgery was < 10 min. The pups were then allowed to recover with their dams for 2 h, and then were placed in a hypoxic chamber for 150 min with O_2 levels and temperature maintained at 8% and 37 °C, respectively. Control animals were separated from the dam for the same period of time. After expose to the hypoxia chamber, all pups were returned to their home cage. Male pups were used for all experiments. Rats were divided into five groups (n = 10 per group): the control group, hypoxia-ischemia group (HI), hypoxia-ischemia group treated with EA (EA), hypoxia-ischemia group treated with TM (TM), and hypoxia-ischemia group treated with combined EA and TM (EA + TM). Body weights were recorded once per week from 3 to 6 weeks after hypoxia-ischemia induction to monitor potential health issues. Experimental schedules are schematically represented in Fig. 1.

2.3. EA stimulation

Starting 3 weeks (P21) after hypoxia-ischemia induction, EA treatment was employed 3 times per week for 3 weeks. EA was applied at two acupoints, Baihui (GV20) and Zusanli (ST36), using sterilized needles with 0.25 mm in diameter (DongBang Medical Co., Ltd., Seoul, Korea). The rats were anesthetized with 1.5% isoflurane in air and stimulated electrically at a frequency of 2 Hz and an intensity of 1 mA for 20 min using a Pulsemaster Multichannel Stimulator SYS-A300 electrical stimulator (World Precision Instruments, Berlin, Germany). Control and HI groups received anesthesia with 1.5% isoflurane for 20 min without EA application.

2.4. TM exercise

Starting 3 weeks after hypoxia-ischemia induction, rats exercised on a motorized treadmill 5 days per week for 3 weeks. The TM exercise paradigm consisted of running at 5 m/min for 10 min in the first week and 15 min in the second week. In the third week, they ran at 5 m/min

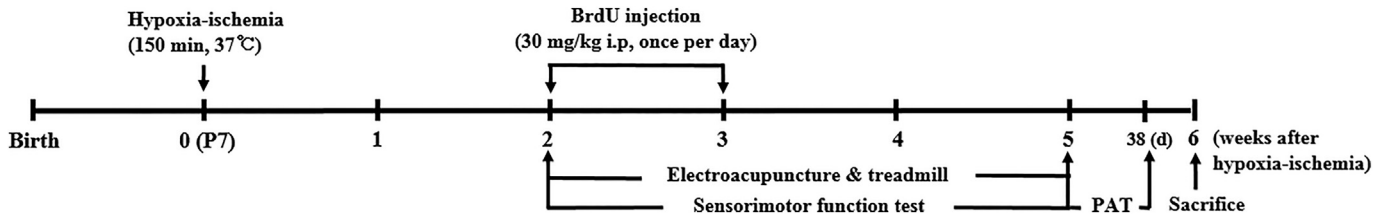

Fig. 1. Schematic diagram of experimental procedures. On postnatal day 7 (P7), Sprague-Dawley rat pups were subjected to left common carotid artery ligation followed by hypoxia-ischemia–inducing conditions. PAT, passive avoidance test.