

Effects of endurance exercise on expressions of glial fibrillary acidic protein and myelin basic protein in developing rats with maternal infection-induced cerebral palsy

Kijeong Kim¹, Mal-Soon Shin², Han-Sam Cho², Young-Pyo Kim^{3*}

¹School of Exercise and Sport Science, University of Ulsan, Ulsan, Korea

²Department of Physiology, College of Medicine, Kyung Hee University, Seoul, Korea

³Faculty of Exercise & Sports Science, Jeju National University, Jeju, Korea

Periventricular leukomalacia (PVL) is a common white matter lesion affecting the neonatal brain. PVL is closely associated with cerebral palsy (CP) and characterized by increase in the number of astrocytes, which can be detected by positivity for glial fibrillary acidic protein (GFAP). Change in myelin basic protein (MBP) is an early sign of white matter abnormality. Maternal or placental infection can damage the neonatal brain. In the present study, we investigated the effects of treadmill walking exercise on GFAP and MBP expressions in rats with maternal lipopolysaccharide (LPS)-induced PVL. Immunohistochemistry was performed for the detection of GFAP and MBP. The present results showed that intracervical maternal LPS injection during pregnancy increased

GFAP expression in the striatum and decreased MBP expression in the corpus callosum of rats. The results also showed that treadmill walking exercise suppressed GFAP expression and enhanced MBP expression in the brains of rats with maternal LPS-induced PVL. The present study revealed that treadmill walking exercise is effective for the suppressing astrogliosis and hypomyelination associated with PVL. Here in this study, we showed that treadmill walking exercise may be effective therapeutic strategy for alleviating the detrimental effects of CP.

Keywords: Periventricular leukomalacia, Cerebral palsy, Glial fibrillary acidic protein, Myelin basic protein, Treadmill walking exercise

INTRODUCTION

Approximately 10% of newborns are born prematurely, and severe disturbances in mental development and perinatal brain injuries have been observed in premature infants (Yesilirmak et al., 2007). More than 10% of these children have adverse neurological outcomes, including cognitive, motor, and behavioral deficits (Wozniak et al., 2004). Periventricular leukomalacia (PVL), a common white matter lesion in neonatal brain, is closely associated with cerebral palsy (CP) (Cai et al., 2000). The exact mechanisms underlying PVL have not been clarified; however, it has been proposed that maternal or placental infection may damage the neona-

tal brain (Adams-Chapman and Stoll, 2006).

Astroglia and myelin are important components of white matter. Reactive astrogliosis, typified by astrocyte proliferation and/or astrocytic hypertrophy, is a common phenomenon in the central nerve system (CNS) following brain trauma or inflammation (Maxwell et al., 1990). Direct injection of cytokines into the neonatal mouse brain increased reactive astrogliosis, indicating that pro-inflammatory cytokines induce astrogliosis (Balasingam et al., 1994). Clinical studies have provided evidences that PVL is closely associated with an increased number of astrocytes in white matter lesions of the neonatal brain (Deguchi et al., 1997; Obonai et al., 1997). Astrogliosis can be detected by positivity for glial fibrillary

*Corresponding author: Young-Pyo Kim

Faculty of Exercise & Sports Science, Jeju National University, 102 Jejudaehak-ro, Jeju 690-756, Korea

Tel: +82-64-754-3584, Fax: +82-64-757-1752, E-mail: kimyp@jejunu.ac.kr

Received: January 15, 2014/ Revised: February 6, 2014/ Accepted: February 15, 2014

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acidic protein (GFAP), an intermediate filament protein specific to astrocytes (Deguchi et al., 1997; Obonai et al., 1997). Myelin basic protein (MBP) is the most abundant protein in the myelin sheath (Kurumatani et al., 1998), and a change in the content of MBP is an early sign of white matter abnormality.

Maternal LPS administration increases the number of GFAP-positive astrocytes, decreases MBP expressions and alters microglia immunoreactivity in the brain of offspring (Cai et al., 2000). CP is caused by damage to the white matter, particularly in the periventricular region, of the developing brain (Bell and Hallenbeck, 2002). PVL is strongly correlated with the development of CP (Bejar et al., 1992). Toso et al. (2005) reported that LPS exposure during pregnancy induce inflammatory model of CP in offspring.

It is well known that exercise induces several physiological and biochemical changes in the brain (Chaouloff, 1997; Sutoo and Akiyama, 1996). Running is known to be an important positive stimulus for neurogenesis (Lee et al., 2013; Ra et al., 2002). Physical exercise is also known to enhance neuronal plasticity and alter the transcription levels of various genes, in a manner associated with increased neuronal activity and synaptic remodeling (Tong et al., 2001). Molteni et al. (2002) reported that voluntary physical activity involving the use of a running wheel increases the expressions of hippocampal genes that are associated with neuronal plasticity. In addition, physical exercise has been reported to upregulate the levels of various neuropeptides and neurotrophins, such as neurotensin, substance P, and brain-derived neurotrophic factor (BDNF) mRNA, in the rat hippocampus (Bucinskaite et al., 1996; Kim et al., 2013; Russo-Neustadt et al., 1999).

In the present study, we investigated whether treadmill walking would have a protective effect on astrogliosis and hypomyelination in rats with PVL. The animal model of PVL was induced by maternal intracervical injection of LPS. In the present study, immunohistochemistry was performed for the detection of GFAP in the striatum and MBP in the corpus callosum.

MATERIALS AND METHODS

Animals and treatments

This study was performed in accordance with the guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. Female Sprague-Dawley rats (180 ± 10 g, 8 weeks old, $n = 20$) were allowed to mate with male rats for a period of 24 h. The female rats were then individually housed in plastic home cages under controlled temperature ($20 \pm 2^\circ\text{C}$) and a light–dark cycle consisting of 12 h of light and 12 h of darkness (lights

on from 07:00 to 19:00 h). Food and water were made available *ad libitum*.

The pregnant rats were divided into two groups: the sham group and the LPS-treated group ($n = 4$ in each group). On the 9th, 13th, and 17th day of pregnancy, the pregnant rats in the LPS-treated group received intracervical injections of 1.0 mg/kg LPS (from *Escherichia coli*, serotype 055:B5, Sigma Chemical Co., St. Louis, MO, USA) suspended in pyrogen-free saline (PFS), and the pregnant rats in the sham group were treated with PFS. After birth, the neonatal rats were divided into three groups: the sham group, the LPS-treated group, and the LPS-treated and exercise group ($n = 4$ in each group).

Exercise protocol

Eight weeks after birth, the rats in the exercise group were forced to walk on a motorized treadmill for 30 min three times a week for 4 weeks. The exercise load consisted of walking at the speed of 8 meters/min at an inclination of 0° .

Tissue preparation

The animals were sacrificed immediately after the completion of the last exercise. The rats were weighed and given an overdose of Zoletil 50[®] (10 mg/kg i.p.; Vibac Laboratories, Carros, France). After a complete lack of response was observed, the rats were transcardially perfused with 50 mM phosphate-buffered saline (PBS) and subsequently fixed with freshly prepared 100 mM phosphate buffer (PB, pH 7.4) containing 4% paraformaldehyde. The brains were removed and fixed in the same fixative overnight and then transferred to a 30% sucrose solution for cryoprotection. Serial coronal sections of 40- μm thickness were obtained using a freezing microtome (Leica, Nussloch, Germany).

GFAP and MBP immunohistochemistry

An average of six sections within the striatum spanning from Bregma 1.20 mm to 0.96 mm and six sections within the corpus callosum spanning from Bregma 0.84 mm to 0.60 mm were obtained from each brain. Tissue sections were incubated overnight with rabbit anti-GFAP antibody or anti-MBP antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:500.

The sections were then incubated with anti-rabbit secondary antibody (1:200, Vector Laboratories, Burlingame, CA, USA). The sections were subsequently incubated with avidin-biotin-peroxidase complex (1:100, Vector Laboratories) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution containing 0.02% 3, 3'-diaminobenzidine tet-

rahydrochloride (DAB) and 0.03% H₂O₂ in 50 mM Tris-HCl (pH 7.6) for approximately 5 min. The sections were then washed three times with PBS and mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature, and coverslips were mounted using Permount® (Fisher Scientific, New Jersey, NJ, USA).

Data analysis

The intensities of GFAP and MBP staining were quantitatively assessed using a microdensitometrical method based on optical density (the mean gray scale) with the use of an image analyzer (Multiscan, Fullerton, CA, USA). Before starting the image analysis, the light source was adjusted to the brightness that generated the best possible contrast between immunopositive and immunonegative cells.

Statistically significant differences were determined by one-way analysis of variance (ANOVA) followed by Duncan's *Post-hoc* analysis. The results were expressed as the mean \pm standard error of the mean (SEM). Differences were considered significant at $P < 0.05$.

RESULTS

Effects of treadmill walking exercise on body weight in rats with maternal endotoxin-induced PVL

Eight weeks after birth, the mean body weight of the rats was 297 ± 3.85 g in the sham group, 246 ± 24.33 g in the LPS-treated group, and 299 ± 2.00 g in the LPS-treated and exercise group. After 4 weeks of exercise, the mean body weight of the rats was 394 ± 4.60 g in the sham group, 347 ± 7.54 g in the LPS-treated group, 342 ± 7.50 g in the LPS-treated and exercise group. There was no significant difference in body weight between the groups.

Effect of treadmill walking exercise on GFAP expression in the striatum of rats with maternal endotoxin-induced PVL

Photomicrographs of GFAP expression in the striatum are pre-

sented in Fig. 1. The optical density of the GFAP-positive cells in the striatum was $56.89 \pm 0.89/\text{mm}^2$ in the sham group, $60.29 \pm 0.36/\text{mm}^2$ in the LPS-treated group, and $57.77 \pm 0.14/\text{mm}^2$ in the LPS-treated and exercise group (Fig. 2). The results of the present study revealed that GFAP expression in the striatum was significantly increased in the LPS-treated group and that treadmill exercise suppressed the LPS-induced increase in GFAP expression.

Effect of treadmill walking exercise on MBP expression in the corpus callosum of rats with maternal endotoxin-induced PVL

Photomicrographs of MBP expression in the corpus callosum are presented in Fig. 3. The optical density of the MBP-positive cells in the corpus callosum was $106.70 \pm 3.49/\text{mm}^2$ in the sham group, $93.30 \pm 2.00/\text{mm}^2$ in the LPS-treated group, and $103.12 \pm 2.16/\text{mm}^2$ in the LPS-treated and exercise group (Fig. 4). The

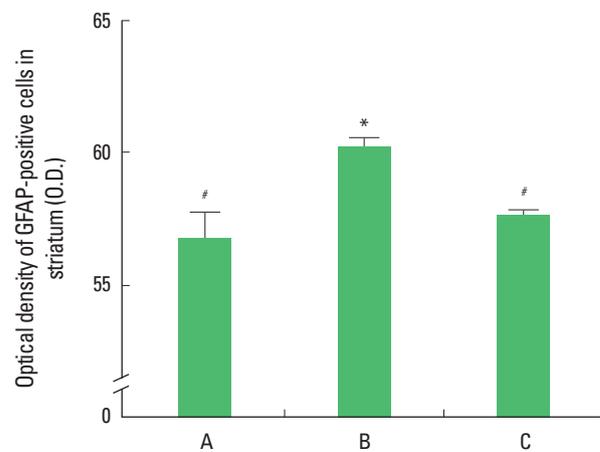


Fig. 2. Effect of treadmill walking exercise on the expression of glial fibrillary acidic protein (GFAP) in the striatum of rats with maternal endotoxin-induced periventricular leukomalacia (PVL). (A) Sham group, (B) lipopolysaccharide (LPS)-treated group, (C) LPS-treated and exercise group. The data are presented as the mean \pm SEM. *Represents $P < 0.05$ compared to the sham group. # $P < 0.05$ compared to the LPS-treated group.



Fig. 1. Photomicrographs of glial fibrillary acidic protein (GFAP)-positive cells in the striatum. The scale bar represents 250 μm .

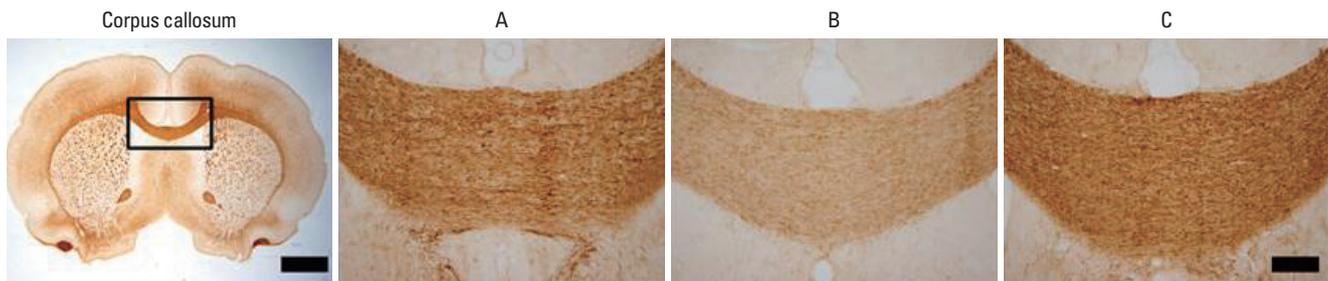


Fig. 3. Photomicrographs of myelin basic protein (MBP)-positive cells in the corpus callosum. The scale bar represents 250 μm .

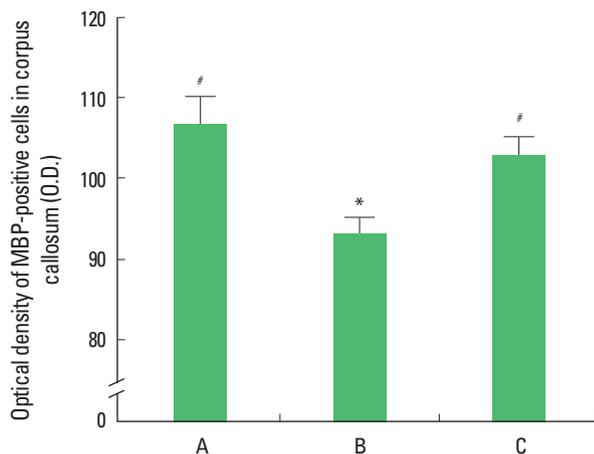


Fig. 4. Effect of treadmill walking exercise on the expression of myelin basic protein (MBP) in the corpus callosum of rats with maternal endotoxin-induced periventricular leukomalacia (PVL). (A) Sham group, (B) lipopolysaccharide (LPS)-treated group, (C) LPS-treated and exercise group. The data are presented as the mean \pm SEM. ^{*}Represents $P < 0.05$ compared to the sham group. [#] $P < 0.05$ compared to the LPS-treated group.

results of the present study showed that the expression of MBP in the corpus callosum was significantly suppressed in the LPS-treated group and that treadmill exercise alleviated the LPS-induced suppression of MBP expression.

DISCUSSION

Maternal infection as well as increased levels of pro-inflammatory cytokines in amniotic fluid and fetal blood is associated with preterm birth and neonatal neurological disorders (Goepfert et al., 2004; Zlokovic et al., 2005). White matter injury, especially such as PVL, is a common form of prenatal brain injury occurring in the immature babies, which results in life-long disorders of movement, posture, and cognition. The pathogenesis of PVL is multifactorial and likely involves in ischemia-reperfusion, formation of reactive oxygen and nitrogen species, glutamate excitotoxicity, im-

pairment of myelination due to loss of oligodendrocytes, apoptotic cell death, microglial activation, and maternal or fetal infection (Adams-Chapman, 2006; Leviton and Dammann, 2004). PVL is known as the major antecedent disorder developing to CP (Yesilirmak et al., 2007). Several animal models have been established to study the mechanisms of PVL and to search the therapeutic potential of candidate agents (Adams-Chapman, 2006). One of the widely used animal models for PVL can be achieved by administration of LPS to pregnant animals (Cai et al., 2004; Hagberg et al., 2002).

In the present results, intracervical injection of LPS into the maternal rats induced astrogliosis in the striatum of rats that was represented by enhanced expression of GFAP in the striatum. Many studies have shown that PVL is closely associated with the increased number of GFAP-positive astrocytes in the white matter lesion of infant brains (Deguchi et al., 1997; Obonai et al., 1997). Therefore, the increased GFAP content in the neonatal rat brain after maternal LPS administration is an indication of potential role of cytokines in mediating maternal infection and induction of PVL in newborn infants.

In the present results, intracervical injection of LPS into the maternal rats induced hypomyelination in the corpus callosum of rats, that was represented by suppressed expression of MBP in the corpus callosum. MBP is the most abundant protein in the myelin sheath. One possible reason for the reduced MBP staining is a reduction MBP production by the oligodendrocyte or a reduction in the number of oligodendrocytes after maternal LPS treatment. Reduced myelination and oligodendrocytes are concomitantly observed in the infant brains with PVL (Wang et al., 2006).

In the present results, we investigated the effects of treadmill walking exercise on astrogliosis and hypomyelination in rats with PVL. Treadmill walking exercise suppressed LPS-induced astrogliosis in the striatum and alleviated LPS-induced hypomyelination in the corpus callosum of the rats with PVL. The present study revealed that treadmill walking exercise is effective for the

suppressing astrogliosis and hypomyelination associated with PVL. In the present study, we showed that treadmill walking exercise may be effective therapeutic strategy for alleviating the detrimental effects of CP.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

This work was supported by the research grant of the Jeju National University in 2011.

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