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The effects of weight- and non–weight-bearing exercise on corticospinal axon sprouting, regeneration-related proteins and functional recovery after spinal cord contusion

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The purpose of this study was to investigate the effects of weight- and non–weight-bearing exercises on the Basso-Beattie-Bresnahan (BBB) locomotor rating scale, corticospinal axon regrowth and regeneration-related proteins following spinal cord injury (SCI). Twenty-four male Sprague-Dawley rats were randomly divided into four groups: control group (n=6), SCI+sedentary group (SED, n=6), SCI+treadmill exercise group (TREAD, n=6), and SCI+swimming exercise group (SWIM, n=6). All rats in the SCI group were given the rest for 2 weeks after SCI, and then they were allowed to engage in low-intensity exercise for 6 weeks on treadmill device. Motor function (BBB score) was improved more in the SWIM group compared to the SED group at 3 and 6 weeks after SCI. The SWIM group also showed higher levels of axonal outgrowth in corticospinal tract and increased expression of phosphorylated extracellular signal-regulated kinase, a marker of axonal regeneration in the dorsal horn of the caudal region, compared to the TREAD group. Additionally, the SWIM group significantly upregulated the expression of regeneration-related proteins. Our findings suggest that non–weight-bearing exercise may be one of several rehabilitation methods for improving locomotor function and corticospinal axon regeneration after SCI.

Keywords: Spinal cord injury, Weight-bearing exercise, Non–weight-bearing exercise, BBB score, Corticospinal tract, Sprouting

INTRODUCTION

Spinal cord injury (SCI) is an incurable condition that causes disability by blocking the transmission of sensory and motor nerve signals, and it has been reported that SCI does not regenerate spontaneously (Wang et al., 2015). SCI is classified into extrinsic injuries (traffic accidents, falls, sports-related injuries, or violence) and non-extrinsic injuries (tumors, infections, or degenerative disc disease), and histological problems in the spinal cord, such as complete or incomplete injuries, are determined based on the degree of damage applied to the spinal cord (Hu et al., 2023).

Neuronal apoptosis and cavity formation around the injury site of the spinal cord are the most common histological and morphological changes. The former is caused by blood infiltration in the gray matter of the injured area and secretion of toxic substances from oligodendrocytes, and the latter results from by the formation of glial scar that block the elongation of corticospinal tract (CST) axons (Keikhaei et al., 2023). Many previous studies have reported that histological changes within the injured spinal cord can lead to problems in behavioral function, such as the Basso-Beattie-Bresnahan (BBB) locomotor rating scale, trunk stability, and tail position, as well as a decline in quality of life due to economic difficulties for treatment (Wang et al., 2015).

Exercise has been known as the most economical way to prevent and improve cancer, obesity, diabetes, and cardiovascular disease, and its effectiveness has recently been proven in the category of nerve regeneration (Cho and Seo, 2023). Especially in the field of spinal rehabilitation, regular low-intensity walking exercise has

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established as a therapeutic approach to upregulate expression levels of central nerve regeneration-related proteins such as neurotrophins, brain-derived neurotrophic factor, phosphorylated extracellular signal-regulated kinase 1 and 2 (p-ERK)1/2, phosphorylated cAMP-responsive element-binding protein (p-CREB), and phosphorylated protein kinase B (p-Akt) (Wu et al., 2016).

The recommended exercise types for spinal rehabilitation include low-intensity exercises such as treadmill walking (33%), weight-bearing exercise (17%), and voluntary wheel running (17%). Zhan et al. (2023) emphasized the necessity of load-bearing exercises after SCI, as weight-bearing exercises might enhance the sensitivity of sensory and motor nerves, promote neuroplasticity, and aid in the recovery of motor function. However, some previous studies have stated that weight-bearing exercise after SCI not only fails to improve movement and pain tolerance but may actually worsen them (Erschbamer et al., 2006). Kumar et al. (2021) presented several findings suggesting that non–weight-bearing exercise might be a new strategy in spinal cord regeneration for improving neuropathic pain, axonal regeneration, and functional recovery (Cho and Seo, 2023).

Taken together, previous studies on non–weight-bearing exercise after SCI are very limited, and there are no studies comparing weight- and non–weight-bearing exercise to identify the optimal exercise type for improving spinal cord nerve damage. Therefore, the purpose of this study is to determine whether weight-bearing and non-weight-bearing exercises might improve regeneration-related protein levels, CST axonal elongation, and functional recovery after SCI. Based on the results of this study, we aim to identify the optimal exercise type for improving outcomes after SCI.

MATERIALS AND METHODS

Experimental animals

Twenty-four male Sprague-Dawley rats (4 weeks old) were housed in constant room temperature of 22°C–24°C and 60% humidity, with a 12/12-hr light-dark cycle. They were provided with commercial rat chow (Samyang Co., Seoul, Korea) and water ad libitum. This experiment was performed according to the guidelines of the approved Institutional Animal Care and Use Committee protocol (2022-0030) from Jeju National University. The experimental rats were randomly divided into four groups: the control group (CONT, n=6), the SCI+sedentary group (SED, n=6), the SCI+treadmill exercise group (TREAD, n=6), and the SCI+swimming exercise group (SWIM, n=6).

Spinal cord injury

For spinal cord contusion injury, all animals were anesthetized by inhalation of isolurane using inhalation anesthesia device (Jeungdo Bio and Plant, Seoul, Korea), and the concentration of isoflurane was maintained at 1.5% to 2.5%. After exposing the spinal cord without damaging the dura mater at the T9–10 level, a contusion injury was induced by freely dropping a 10 g impactor from a height of 2.5 cm on the dorsal surface of the exposed spinal cord using the New York University Impactor System (NYU impactor, New York, NY, USA) (Kim et al., 2017). Afterwards, the muscles and skin were sutured, and the rats were kept on a heating pad at 36°C–37°C on the rectum until they woke up from anesthesia. All rats were sacrificed using CO_2 gas 24 hr after the end of exercise for tissue extraction. The extracted tissue was the spinal cord at the T5–12 level of the thoracic vertebrae, and was stored in a -80°C freezer.

Weight- and non–weight-bearing exercise program

Two weeks after SCI, weight- and non–weight-bearing exercise were applied 6 times a week for 6 weeks. The weight-bearing exercise was based on the previous study of Jung et al. (2016), and walking exercise was applied on a treadmill (Jeungdo Bio and Plant) at a slope of 0° and a speed of 6 m/min. The exercise consisted of 4 repetitions of 3-min walking in the first week and 6 repetitions of 5-min walking from the second to the sixth week. The rest time between each session was set to 5 min. Non–weight-bearing exercise (swimming exercise) was performed in a self-made acrylic swimming pool (80 cm×50 cm×50 cm) by attaching a stopper (10 g) to the tail to prevent the front feet from supporting the wall, and forced exercise was performed. The room temperature was maintained at 25°C, and the water temperature was maintained at 32°C–35°C. The swimming exercise performed by Harman et al. (2021) was modified, and consist of four 3-min swims in the first week and 6 repetitions of 5-min swims in the second to sixth weeks. The rest time between each session was set to 5 min.

Locomotor assessment

Functional recovery was assessed using the BBB locomotor scale in an open area, and we recorded locomotion by digital video camera at 0, 1, 3, and 6 weeks after exercise application (Jung et al., 2016). Video analysis was performed by two researchers who were not aware of the experiment. The BBB scale is composed of 1 point (no observable hindlimb movement) to 21 points (consistent gait, gait and step intervals, parallel foot position, consistent trunk stability, and consistently raised tail). Scores were divided into left

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and right sides from the early to late recovery stages, and in order to increase the reliability of the scale scores, two researchers who were not aware of the experiment analyzed the videos recorded for 6 weeks, and we used the average value.

Immunofluorescence staining

The spinal cord of the injured area was embedded in frozen section compound (Leica, Biosystems Richmond, Inc., Richmond, IL, USA) for analysis, and frozen sections were performed using a cryostat (Leica CM 1860, Leica, Nussloch, Germany). Double immunofluorescence staining was performed to observe motor neurons and nucleus in the spinal cord. The ventral horn of the caudal region of the injured spinal cord was frozen into 10-μm-thick cross-sections and attached to slides. After that, it was fixed in 4% paraformaldehyde and 4% sucrose mixed in phosphate-buffered saline (PBS) for 40 minutes, treated with 0.5% Nonidet P-40 in PBS, and cultured in 2.5% bovine serum albumin for 4 hr. Afterwards, the cells were incubated with anti-rabbit p-ERK1/2 antibody (1:200, Cell Signaling Biotechnology, Danvers, MA, USA), followed by fluorescein-goat anti-mouse antibody (1:400, Molecular Probes, Eugene, OR, USA) for 1 hour at room temperature, coverslipped with gelatin mounting medium, and observed under a fluorescence microscope (Nikon model E-600, Nikon, Tokyo, Japan). All images captured with a digital camera were reviewed using Adobe Photoshop Software (ver. CS6, Adobe, San Jose, CA, USA).

Anterograde DiI tracing technique

To observe CST axons, fluorescent lipophilic carbocyanine dye l,l'-dioctodecyl-3,3,3´,3´tetramethylindocarbocyanine perchlorate (DiI, Molecular Probes; 3 μL of 3% in dimethyl sulfoxide) was injected into the motor cortex (2.0 mm posterior to bregma, 2.3 mm lateral to midline, 1.5 mm ventral to the dura surface) that controls lower limb movements using an injector device, PLI-100 PLUS Pico injector at 3 days before tissue preparation (Seo et al., 2013). The T9–10 region was extracted from each group, and fluorescence images of DiI-labeled axons were observed after cross and horizontal section of the injured spinal cord.

Western blot analysis

The extracted spinal cord was rinsed with PBS and lysed using Triton solution. After electrophoresis on a sodium dodecyl sulfate polyacrylamide gel, it was transferred to polyvinylidene difluoride membrane for 2 hr. The transferred membranes were reacted with 5% skim milk/tris-buffered saline with 0.1% Tween 20 solution at room temperature for 1 hr, and then incubated with primary antibody at 4°C for 24 hr. The primary antibodies were anti-rabbit p-ERK1/2 antibody (Cell Signaling Biotechnology), anti-rabbit p-CREB antibody (Cell Signaling Biotechnology), and anti-rabbit p-Akt antibody (Cell Signaling Biotechnology), anti-rabbit caspase-3 antibody (Cell Signaling Biotechnology), anti-mouse β-actin (1:2,000, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and secondary antibodies were horseradish peroxidase conjugated anti-mouse and rabbit IgG antibodies (1:1,000, GeneTex, Irvine, CA, USA). at room temperature using The reaction was performed for 1 hr. Proteins were detected using Westar ECL (Cyanagen, Bologna, Italy), and the detected bands were analyzed using Chemidoc (Bio-Rad, Hercules, CA, USA).

Statistical analysis

All the data are presented as the mean±standard error. Statistical analysis was performed using one-way analysis of variance, followed by the Duncan *post hoc* test. The significance level was set at $P<0.05$. Data analysis and graphs were generated using Prism 6 software (GraphPad, La Jolla, CA, USA).

RESULTS

BBB locomotor scale after SCI

The changes in BBB scores over 6 weeks after SCI are shown in Fig. 1. All rat after SCI had low BBB scores, but the average BBB scores showed different pattern over time in three groups. In particular, the SWIM group significantly showed higher BBB scores than SED after 3 weeks ($P<0.022$) and 6 weeks ($P<0.008$). How-

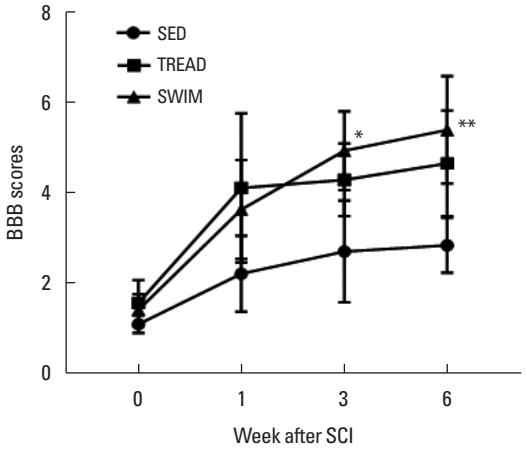

Fig. 1. Changes in Basso-Beattie-Bresnahan (BBB) locomotor scores after spinal cord injury (SCI). SED, sedentary group; TREAD, treadmill exercise group; SWIM, swimming exercise group. *$P<0.05$, **$P<0.01$ vs. SED.

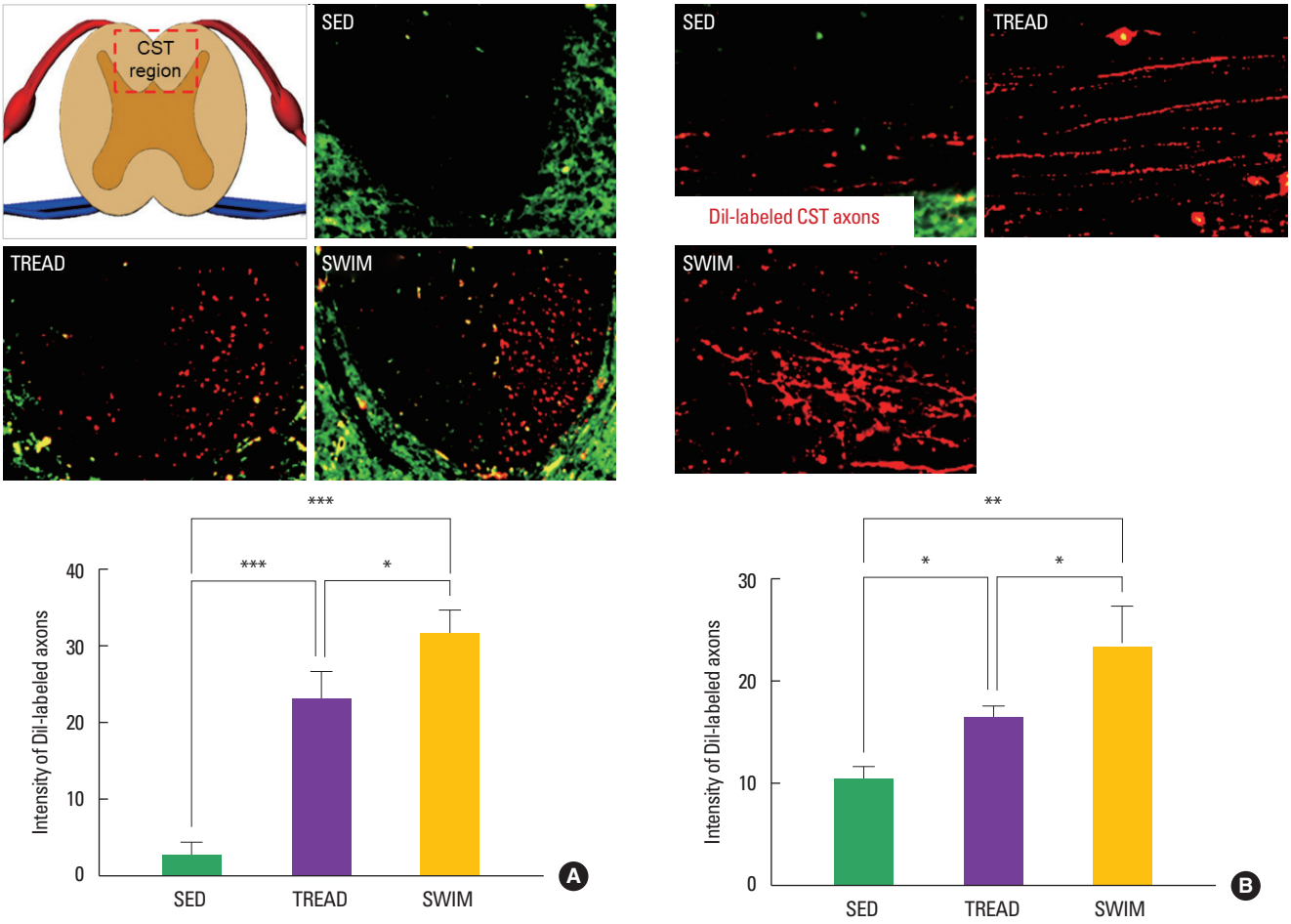

Fig. 2. DiI (I,I'-dioctodecyl-3,3,3',3'tetramethylindocarbocyanine perchlorate)-labeled corticospinal tract (CST) axons around the injury site at 6 weeks after spinal cord injury (SCI). (A) Cross-section images and quantitative graph on intensity of DiI-labeled CST axons. (B) Horizontal section images and quantitative graph on intensity of DiI-labeled CST axons. SED, sedentary group; TREAD, treadmill exercise group; SWIM, swimming exercise group. Red is DiI-labeled CST axons. *P<0.05. **P<0.01. ***P<0.001.

ever, there was no significant difference from the TREAD group.

CST axonal elongation after SCI

As shown in Fig. 2, CST axon elongation was confirmed at the injury site 6 weeks after SCI through DiI tracing technique. In the cross-section of the spinal cord (Fig. 2A), the two exercise groups showed a significant increase in DiI-labeled CST axon elongation compared to SED (P < 0.001). In particular, the SWIM group further increased the mean length of DiI-labeled CST axon than the TREAD group (P < 0.016). The result in the horizontal section of the spinal cord (Fig. 2B) were similar to those in Fig. 2A. The SWIM group showed a significant increase in DiI-labeled CST axon elongation compared to both TREAD (P < 0.027) and SED groups (P < 0.002).

Expression of p-ERK1/2 in motor neuron after SCI

Immunofluorescence staining was performed to examine expression of p-ERK1/2 in motor neurons of the caudal region 6 weeks after SCI. As shown in Fig. 3, the number of ERK1/2-expressed motor neurons located in the ventral horn of the caudal region was significantly increased in the TREAD group than the SED group (P < 0.002). In particular, the SWIM group further increased the intensity of p-ERK1/2-labled neurons than the TREAD group (P < 0.001).

Expression of regeneration-related proteins after SCI

In the field of spinal cord regeneration, p-CREB and caspase-3 are specific markers to examine the neuropathic pain and apoptosis, respectively. And p-ERK1/2 and p-Akt are closely associated with axonal sprouting around injury site of the contused spinal cord.

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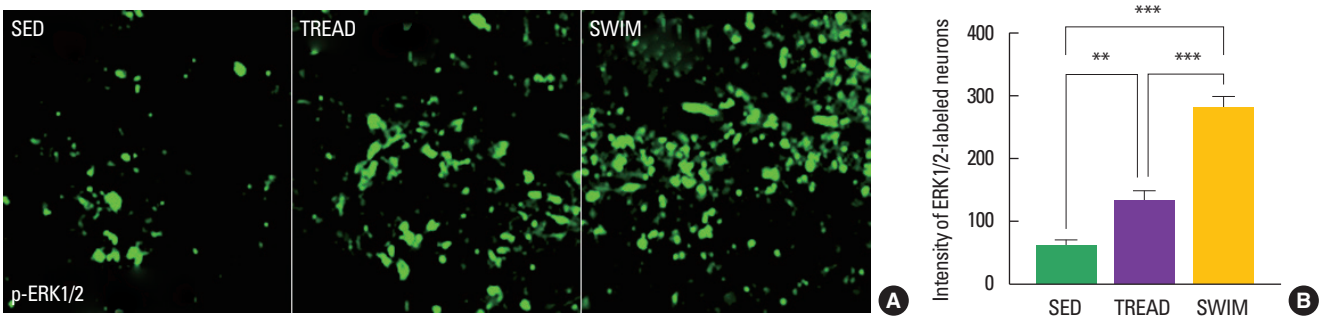

Fig. 3. Phosphorylated extracellular signal-regulated kinases 1 and 2 (p-ERK1/2)-labeled motor neurons in ventral horn of the caudal region after spinal cord injury (SCI). (A) Images of p-ERK1/2-expressed motor neurons at the thoracic 11 level after SCI. (B) Quantitative graph on intensity of p-ERK1/2-labeled neurons. SED, sedentary group; TREAD, treadmill exercise group; SWIM, swimming exercise group. $^{**}P<0.01$. $^{***}P<0.001$.

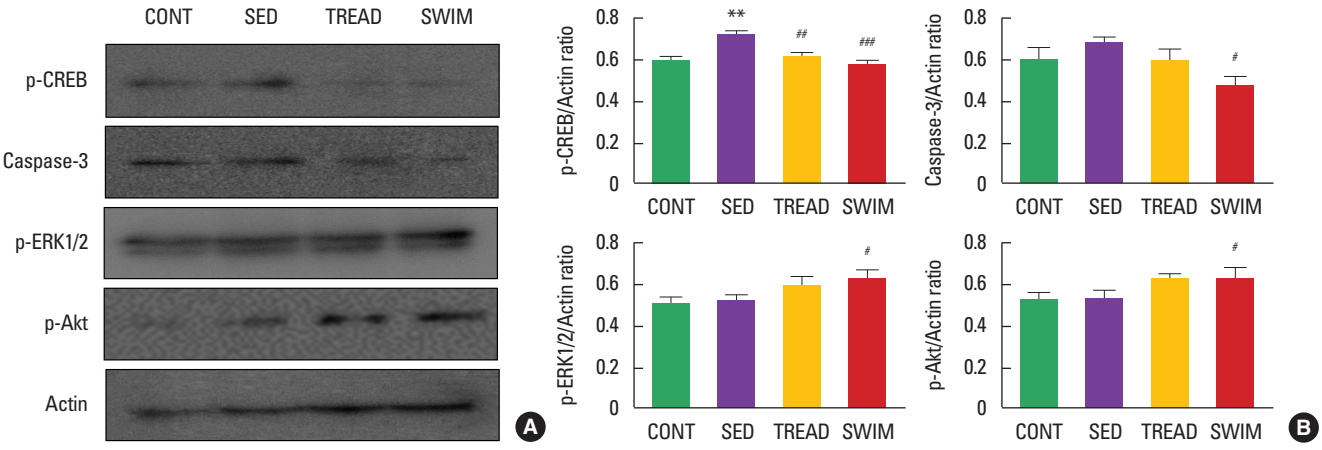

Fig. 4. Expression of regeneration- and apoptosis-related proteins in the contused spinal cord. (A) Representative expression of phosphorylated cAMP response element-binding protein (p-CREB), caspase-3, phosphorylated extracellular signal-regulated kinases 1 and 2 (p-ERK1/2) and phosphorylated protein kinase B (p-Akt). (B) The density ratio of target protein to actin band. CONT, control group; SED, sedentary group; TREAD, treadmill exercise group; SWIM, swimming exercise group. $^{**}P<0.01$ vs. CONT. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. SED.

As shown in Fig. 4, p-CREB was expressed higher in the SED than the CONT group ($P<0.01$), but the TREAD ($P<0.004$) and SWIM ($P<0.001$) groups significantly decreased induction levels of p-CREB in the contused spinal cord compared to the SED group. Caspase-3, an apoptosis indicator, was expressed lower in the SWIM group than the SED group ($P<0.037$). p-ERK1/2 and p-Akt were expressed higher only in the SWIM group than those in the SED group ($P<0.029$, $P<0.044$).

DISCUSSION

In patients with SCI, improving locomotor function is very important for achieving a higher level of physical fitness and a better quality of life (Pedrinelli et al., 2015). BBB locomotor scale has been widely used to confirm the functional recovery in animal study on SCI, which has high sensitivity for hind limb motor skills test (Scheff et al., 2002). Thus, we applied BBB locomotor scale to analyze effect of weight- and non–weight-bearing exercise for 30 min on functional recovery after SCI, and confirmed that the SWIM group significantly improved motor function at 3 and 6 weeks after SCI compared to the SED group. But, the TREAD group did not induce significant changes in motor function. It has been well known that long-term treadmill exercise could cause fatigue and muscle damage through overtraining syndrome, and Shibata et al. (2021) provided key information that the optimal exercise time to maximize the effect of exercise rehabilitation might be 15 to 17.5 min regardless of the severity of paralysis, after which symptoms of exhaustion might appear (Kreher and Schwartz, 2012). In our study, treadmill exercise was gradually increased until a maximum of 30 min, and this is longer than the exercise time claimed by Shibata et al. (2021) and Kreher and Schwartz (2012), which may be a potential reason for not significantly inducing improve-

ment in locomotor function. Another previous study reported the role of swimming in exercise rehabilitation suggested that swimming exercise belongs to non–weight-bearing exercise and might be effective in improvement of neuropathic pain and axonal sprouting in animal model with SCI. These findings support the results of our study in which swimming exercise significantly increased BBB locomotor score. Therefore, we believe that non–weight-bearing exercise is positive for spinal cord regeneration, but weight-bearing exercise required further study regarding intensity and time of exercise during regeneration period.

The CST is the pyramidal tract and axon bundle that carry locomotor-related information from motor cortex in the cerebral to motor neuron in the spinal cord. SCI leads to extensive dieback of CST axons with retraction bulbs present from 1 day after contusion, and the number of sprouting CST axons around injury site of the contused spinal cord has been used as an important indicator to verify improvement in motor function during regeneration stage (Hou et al., 2015). Therefore, we applied DiI tracing technique to examine whether CST axonal sprouting can be occurred according to the exercise types after SCI. As a result, CST axonal sprouting were significantly higher in both SWIM and TREAD groups than the SED group, and the intensity of DiI-labeled CST axons was the highest in the SWIM group. Previous study on spinal cord regeneration suggested that weight-bearing exercise could accelerate CST axonal elongation and sprouting around and in caudal region of the injury site, and 3-week swimming exercise was effective for cell survival and axonal regrowth around and within the cavity of the contused spinal cord (Jung et al., 2016). These previous studies support our findings that exercise itself may be a therapeutic approach for spinal cord regeneration, regardless of the type of exercise. Additionally, although further studies are needed, we believe that non–weight-bearing exercise is more effective in CST axonal sprouting after SCI.

ERK1/2 is known as a representative marker for spinal axon regeneration (Oh et al., 2009; Ono et al., 2014). This study performed immunofluorescence staining to investigate p-ERK1/2-expressed motor neurons in the ventral horn of the caudal region after SCI. As a result, both TREAD and SWIM groups significantly increased the number of p-ERK1/2-labeled motor neurons in the caudal level of the cord than the SED group, and the SWIM group showed the highest expression level of p-ERK1/2 in motor neurons compared to the TREAD group. Oh et al. (2009) reported that inhibition of ERK1/2 decreased axonal sprouting in CST around injury site and treadmill exercise after SCI upregulated CST axonal sprouting through activation of ERK1/2 in motor neurons. In addition, Jang et al. (2018) suggested that swimming exercise had attenuated nerve injury-induce neuropathic pain and mechanical allodynia in the hind paw with increment of p-ERK1/2. These previous studies are consistent with our findings providing information that non-weight-bearing exercise after SCI can further upregulate the expression level of p-ERK1/2 than weight-bearing exercise.

Activation of p-CREB signaling in the injured spinal cord contributes to chronic neuropathic pain in rats (Li et al., 2020), and caspase-3 is an apoptotic molecule induced in the injured spinal cord (Citron et al., 2000). Although phosphorylation of ERK1/2 has been known as a double-edged sword involved in both facilitating and inhibiting central nerve regeneration, the ERK-AKT signaling pathway, which activates AKT downstream molecules, can improve sensory-motor function in animal model with SCI (Fakhri et al., 2019). In this study, spinal axon regeneration- and apoptosis-related protein expression according to exercise types after SCI were confirmed by Western blot analysis. As a result, p-ERK1/2 and p-Akt in the contused spinal cord was dramatically higher in the SWIM group than the SED group. This can be interpreted as a result that non–weight-bearing exercise may be the most effective in improving neuropathic pain and neuronal death after SCI. Previous studies reported that exercise-activated PI3K-Akt-ERK1/2 signaling pathway could respond cell survival and nerve regeneration (Kiss et al., 2022). In addition, Lee et al. (2020) emphasized the importance of exercise to facilitate nerve regeneration by reducing the expression of caspase-3 and CREB in the contused spinal cord.

Considering all these results, our study suggests potential information that non–weight-bearing exercise may be an effective therapeutic method for improving nerve regeneration and functional recovery after SCI.

CONFLICT OF INTEREST

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

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REFERENCES

Cho YH, Seo TB. The role of walking exercise on axonal regrowth and neuropathic pain markers in dorsal root ganglion after sciatic nerve injury. J Exerc Rehabil 2023;19:320-326.

Citron BA, Arnold PM, Sebastian C, Qin F, Malladi S, Ameenuddin S, Landis ME, Festoff BW. Rapid upregulation of caspase-3 in rat spinal cord after injury: mRNA, protein, and cellular localization correlates with apoptotic cell death. Exp Neurol 2000;166:213-226.

Erschbamer MK, Pham TM, Zwart MC, Baumans V, Olson L. Neither environmental enrichment nor voluntary wheel running enhances recovery from incomplete spinal cord injury in rats. Exp Neurol 2006; 201:154-164.

Fakhri S, Dargahi L, Abbaszadeh F, Jorjani M. Effects of astaxanthin on sensory-motor function in a compression model of spinal cord injury: Involvement of ERK and AKT signalling pathway. Eur J Pain 2019;23: 750-764.

Harman KA, DeVeau KM, Squair JW, West CR, Krassioukov AV, Magnuson DSK. Effects of early exercise training on the severity of autonomic dysreflexia following incomplete spinal cord injury in rodents. Physiol Rep 2021;9:e14969.

Hou TT, Yang XY, Xia P, Pan S, Liu J, Qi ZP. Exercise promotes motor functional recovery in rats with corticospinal tract injury: anti-apoptosis mechanism. Neural Regen Res 2015;10:644-650.

Hu X, Xu W, Ren Y, Wang Z, He X, Huang R, Ma B, Zhao J, Zhu R, Cheng L. Spinal cord injury: molecular mechanisms and therapeutic interventions. Signal Transduct Target Ther 2023;8:245.

Jang Y, Lee B, Kim EK, Shim WS, Yang YD, Kim SM. Involuntary swimming exercise in pregnant rats disturbs ERK1/2 signaling in embryonic neurons through increased cortisol in the amniotic fluid. Biochem Biophys Res Commun 2018;495:1208-1213.

Jung SY, Seo TB, Kim DY. Treadmill exercise facilitates recovery of locomotor function through axonal regeneration following spinal cord injury in rats. J Exerc Rehabil 2016;12:284-292.

Keikhaei R, Abdi E, Darvishi M, Ghotbeddin Z, Hamidabadi HG. Combined treatment of high-intensity interval training with neural stem cell generation on contusive model of spinal cord injury in rats. Brain Behav 2023;13:e3043.

Kim YH, Ha KY, Kim SI. Spinal cord injury and related clinical trials. Clin Orthop Surg 2017;9:1-9.

Kiss Bimbova K, Bacova M, Kisucka A, Galik J, Zavacky P, Lukacova N. Activation of three major signaling pathways after endurance training and spinal cord injury. Mol Neurobiol 2022;59:950-967.

Kreher JB, Schwartz JB. Overtraining syndrome: a practical guide. Sports Health 2012;4:128-138.

Kumar S, Behera S, Basu A, Dey S, Ghosh-Roy A. Swimming exercise promotes post-injury axon regeneration and functional restoration through AMPK. eNeuro 2021;8:ENEURO.0414-0420.2021.

Lee SS, Kim CJ, Shin MS, Lim BV. Treadmill exercise ameliorates memory impairment through ERK-Akt-CREB-BDNF signaling pathway in cerebral ischemia gerbils. J Exerc Rehabil 2020;16:49-57.

Li J, Shi H, Liu H, Dong F, Liu Z, Lu Y, Chen L, Bao L, Zhang X. Nerve injury-induced neuronal PAP-I maintains neuropathic pain by activating spinal microglia. J Neurosci 2020;40:297-310.

Oh MJ, Seo TB, Kwon KB, Yoon SJ, Elzi DJ, Kim BG, Namgung U. Axonal outgrowth and Erk1/2 activation by training after spinal cord injury in rats. J Neurotrauma 2009;26:2071-2082.

Ono Y, Tanaka H, Takata M, Nagahara Y, Noda Y, Tsuruma K, Shimazawa M, Hozumi I, Hara H. SA4503, a sigma-1 receptor agonist, suppresses motor neuron damage in in vitro and in vivo amyotrophic lateral sclerosis models. Neurosci Lett 2014;559:174-178.

Pedrinelli A, Garcez-Leme LE, Azul Nobre Rdo S. The effect of physical training on the locomotor apparatus in elderly people. Rev Bras Ortop 2015;44:96-101.

Scheff SW, Saucier DA, Cain ME. A statistical method for analyzing rating scale data: the BBB locomotor score. J Neurotrauma 2002;19:1251-1260.

Seo TB, Chang IA, Lee JH, Namgung U. Beneficial function of cell division cycle 2 activity in astrocytes on axonal regeneration after spinal cord injury. J Neurotrauma 2013;30:1053-1061.

Shibata T, Tashiro S, Shinozaki M, Hashimoto S, Matsumoto M, Nakamura M, Okano H, Nagoshi N. Treadmill training based on the overload principle promotes locomotor recovery in a mouse model of chronic spinal cord injury. Exp Neurol 2021;345:113834.

Wang H, Liu NK, Zhang YP, Deng L, Lu QB, Shields CB, Walker MJ, Li J, Xu XM. Treadmill training induced lumbar motoneuron dendritic plasticity and behavior recovery in adult rats after a thoracic contusive spinal cord injury. Exp Neurol 2015;271:368-378.

Wu Q, Cao Y, Dong C, Wang H, Wang Q, Tong W, Li X, Shan C, Wang T. Neuromuscular interaction is required for neurotrophins-mediated locomotor recovery following treadmill training in rat spinal cord injury. PeerJ 2016;4:e2025.

Zhan Z, Pan L, Zhu Y, Wang Y, Zhao Q, Liu Y, Li S, Wang H, Yang C, Yu L, Yin Y, Tan B. Moderate-intensity treadmill exercise promotes mTOR-dependent motor cortical neurotrophic factor expression and functional recovery in a murine model of crush spinal cord injury (SCI). Mol Neurobiol 2023;60:960-978.