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Histopathological change following exercise time course in spinal cord injured rats with G-CSF

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Objective
Many experimental studies have been performed for motor functional recovery in rats with spinal cord injury (SCI). In previous study, we observed the combined effect of G-CSF treatment and aerobic exercise for motor function recovery in rats with spinal cord injury. The purpose of this study was histopathological change following exercise time course in SCI rats with G-CSF.

Methods
SCI rats treated with G-CSF were divided into 2 groups: a group treated with treadmill exercise plus G-CSF (intervention group, n=12) and a group without exercise (control group, n=6). Laminectomy at the T8–10 spinal levels with compression injury of the spinal cord was performed in all rats. G-CSF (20μg/ml) was administered via intraperitoneally for 5 consecutive days after SCI in intervention groups. From one week after surgery, intervention group received 30 minutes of exercise 5 days per week for 4 weeks. Functional recoveries were assessed using the Basso, Beattie, and Bresnahan (BBB) scale. 5 days (n=4), 3 weeks (n=4), and 5 weeks (n=4) after SCI in intervention group, hematoxylin and eosin staining for cavity size and immunohistochemistry for glial scar formation and neuro-regeneration factor expression were assessed. Statistical analysis was performed with SPSS version 22.0 (SPSS, Chicago, IL). The nonparametric Kruskal-Wallis test was used to identify statistically significance to evaluation values between each group. Post hoc analysis used Mann-Whitney U test. P-values of < 0.05 were considered statistically significant.

Results
BBB scores showed better locomotory ability in intervention group the longer the exercise preod (Fig.1). H&E Results showed the destructive nature of the injuries, and the longer the exercise period, cavity size was reduced continuously in intervention group (Fig 2). Immunohistochemical analysis was also performed at 5 days, 3 weeks, and 5 weeks post-SCI. No expression of VEGF and BDNF at 5 days post-SCI was shown. The expression of GFAP patterns showed that glia cell formation was suppressed continuously with longer exercise period. The expression of BDNF on neurogenesis and VEGF on angiogenesis initiated after 5 days post SCI. While the expression of VEGF was the highest at 3 weeks of SCI and declined thereafter, BDNF were more expressed continuously with longer exercise period ($p<0.05$).

Conclusion

In this study, we found the mechanism of motor function recovery that angiogenesis first occurred and neurogenesis was observed later using immunohistology in SCI rats with exercise and G-CSF. Thus, exercise time course was important for restoring motor function.

Key Words

G-CSF, spinal cord injury, exercise, BDNF, VEGF, GFAP Abbreviation: BDNF: brain derived neurotropic factor, VEGF: vessel endothelial growth factor, GFAP: glia fibrillary acidic protein

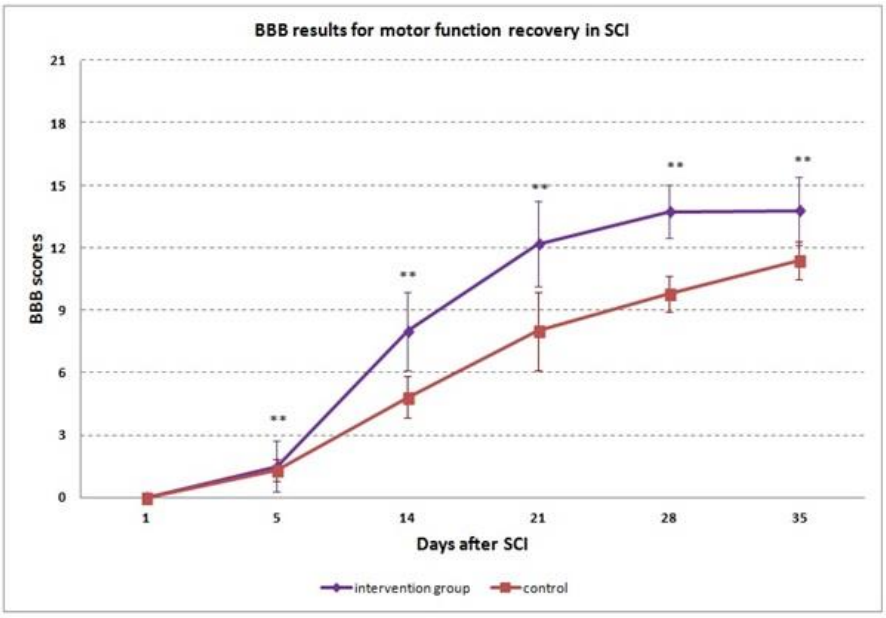

Figure 1 The therapeutic activities of intervention group and control group were examined with the BBB scale analysis. Rats showed more effective motor function recovery in intervention group than that in control group (double asterisks indicate $P<0.01$).

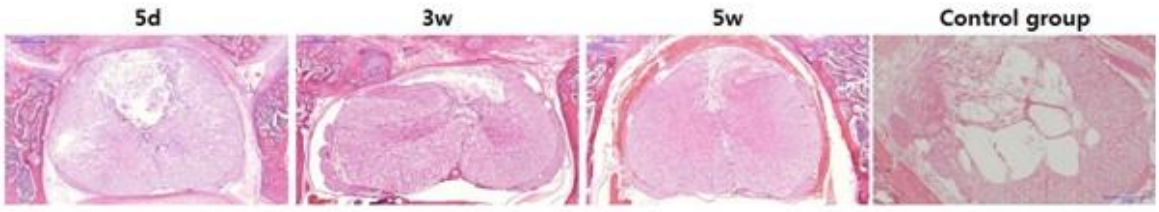

Figure 2 H & E stained spinal cord cross sections (×20) 5 days, 3 weeks, and 5 weeks after injury in intervention group showed that the cavity size continues to decrease over time. Image of control group was after 5 weeks in rats only treated with G-GCF. Scale bar represents 500μm (d: days, w: weeks).

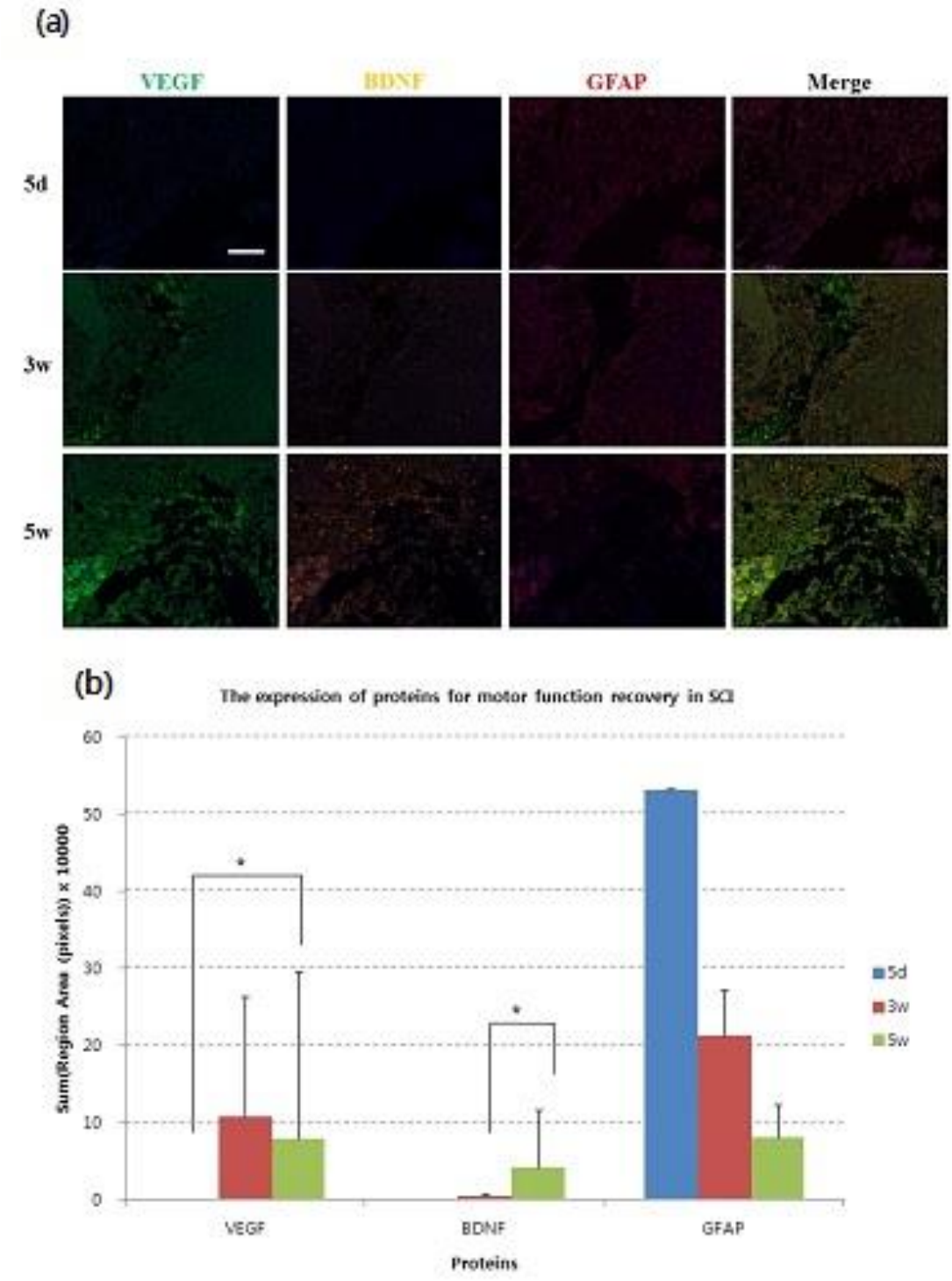

Figure 3 Spinal cord tissues were immunostained for BDNF, VEGF, and GFAP 5days, 3 weeks, and 5 weeks after the injury. (a) BDNF, GFAP, and VEGF immunoreactivities in spinal cord tissues at 5 weeks after injury. Sections were imaged at ×200 using a PerkinElmer Vectra. (b) Areas (pixels) of BDNF, GFAP, and VEGF immunostaining in each group was represented with inForm analysis software (both from PerkinElmer). Scale bar represents 118.7 μm (asterisks indicate $P<0.05$, d: days, w: weeks).