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Original Article

Nerve growth factor expression in stroke induced rats after shock wave

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Abstract. [Purpose] This study investigates effects of extracorporeal shock wave therapy on nerve growth factor expression in stroke in rats. [Subjects and Methods] Sixteen eight-week-old Sprague-Dawley rats were used and randomly divided into two groups: an experimental group, a control group. The experimental group received extracorporeal shock wave therapy after the stroke. The spinal cord encompassing the lumbar 4-lumbar 5 level was then removed for Western blot analysis. [Results] There was a significant difference in nerve growth factor expression between the groups after the impairment. [Conclusion] Application of extracorporeal shock wave therapy increased the expression of nerve growth factor and nerve growth factor postulated to promote nerve growth factor upregulation through formation of a microenvironment at the spinal cord level related to the injured area.

Key words: Stroke, Shock wave, NGF

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INTRODUCTION

Although 80% of patients are capable of walking after a stroke, they experience walking impairment that strays from a normal walking pattern. Due to diminished walking function of the affected side, the stance phase time of the affected side lower limb decreases and the swing phase time of the unaffected side lower limb decreases in patients with a stroke. Moreover, they experience walking asymmetry and decreased gait speed[1)].

Most patients with stroke develop contracture in the ankle joint due to spasticity. They also experience dragging symptoms of the ankle joint in the early swing phase and difficulties in heel striking in the early stance phase. Patients with stroke sometimes exhibit typical abnormal walking patterns, such as excessive hip joint flexion or circumduction walking as a means of compensating for the walking impairment. Patients with stroke that have foot drop symptoms due to spasticity must secure a decreased angle of ankle joint dorsiflexion to prevent foot drop symptoms[2)].

The nerve growth factor (NGF) promotes regeneration after injury to nerves in the central nerve system. Some study reported that combination with the NGF acceptor increases following therapeutic intervention and promotes the expression of the cytoskeletal protein of motor nerves and the neuronal growth-associated proteins that are related to axonal growth of nerve recovery, eventually promoting the maturation, growing, differentiation, and axonal plasticity of nerve cells[3)].

In a study by Moon et al. that examined the effects of ESWT on lower limb spasticity of subacute patients with stroke, instant improvement was observed after ESWT in the modified Ashworth scale[4)]. This study examined the effects of applying extracorporeal shock wave therapy (ESWT) to the affected side gastrocnemius muscle of stroke rats. The NGF expressions were assessed.

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SUBJECTS AND METHODS

In this study, sixteen eight-week-old Sprague-Dawley rats were used and randomly divided into two groups: an experimental group, a control group. The experimental group received extracorporeal shock wave therapy after the stroke. During the experimental period, the rats were bred under the temperature and humidity conditions of 23 ± 2 °C and 50 ± 5% in a breeding room where each cage contained four rats. A 12-hour light cycle from 7am to 7pm and 12-hour dark cycle from 7pm to 7am were applied.

The collagenase-induced model was used to produce ICH inside the left striatum[5]. All surgical procedures and experimental protocols followed Daegu University's guidelines and were approved by the Institution of Animal Care and Use Committee (IACUC).

Histologic evaluation was conducted 30 days after ICH. For NGF among neurotrophic factors, only post-evaluation was conducted 30 days after ICH. For ESWT, initial therapy began the second day after ICH, and ESWT was applied three times a week for five weeks from the next day.

For ESWT, a magnetic-type ESWT device (HAEMIL, Soltar, Korea) was used and it was applied to the gastrocnemius muscle of the affected side hind limb with low intensity using the PAD5 head.

Western blot analysis was conducted for the evaluation of the NGF. To collect the spinal cord from the experimental animal subjected to the completed experimental treatment, Zoletil (Virbac, France) and Rompun (Bayer Korea, Seoul) was mixed at a ratio of 1:1 and injected into the visceral cavity for general anesthesia, at a rate of 2 ml/kg. Myocardiac perfusion was performed using 0.9% NaCl to remove blood, and the spinal cord tissue was extracted.

The results obtained from each experiment were reported as mean ± standard deviation (mean ± SD). The independent t-test was conducted to examine the between-group differences of the effects before and after the intervention. SPSS version 20.0 was used for data analysis, and the statistical significance level was set at 0.05.

RESULTS

Western blot analysis was used for quantitative analysis in the time course evaluation of NGF expression. A significant between-group difference existed in the NGF expression in western blot analysis ($p<0.05$). NGF expression was statistically significantly higher in the experimental group that received ESWT compared to the control group (Table 1).

DISCUSSION

In a study that examined the impact of low-energy ESWT on pain and walking of patients with polyneuropathy, ESWT was applied three times a week for two weeks. According to the study results, pain decreased most prominently after two weeks, and the effects persisted up to eight weeks. As for the index for walking, step length and gait speed increased 14.6% and 24.8%, respectively, compared to before the experiment. The dual support duration also statistically significantly decreased by 12.2%[6].

A number of studies have been conducted that investigated promotion of nerve regeneration of damaged nerves, survival of nerve cells, and maintenance of neurotrophic factors. Increase of neurotrophic factors after nerve injury develops as a natural recovery method for restoration that can promote nerve regenerative response with stimulation that is additionally provided from an exogenous supply or injection of neurotrophic factors[7]. ESWT is one of the exogenous stimulations that promote expression of neurotrophic factors. Because high-energy ESWT can destroy nerve tissues, low-energy shock waves should be applied for the regeneration of nerves[8, 9].

This study examined the effects of applying ESWT to rats with central nerve injury on the expression of the neurotrophic factor, NGF. NGF expression of spinal cord level L4–L5 was investigated 30 days after nerve injury using western blot analysis. In the study results, NGF expression was statistically significantly higher in the experimental group compared to the control group. That is, ESWT promoted expression of NGF that affected the regeneration, survival, and remodeling of nerves. The application of ESWT is postulated to promote NGF upregulation through formation of a microenvironment at the spinal cord level related to the injured area.

This study has limitations in that it is difficult to generalize the results to human subjects, because the experiment was conducted using rats. As the post-evaluation was conducted four weeks after the initial evaluation, the locomotion ability

Table 1. Comparison of NGF expression between groups (Mean ± SD)

	Experimental group (n=8)	Control group (n=8)
NGF (%)	112.7 ± 6.8	100.0 ± 0.0*

*$p<0.05$, NGF: nerve growth factor

changes in the rats that occurred after nerve injury was not observed during smaller segments of time.

The impacts of ESWT on manifestation of neurotrophic factors among patients with stroke should be examined according to various application methods of shock waves.

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