

Ganglioside with nerve growth factor for the recovery of extremity function following spinal cord injury and somatosensory evoked potential

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Abstract. – **OBJECTIVE:** To investigate the effect ganglioside with nerve growth factor on the recovery of extremity functionality following spinal cord injury and somatosensory evoked potential.

PATIENTS AND METHODS: A total of 62 patients with spinal cord injury admitted to our hospital from February 2012 to October 2013 were selected and randomized to treatment (N = 31) and control groups (N = 31). The combination of systematic rehabilitation training and GM-1 intervention were prescribed to patients in the control group, while an additional intervention of mNGF (mouse nerve growth factor) was prescribed to patients in the treatment group. All patients were subject to Functional Independence Measure (FIM), Modified Barthel Index (MBI) and P- and N- wave latency of bilateral lower extremities by SEP method evaluations at 3 months before and after the intervention.

RESULTS: Three months after the intervention, the FIM and MBI scores improved significantly in both groups, with significant recovery in the P- and N-wave latencies. ($p < 0.05$). The improvements in the FIM and MBI evaluations and P-, N-wave latencies of the treatment group were better than those of the control group. The post-treatment inter-group difference was statistically significant ($p < 0.05$).

CONCLUSIONS: The combination of systematic rehabilitation training and GM-1 intervention plus mNGF is more effective in restoring extremity function following spinal cord injury. Somatosensory evoked potential can be an excellent index to evaluate rehabilitation efficacy and accurately reflect changes in neurological function.

Key Words:

Mouse nerve growth factor, Spinal cord injury, Ganglioside, Somatosensory evoked potential, Rehabilitation.

Introduction

Spinal cord injury (SCI) is recognized as a transverse injury induced by various risk factors

(trauma, inflammation, malignancy, etc.), resulting in neurological dysfunction. It is a disease with high disability. Restoring functions post spinal cord injury remains to be a medical challenge. Despite great progress in diagnosing and treating spinal cord injury, it is still difficult to cure, leading to a heavy social and family burden. To resolve and improve the prognosis and outcome of spinal cord injury, continuous multidisciplinary investigations are conducted and are a priority for rehabilitation medicine. Systematic rehabilitation training and GM-1 intervention, in combination, is widely used¹⁻³. To promote restoration of neurological function and improve prognosis of SCI patients, the administration of mouse Nerve Growth Factor (mNGF) was used to provide an intensive neurotrophic support, resulting in favorable results. Somatosensory evoked potential (SEP) are widely used in clinical practice, but its utility is restricted to the diagnosis of neurological disorders and neurosurgery monitoring. Furthermore, the use of somatosensory evoked potential (SEP) were rarely reported as an index of SCI restoration. Therefore, somatosensory evoked potential was introduced in this study to evaluate the restoration profile of SCI patients.

Patients and Methods

Clinical Data

Inclusion criteria: (1) compliance with the SCI diagnostic criteria of the American Spinal Injury Association (ASIA)⁴; (2) post-interventions of spinal decompression and/or fixation, bone graft fusion; (3) spine fracture concomitant with SCI, confirmed by CT and/or MRI; (4) in good condition before injury and no prior history of neck, shoulder, waist and leg pains; and (5) informed consent signed by the patient or family members.

Exclusion Criteria: (1) comorbidities of cardiac, hepatic and renal dysfunction; (2) prior histories of cerebrovascular disease or mental disorders; and (3) non-compliance to study treatment.

A total of 62 patients with spinal cord injury admitted to our department from February 2012 to October 2013 were selected and randomized into treatment and control groups according to the Random Number Table, with 31 patients for each group. The general characteristics were balanced and comparable between these two groups. No statistically significant differences were observed ($p > 0.05$) (Table I).

Intervention

For patients in the control group, the combination of rehabilitation training and GM-1 intervention were prescribed based on the injury level, while an additional intervention of mNGF (mouse nerve growth factor) was prescribed to patients of the treatment group.

1. Comprehensive rehabilitation interventions: Physical therapies: ROM (range of motion) training, residual strength training, turning and sitting, standing, balancing and walking; application of functional electrical stimulation and orthopedic brace. Occupational therapies: training of daily activities are important; self-care activities such as eating, grooming, dressing, moving in bed, a transition from bed to wheelchair, concomitant with acupuncture, physical factor therapy, massage, psychological and symptomatic supportive treatments. The rehabilitation interventions described above were conducted using the one to one pattern, with 45 min in each session, bid (morning and afternoon), six times per week for three months. During treatment, immediate rehabilitation intervention could be adjusted in response to the restored function.
2. GM-1 intervention: The regimen was GM-1 60 mg + NS 100 mL iv gtt qd, with medication for four weeks and off medication for two weeks for one cycle, followed by another two cycles.
3. mNGF intervention: The regimen was mNGF 30 μ g + NS 2 mL im qd, with medication for four weeks and off medication for two weeks for one cycle, followed by another two cycles.

Evaluation

Patients in both groups were evaluated three months before and after the intervention.

Table I. Comparison of the general conditions between two groups.

Group	n	Gender		Average age	Course of disease	ASIA classification of spinal cord injury					Spinal cord injury level				
		Men	Women			A	B	C	D	E	C	T	L	S	
Intervention group	31	24	7	33.21 ± 12.54	19.87 ± 11.23	4	12	10	5	0	5	10	12	4	
Control group	31	23	8	33.36 ± 11.79	20.31 ± 12.35	5	11	9	6	0	6	11	11	3	

1. The ADL (activity of daily living) and independent living capacity of patients were evaluated by the Modified Barthel Index (MBI) and activity of daily living (ADL) under double-blind study.
2. SEP measurements: SEP examinations were conducted using a medical evoked potential instrument (Neuron Oxford, Oxford, UK). Specific examination procedures were described as follows: take a supine position, remain quiet and calm. Next, take the posterior tibial nerve inferior to medial malleolus of bilateral lower extremities and stimulate with a square wave pulse for 0.2 ms and at a frequency of 10 Hz. Next, record the electrode placement, with International EEG Classification System 10-20 as the signal and Cz' as the record point, and place the reference electrode over the FPz point. Keep a close contact between the electrode and skin with the conductive adhesive tape. A computer was used to integrate and record each pulse under a filter of 100-2000 Hz, with 100-500 times of superposition for 100 ms and at least two replicates. Results included average bilateral lower extremities values and the P-wave and N-wave for the tibial nerve of the lower extremities. Neurophysiological experts examined the patients from the treatment and control groups under double-blinded pattern at enrolment and at three months after intervention. During the examination, quiet circumstance and constant room temperature (from 18-25°C) is required and patients were asked to stay awake.

Statistical Analysis

Descriptive data with normal distribution were analyzed by t-test and without normal distribution with rank sum test. The numeration data were analyzed using chi square test. Statistical analyses were implemented by using SPSS11.0 software package (SPSS Inc., Chicago, IL, USA) and $p < 0.05$ was statistically significant.

Results

There were no statistically significant inter-group differences in the results of FIM and MBI evaluations between the treatment and the control group before interventions ($p > 0.05$). After the intervention, the FIM and MBI scores improved significantly in both groups, and P- and N-wave latencies were recovered significantly. These differences were statistically significant in comparison to those before treatment ($p < 0.05$). The extent of improvement in the FIM and MBI evaluations and P-, N-wave latencies of treatment group were significantly better than those of the control group. The post-treatment inter-group difference was statistically significant ($p < 0.05$) (Tables II and III).

Discussion

Current investigations of post-nerve injury restoration and regeneration focused on the following two aspects: (1) Anatomy reconstruction of nerve morphology; (2) Development of neurobiology features. As microsurgical techniques advance, neurosurgery accuracy and postoperative recovery will improve. Research interests change from microsurgical techniques to cellular and molecular modifications during nerve regeneration⁵. The application of pharmaceutical drugs induce nerve restoration and rehabilitation intervention.

There are two pathological mechanisms involved in the process of nerve damage acute spinal cord injury. (1) Primary injury, including mechanical pressure and bleeding, occurring 4 hours after injury; (2) Secondary injury, including edema, inflammation, ischemia, excessive activation of glutamate receptors, calcium overflow, peroxidation and other toxic effects, is a reversible and active regulation mechanism at the cellular and molecular levels. Currently, there is

Table II. Comparison of the MBI and FIM scores before and after treatment between two groups ($\bar{x} \pm s$).

Group	n	FIM score		MBI score	
		Before intervention	After intervention	Before intervention	After intervention
Intervention group	31	35.53 $\pm$ 11.32	84.76 $\pm$ 11.36 ^{b,c}	38.42 $\pm$ 11.28	81.49 $\pm$ 8.17 ^{b,c}
Control group	31	36.17 $\pm$ 11.41	71.93 $\pm$ 11.84 ^a	37.72 $\pm$ 11.63	81.49 $\pm$ 8.17 ^{b,c}

Note: ^a $p < 0.05$, ^b $p < 0.05$, compared with the pre-intervention level; ^c $p < 0.05$, compared with the control group. FIM: Functional Independence Measure; MBI: Modified Barthel Index.

Table III. Comparison of the lower-limb SEP latencies before and after intervention between two groups (ms $\bar{x} \pm s$).

Group	n	N-wave latency		P-wave latency	
		Before intervention	After intervention	Before intervention	After intervention
Intervention group	31	36.4 $\pm$ 11.3	30.1 $\pm$ 8.7 ^{b,c}	41.3 $\pm$ 10.5	33.3 $\pm$ 9.8 ^{b,c}
Control group	31	36.9 $\pm$ 12.1	33.2 $\pm$ 8.6 ^a	41.4 $\pm$ 10.6	37.1 $\pm$ 9.5 ^a

Note: ^a $p < 0.05$, ^b $p < 0.05$, compared with the pre-intervention level; ^c $p < 0.05$, compared with the control group.

no effective treatment for primary spinal cord injury. Surgical intervention restores the residual function of the spinal cord, stabilizes and minimizes injury to the spinal cord. However, due to factors which might induce secondary injury, surgical intervention is not sufficient, which brings an opportunity to medical interventions:

GM-1 is used in clinical practice and is recognized to be a well-established drug like methylprednisolone in SCI medical interventions^{2,3}. GM-1 could penetrate blood-brain barrier (BBB) with the highest concentration of GM-1 observed in nerve injury areas. It has a high binding affinity to neural tissue of the affected areas. GM-1 administration could improve animal nerve functions. Its possible mechanisms⁴ include: antagonism of excitatory amino acid toxicity; activity reservation of Na⁺-K⁺-ATPase and Ca²⁺-ATPase; correction of electrolyte imbalances and prevention of cellular edema; prevention of intracellular calcium accumulation; reduction of lipid peroxidation and free radical damage; prevention of lactic acidosis; direct restoration of damaged nerve cell membranes; promotion of multiple nerve growth factors; regulation of the expression profiles of various inflammatory factors and cytokines; blockage of nerve cell apoptosis.

Re-exposure of pre-embedded receptors of neurotrophic factors (NTFs) has been demonstrated after CNS injury, which suggests the support of neurotrophic factors is required for the regeneration of damaged neurons^{6,7}. Intervention of neurotrophic factor is also one of the mechanism to boost rehabilitation mediated spinal cord plasticity. After CNS injury, the restricted expression profile of NTF in damaged tissues plays an active role in prophylaxis of secondary injury induced by microenvironment changes. However, the effects of restoration and nerve function recovery were limited due to insufficient NTF presentation. Therefore, the use of exogenous NTFs is considered to promote restoration of damaged spinal cord. As one of the main members of the

neurotrophin family, nerve growth factor (NGF) is the first identified neurotrophic factor⁵. It is not one of the most important bioactive proteins in the nervous system of human beings, but also required for the differentiation, development and maintenance of the central and peripheral nervous system. It has dual functions similar to those of neurotrophic factors and nerve growth factors and promotes post-nervous system damage restoration. In case of physical, chemical, and other damages to the nervous system, exogenous nerve growth factor could protect sensory neurons and sympathetic neurons from further damages to boost growth of nerve fiber regeneration and restoration of neurological function. Moreover, NGF is also associated with induction of oriented growth of neural cells, promotion of axonal regeneration and remyelination. NGF has an effect on sympathetic, sensory and motor nerves, as indicated by numerous studies.

As reported in earlier studies, NGF induced neuronal differentiation might be correlated to the effect of ganglioside GM-1^{8,9}. GM-1 could amplify the neurotrophic effect of NTFs and reduce damaged cell death^{10,11}. GM1 is associated with boosting of the NGF-mediated motor neuron regeneration, acceleration of axon myelination, restoration of motor conduction function and has favorable biological effects. In the early stages of injury, the administration of exogenous NGF and GM1 in combination was associated with acceleration of nerve recovery, promotion of neuronal function recovery, reduction of cell death in earlier stages, shortening restoration duration of damaged nerves, resulting in significant improvement of quality of life.

In this study, mNGF yielded from the mouse submandibular gland was identified to be a biological protein with a molecular weight of 26.5 KD. As an exogenous nerve growth factor, mNGF for injection, was combined with GM-1 to produce a synergic effect other than a simple additive effect of both medications. Therefore,

the outcome of the treatment group was significant in comparison to that of the control group. Moreover, the treatment outcome of the GM-1 combined with different doses and mNGF has not been investigated in this study and warrants further clinical investigations.

The neurological evaluation post-spinal cord injury has relied on evidence-based medicine for a long time. This standard is based on the ASIA scale, Damage Classification System, FIM and MBI scales, which is affected by patient compliance, subjective sensation, physician's judgment and other factors. Moreover, radiological examinations (such as X-ray, CT and MRI, etc.) are helpful when determining the site, segment, scope and damage extent of the spinal cord injury, but changes in spinal anatomy and radiological findings could not reflect the functional outcome. In recent years, electrophysiologists and clinicians described SEP changes in-parallel to pathological and functional changes, which provides a better reflection to spinal cord function. Therefore, SEP is used as an index to evaluate the efficacy of the interventions. SEP is a noninvasive electrophysiological technique which reflects the conduction functions of somatosensory pathways and upstream thick myelinated nerve fibers at the different nerve levels. This examination covers the full-somatosensory pathway of the nervous system, which reflects the integrity and functionality of this pathway. Moreover, the neurological damage profile could be used as a diagnostic tool to identify the functions of the neural pathways, especially CNS. In this study, significant improvement of P- and N-wave latencies were observed for both groups ($p < 0.05$), while P- and N-wave latencies improvements of the tibial nerve were superior to those of the controls ($p < 0.05$). The Nerve Growth Factor (NGF) is effective, and SEP was proposed as an excellent index to evaluate rehabilitation efficacy, consistent with the results of FIM and MBI evaluations. Since SEP is independent of aphasia, unconsciousness, sleep or central nervous system depressants and could electrophysiologically reflect the functionality of the nervous system. This tool could be used to confirm spinal cord injury, damage extent, prognosis prediction, choice of intervention regimen, and assessment of efficacy, and is of clinical application value.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

- 1) HALL ED, SPRINGER JE. Neuroprotection and acute spinal cord injury: a reappraisal. *NeuroRx* 2004; 1: 80-100.
- 2) GEISLER FH, COLEMAN WP, GRIECO G, POONIAN D; SYGEN STUDY GROUP. The Sygen multicenter acute spinal cord injury study. *Spine (Phila Pa 1976)* 2001; 26(24 Suppl): S87-98.
- 3) GEISLER FH, COLEMAN WP, GRIECO G, POONIAN D; SYGEN STUDY GROUP. Measurements and recovery patterns in a multicenter study of acute spinal cord injury. *Spine (Phila Pa 1976)* 2001; 26(24 Suppl): S68-86.
- 4) KIRSHBLUM SC, BURNS SP, BIERING-SORENSEN F, DONOVAN W, GRAVES DE, JHA A, JOHANSEN M, JONES L, KRASSIOUKOV A, MULCAHEY MJ, SCHMIDT-READ M, WARRING W. International standards for neurological classification of spinal cord injury (revised 2011). *J Spinal Cord Med* 2011; 34: 535-546.
- 5) STÖCKEL K, SCHWAB M, THOENEN H. Comparison between the retrograde axonal transport of nerve growth factor and tetanus toxin in motor, sensory and adrenergic neurons. *Brain Res* 1975; 99: 1-16.
- 6) BROWN A, RICCI MJ, WEAVER LC. NGF message and protein distribution in the injured rat spinal cord. *Exp Neurol* 2004; 188: 115-127.
- 7) QIAO L, VIZZARD MA. Up-regulation of tyrosine kinase (Trka, Trkb) receptor expression and phosphorylation in lumbosacral dorsal root ganglia after chronic spinal cord (T8-T10) injury. *J Comp Neurol* 2002; 449: 217-230.
- 8) SCHWARTZ M, SPINNAN N. Sprouting from chicken embryo dorsal root ganglia induced by nerve growth factor is specifically inhibited by affinity-purified anti ganglioside antibodies. *Proc Natl Acad Sci USA* 1982; 79: 6080-6083.
- 9) CUELLO AC, GAROFALO L, KENIGSBERG RL, MAYSINGER D. Gangliosides potentiate *in vivo* and *in vitro* effects of nerve growth factor on central cholinergic neurons. *Proc Natl Acad Sci USA* 1989; 86: 2056-2062.
- 10) DUCHEMIN AM, NEFF NH, HADJICONSTANTINO M. GM 1 increase the content and mRNA NGF in the brain aged rats. *Neuro Report* 1997; 8: 3823-3832.
- 11) BAUMGARTNER WA, REDMOND JM, ZEHR KJ, BROCK MV, TSENG EE, BLUE ME, TRONCOSO JC, JOHNSTON MV. The role of the monosialoganglioside, GM1 as a neuroprotectant in an experimental model of cardiopulmonary bypass and hypothermic circulatory arrest. *Ann N Y Acad Sci* 1998; 845: 382-398.