Thesis of Doctor Degree

Study in the Chiropractic Manipulation Facilitating Regeneration of the Sciatic Nerve in Rats

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Abstract

The purpose of this study was to elucidate the effects of impulsive spinal manipulation on the regeneration of the sciatic nerve crush injury in the rats. Thirty-five adult female Sprague-Dawley rats were randomly assigned into 5 groups (n=7): a sham operation group, a sciatic crushed nerve injury group, a sciatic crushed nerve injury and single impulse thrust treated group, a sciatic crushed nerve injury and five impulse thrusts treated group, and a sciatic crushed nerve injury and ten impulse thrusts treated group. Sciatic nerve crush injury was induced using a validated method, and then after one day, we applied the impulsive spinal manipulation at the level of L6-S1 using an Impulse Adjusting Instrument®, once a day for 7 consecutive days. To measure the functional recovery of the sciatic nerve, sciatic function index (SFI), western blot for the myelin basic protein (MBP) and growth associated protein 43 (GAP-43), and immunohistochemistry for neurofilaments were analyzed. Sciatic nerve crush injury significantly decreased the SFI and the expression of MBP in the crush injured nerve ($p<0.05$). Moreover, some fiber misalignment and decreased neurofilament were found in sciatic nerve crush injury-induced groups. On the other hand, impulsive spinal manipulation significantly improved the SFI and increased the expression of MBP ($p<0.05$). Moreover, the five impulsive spinal manipulation significantly strengthen the expression of GAP-43 ($p<0.05$) and the expression of neurofilaments in the sciatic nerve as compared with SNI. This study showed that the impulsive spinal manipulation facilitated the functional recovery after sciatic nerve injury by increasing the expression of neurofilaments and enhancing the expressions of proteins related to Schwann-related remyelination and axonal growth.

Key Words: Sciatic Nerve Crush Injury, Sciatic Function Index, Myelin Basic Protein,

Growth Associated Protein 43, Neurofilaments

I. Introduction

In daily life, peripheral nerve injury can easily occur due to accidental trauma, acute compression, or deliberate surgery (Wang et al., 2012). Such peripheral nerve injury results in partial or total loss of motor, sensory and autonomic functions. Substantially, injury to the peripheral nervous system can cause functional loss and decrease the quality of life due to permanently impaired sensory/motor functions and secondary problems, such as neuropathic pain (Jaquet et al., 2001; Rosberg et al., 2005). Among various types of peripheral nerve injuries, crush injury is induced by nerve compression, and such nerve crush injury in rats is especially used to study regeneration after nerve damage as an experimental model in which injury has the potential to recover completely (Roglio et al., 2008).

Nerve injury initiates many responses in cells around the injury site and has an effect on the progression of axonal degeneration and regeneration during the early period of 3 to 7 days after injury (Torigoe et al., 1996; Omura et al., 2004). Especially, the regulation of cellular signaling execution and gene expression in controlling degenerative and regenerative potential is one of the important events because their alterations after injury are critical to the mechanism of nerve regeneration (Pan et al., 2010). Generally, the peripheral nerve system has a considerable capacity for regeneration. However, the regeneration process requires the sequential expression of specific growth-associated and function-associated genes in both sheath cells and neurons (Chen et al., 2007a).

Growth associated protein 43 (GAP-43) is associated with the development and plasticity of the nervous system. During the nerve development or injury process, the expression of GAP-43 varies from very low levels to high levels (Wang et al., 2012). Substantially, many studies have reported that peripheral nerve injuries including sciatic nerve injury cause the change of GAP-43 expression (Seo et al., 2006; Pan et al. 2010;

Wang et al., 2012). The expression of myelin basic protein (MBP) also is affected by sciatic nerve injury (Yin et al., 2001; Pan et al. 2010). MBP, the second most abundant protein in the peripheral nervous system, is naturally expressed in oligodendrocytes and Schwann cells (Stahl et al., 1990; Pedraza et al., 1997). Especially, MBP is known to subserve a regulatory function in implementing the myelination program (Pedraza et al., 1997). Pan et al. (2010) suggested that augmentation of GAP-43 and MBP might produce a microenvironment favorable to nerve regeneration, including promotion of axonal outgrowth and remyelination.

Recovery from nerve crush injury is still a challenging medical problem and mainly depends on post-injury treatment. In general, surgical treatment is not used for nerve crush injury. Therefore, many alternative therapeutic approaches have been developed and proposed to have a beneficial effect on peripheral nerve regeneration (Pan et al., 2010; Wang et al., 2012).

Spinal manipulation is a commonly used nonoperative treatment modality and a mechanical input to tissues of the vertebral column (Pickar, 2002; Dishman & Burke, 2003). The practitioner delivers a dynamic thrust impulse to a specific vertebra during spinal manipulation. The practitioner controls the velocity, magnitude, and direction of the impulse (Bergmann, 1992; Pickar, 2002). Therefore, the skill of spinal manipulation lies in the practitioner's ability to control these three factors once the specific contact with a vertebra is made (Pickar, 2002). Many studies have reported the beneficial effects of spinal manipulation. Especially, spinal manipulation therapy has been proved effective in alleviating acute low back pain and thus it has been suggested to improve neck pain, sciatica, and chronic low back pain (Koes et al., 1996; Hurwitz et al., 1996). In addition, according to Pickar's review (2002), spinal manipulation has the effects on sensory receptors in paraspinal tissues, central sensitization, and muscle reflexes. Recently, the instrumented-adjusting using the Activator Adjusting Instrument is commonly used by chiropractors, and assessing clinical effectiveness of Activator

Adjusting Instrument and manual manipulation reveals that both of treatment interventions result in equally statistically significant patient outcomes (Huggins et al., 2012).

The neurophysiological evidences have gradually demonstrated the physiological effects produced by spinal manipulation (Pickar, 2002). However, there are few studies that present the molecular-biological evidences on neurophysiological effects of spinal manipulation. Therefore, we investigated the effects of impulsive spinal manipulation on sciatic nerve regeneration of rats using a mechanical adjusting device, Impulse Adjusting Instrument®. In this study, we performed behavioral test, immunohistochemistry, and western blot to ascertain the effects of impulsive spinal manipulation on sciatic nerve regeneration.

II. Materials and Methods

1. Animals

Female Sprague-Dawley rat weighing 280±10g (12 weeks of age) were used. The experimental procedures were performed in accordance with the animal care guideline of National Institutes of Health (NIH) and the Korea Academy of Medical Sciences. The animal were house at controlled temperature (23±2°C) and maintained under light-dark cycles, each consisting of 12h of light and 12h of darkness (lights on from 07:00 to 19:00h), with food and water made available ad libitum. The rats were randomly divided into five groups (n=7 in each group): a sham operation group (Sham), a sciatic crushed nerve injury group (SNI), a sciatic crushed nerve injury and single impulse thrust treated group (SNI+Impulse 1), a sciatic crushed nerve injury and five impulse thrusts treated group (SNI+Impulse 5), and a sciatic crushed nerve injury and ten impulse thrusts treated group (SNI+Impulse 10).

2. Surgical Procedure

To induce crush injury on the sciatic nerve in rats, the previously described surgical procedure was performed (Byun et al., 2005) (Fig. 1). In brief, the right sciatic nerve was exposed by incision on the gluteal muscle under anesthesia using Zoletil 50® (50 mg/kg; Virbac Laboratories, Carros, France). The sciatic nerve was carefully exposed and crushed for 30sec using a surgical clip (pressure: 125g; Fine Science Tool Inc., San Francisco, CA, USA). The crushed location was between the sciatic notch and the point of trifurcation. At the end, the surgical wound was sutured and recovered. In the sham operation rats, the sciatic nerve exposed, however the nerve was not crushed.

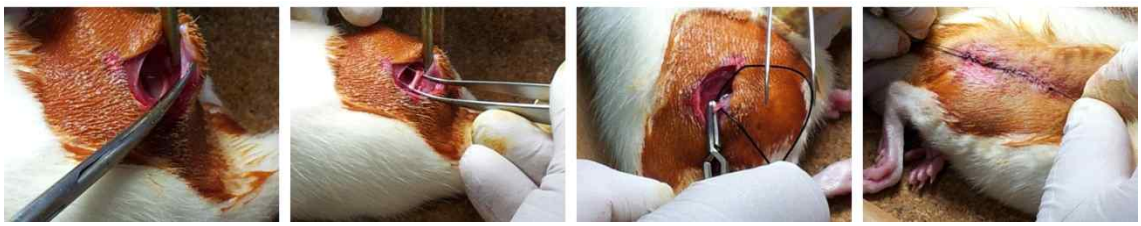

Fig. 1. Induction of Sciatic Nerve Crush Injury.

3. Chiropractic Manipulation Using the Adjusting Instrument

In this study, chiropractic adjusting instrument (Impulse Adjusting Instrument®, Neuromechanical Innovations, LLC, Phoenix, AZ, USA) (Fig. 2a) was used to model manipulation. Manipulation was applied to the between the level of L6-S1 at an angle of approximately 90°, with the animal held in prone position by an assistant (Fig. 2b). The spinal manipulation interventions were conducted once a day for 7 consecutive days consisting of a single impulse thrust, 5 impulse thrusts (6Hz), and 10 impulse thrusts (6Hz) in the respective groups. Each impulse thrust of chiropractic adjusting instrument includes the force 2N for 2msec. The force 2N corresponds to the about 70 percentages of animal body weight. Sham received no treatment.

Fig. 2. Instrument-Assisted Spinal Manipulation.; (a) Impulse Adjusting Instrument® (Neuromechanical Innovations, LLC, Phoenix, AZ, USA) (b) Illustration of the adjustments applied to the level of L6-S1.

4. Walking Tract Analysis

Functional recovery rate after nerve injury was analyzed using a walking tract assessment, which can be quantified with sciatic function index (SFI). Examination of the walking patterns was performed 3 times 1 day with intervals through the course of the experiment as the previously described method (Byun et al., 2005). Footprint was recorded in a wooden walking alley (8.2×42 cm) with a darkened goal box at the end. The floor of the alley was covered with white paper. The anatomical landmarks on the hind feet of the rats were smeared with finger point. The rat was allowed to walk down the tract, leaving its footprints on the paper.

From the footprints the following parameter were calculated: distance from the heel to the top of the 3rd toe (print length, PL), distance between the 1nd to the 5th toe (toe spread, TS), and distance from the 2nd to the 4th toe (intermediary toe spread, IT). These parameters were taken both from the intact left (non-operated) foot (NPL, NTS, and NIT) and from the injured right (experimental) foot (EPL, ETS, and EIT). SFI values were obtained using following equation: SFI=-38.3 · PLF+109.5 · TSF+13.3 · ITF-8.8. Print length factor (PLF)=(EPL-NPL)/NPL; toe spread factor (TSF)=(ETS-NTS)/NTS; intermediary toe spread factor (ITF)=(EIT-NIT)/NIT. Interpolating identical values of PL, TS, and IT from the right and left hind feet are close to zero in normal rats. A value of -100 indicates complete impairment.

5. Tissue Preparation

The animals were sacrificed at the 8th day after behavioral tests. The animals were anesthetized using Zoletil 50® (10mg/kg, i.p.; Virbac Laboratories), transcardially perfused with 50mM phosphate-buffered saline (PBS), and fixed with a freshly prepared solution consisting of 4% paraformaldehyde in 100Mm phosphate buffer (PB, pH 7.4).

For immunohistochemistry, the sciatic nerves were dissected and postfixed in the same fixative overnight and transferred into a 20% sucrose solution for cryoprotection. Coronal section of 20μm thickness was made with a freezing microtome (Leica, Nussloch, Germany). For western blot, the sciatic nerves were dissected and then were immediately frozen at frozen at -70°C.

6. Immunohistochemistry for Neurofilment-200

To detect expression of neurofilaments in the sciatic nerve, immunohistochemistry was performed. Briefly, the sections onto slides were incubated overnight with mouse monoclonal anti-neurofilament-200 antibody (Sigma-Aldrich, St. Louis, MO, USA). The sections were then incubated for 1h with anti-mouse secondary antibody (Vector Laboratories Inc., Burlingame, CA, USA). The sections were subsequently incubated with an avidin–biotin–peroxidase complex (Vector Laboratories) for 1h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution consisting of 0.02% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich) and 0.03% H_2O_2 in 50 mM Tris–HCl (pH7.6). The slides were then washed three times with PBS and air-dried overnight at room temperature. Finally, the coverslips were mounted by using Permount®.

7. Western Blot Analysis for MBP and GAP-43

The sciatic nerves were homogenized on ice, and lysed in a lysis buffer containing 50mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1mM PMSF, 1mM EGTA, 1.5mM MgCl2・6H2O, 1mM sodium orthovanadate, and 100mM sodium flouride. Protein content was measured using a Bio-Rad colorimetric protein assay kit (Hercules, CA, USA). Protein (20μg) was separated on SDS-polyacrylamide gels and

transferred onto a nitrocellulose membrane. Mouse beta-actin antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse growth associated protein 43 antibody (GAP-43) (1:1000; Santa Cruz Biotechnology), and goat myelin basic protein (MBP) (1:1000; Santa Cruz Biotechnology) were used as the primary antibodies. Horseradish peroxidase-conjugated anti-mouse antibody for beta-actin and GAP-43 (1:3000; Vector Laboratories), and horseradish peroxidase-conjugated anti-goat antibody (1:5000; Santa Cruz Biotech) for MBP were used as the secondary antibodies.

Experiments were performed in normal laboratory conditions and at room temperature, except for the transferred membranes. Transferred membranes were performed at 4ºC with the cold pack and pre-chilled buffer. Band detection was performed using the enhanced chemiluminescence (ECL) detection kit (Santa Cruz Biotechnology). To compare the relative expression of proteins, the detected bands were calculated densitometrically using Molecular AnalystTM, version 1.4.1 (Bio-Rad).

8. Statistical Analysis

The results were expressed as the mean±standard error of the mean (S.E.M.). The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test. Differences among groups were considered statistically significant at $p<0.05$.

III. Results

1. Effect of Impulsive Spinal Manipulation on Functional Recovery of Crushed Sciatic Injury

As shown in figure 3, the SFI value in Sham was close to zero, indicating that intact animals had completely normal function of sciatic nerve. Before impulsive spinal manipulation, there was no significant difference among the sciatic crush injury-induced 4 groups; SNI, SNI+Impulse 1, SNI+Impulse 5, and SNI+Impulse 10. From 4 to 7 days Brain Res.

After the induction of sciatic crush injury, the SFI value in SNI spontaneously increased. However, the impulsive spinal manipulation significantly further increased the SFI values in all manipulation treated groups ($p<0.05$). Especially, the five impulsive manipulation was the most effective in increasing the SFI value. This result implies that the impulsive spinal manipulation may be beneficial for the functional recovery from crushed sciatic injury.

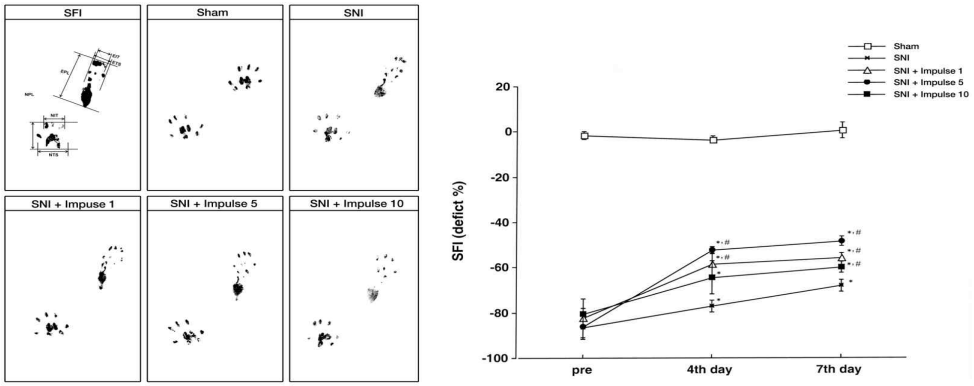

Fig. 3. Effect of Impulsive Spinal Manipulation on Sciatic Functional Index (SFI). Above: Walking track footprints sciatic nerve injury in rat. Below: The plot showing the time-dependent change in the SFI value of rats in each group. All data are represented as mean±standard error of the mean (S.E.M.). *represents $p<0.05$ compared to Sham. #represents $p<0.05$ compared to SNI. (□) Sham: a sham operation group, (✚) SNI: a sciatic crushed nerve injury group, (△) SNI+Impulse 1: a sciatic crushed nerve injury and single impulse thrust treated group, (●) SNI+Impulse 5: a sciatic crushed nerve injury and five impulse thrusts treated group, (■) SNI+Impulse 10: a sciatic crushed nerve injury and ten impulse thrusts treated group.

2. Effect of Impulsive Spinal Manipulation on the Expression of Neurofilament-200 in Crushed Sciatic Injury

Neurofilament-200 immunohistochemistry was performed on crushed sciatic nerve and illustrated in figure 4. After inducing sciatic nerve crush injury, some fiber misalignment and decreased neurofilaments were found in the sciatic crush injury-induced groups. However, the five impulsive manipulation conspicuously increased the expression of neurofilaments.

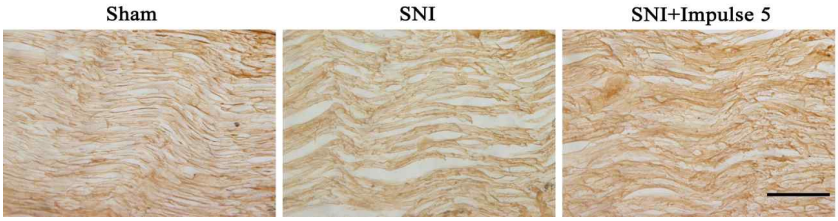

Fig. 4. Effect of Impulsive Spinal Manipulation on the Expression of Neurofilament-200 in Crushed Sciatic Injury. he scale bar represents 100μm. Sham: a sham operation group, SNI: a sciatic crushed nerve injury group, SNI+Impulse 5: a sciatic crushed nerve injury and five impulse thrusts treated group.

3. Effect of Impulsive Spinal Manipulation on the Expression of MBP in Crushed Sciatic Injury

To ascertain the effect of impulsive spinal manipulation on Schwann-related remyelination, protein lysates prepared from proximal portions of the crushed sciatic nerve were analyzed by western blot. Crush nerve injury remarkably decreased the expression of MBP in proximal region of injury site. On the other hand, the expressions of MBP in the impulsive spinal manipulation-treated groups were significantly increased (p<0.05), and the five impulsive manipulation was the most effective in enhancing the MBP expression (Fig. 5). From this result, it can be inferred that the impulsive spinal manipulation may have the beneficial effect on Schwann-related remyelination.

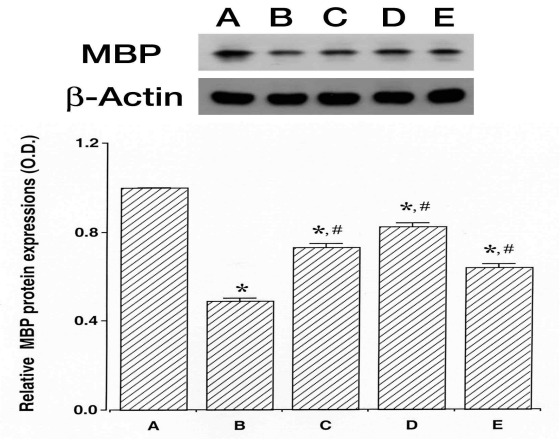

Fig. 5. Effect of Impulsive Spinal Manipulation on the Expression of MBP in Crushed Sciatic Injury. β-Actin was used as an internal control (43kDa). (A) Sham: a sham operation group, (B) SNI: a sciatic crushed nerve injury group, (C) SNI+Impulse 1: a sciatic crushed nerve injury and single impulse thrust treated group, (D) SNI+Impulse 5: a sciatic crushed nerve injury and five impulse thrusts treated group, (E) SNI+Impulse 10: a sciatic crushed nerve injury and ten impulse thrusts treated group. Upper: The results of band detection using the enhanced chemiluminescence (ECL) detection kit. Lower: The relative expression of MBP protein. All data are represented as mean ± standard error of the mean (S.E.M.). *represents $p<0.05$ compared to Sham. #represents $p<0.05$ compared to SNI.

4. Effect of Impulsive Spinal Manipulation on the Expression of GAP-43 in Crushed Sciatic Injury

In order to examine the effect of impulsive spinal manipulation on facilitated axonal regeneration, the expression of GAP-43 in proximal region of the crushed sciatic nerve was analyzed. In Sham, GAP-43 was slightly expressed. Namely, GAP-43 was somewhat expressed in the intact sciatic nerve, but crush nerve injury significantly increased the expression of GAP-43. In SNI+Impulse 5 and SNI+Impulse 10, GAP-43 expressions were slightly further elevated, and the five impulsive spinal manipulation significantly further enhanced the expression of GAP-43 ($p<0.05$) (Fig. 6). This result means that the impulsive spinal manipulation can contribute to facilitating axonal

regeneration of injured nerve.

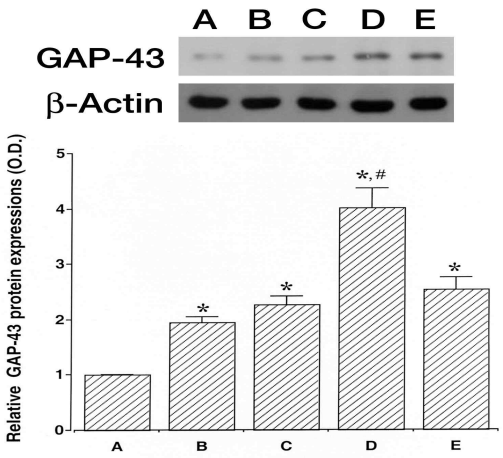

Fig. 6. Effect of Impulsive Spinal Manipulation on the Expression of GAP-43 in Crushed Sciatic Injury. β-Actin was used as an internal control (43kDa). (A) Sham: a sham operation group, (B) SNI: a sciatic crushed nerve injury group, (C) SNI+Impulse 1: a sciatic crushed nerve injury and single impulse thrust treated group, (D) SNI+Impulse 5: a sciatic crushed nerve injury and five impulse thrusts treated group, (E) SNI+Impulse 10: a sciatic crushed nerve injury and ten impulse thrusts treated group. Upper: The results of band detection using the enhanced chemiluminescence (ECL) detection kit. Lower: The relative expression of GAP-43 protein. All data are represented as mean±standard error of the mean (S.E.M.). *represents $p<0.05$ compared to Sham. #represents $p<0.05$ compared to SNI.

IV. Discussion

Peripheral nerve injuries vary widely in extent and severity (Wood et al., 2011). Among various types of peripheral nerve injury, crush injury is typically induced by applying a crush across the nerve with forceps for 30s (Bridge et al., 1994). Such nerve injury triggers many responses in cells around the injury site, resulting in functional loss of sciatic nerve (Torigoe et al., 1996; Omura et al., 2004). Many studies have reported that crush nerve injury induces changes in neuronal protein synthesis necessary to sustain subsequent peripheral regeneration, and functional deficits (Yin et al., 2001; Seo et al., 2006; Pan et al. 2010; Wang et al., 2012). In the present study, crush nerve injury induced the significant functional deficit, compared with Sham (Fig. 3). Walking track analysis is commonly used to measure global functional recovery following sciatic nerve injury, and is based on typical run patterns which are consistent and relatively independent of the speed, size, and age of untreated rats (Hruska et al., 1979). Therefore, walking track analysis can provide information about both functional deficits and improvement in nerve recovery (Rustemeyer & Dicke, 2009). Pan et al. (2010) reported that the SFI value measured in walking track analysis is nearly completely recovered 4 weeks after crush injury. Likewise, in this study, the SFI value in SNI spontaneously increased as time went on. However, the impulsive spinal manipulation significantly facilitated the functional recovery from crushed sciatic injury (Fig. 3).

In the present study, crush nerve injury triggered misalignment of neurofilaments and decreased the neurofilament expression, as well as functional loss of sciatic nerve (Fig. 4). Reduced expression of neurofilaments by nerve injury is also found in other many studies (McLean et al., 2002; Pan et al., 2009). Neurofilaments, consisting of three subunits-NFL, NFM, and NFH-, are intermediate filaments of neurons and one of the major components of the neuronal cytoskeleton, responsible maintaining the caliber of

axons. Therefore, the correct formation of an axonal network of neurofilaments is important for the conduction velocity of impulses because the speed of conductivity of an impulse down the axon is partially proportional to axon's calibre (Al-Chalabi & Miller, 2003). During development, neurofilament subunit composition controls axon outgrowth in that these subunits assemble into a 10nm filamentous structure running the length of the axon. Moreover, neurofilaments might serve as scaffolding to anchor a various intracellular organelles and cytoplasmic proteins (Goldman et al., 2008; Perrot et al., 2008). Many studies have reported that neurodegenerative disorders have significant neurofilament pathologies, emphasizing the importance of neurofilaments for maintaining a healthy, functioning nervous system (Szaro & Strong, 2010). Szaro and Strong (2010) also suggested that neurofilament protein expression is closely connected to the successive phases of axonal outgrowth and maturation that occur both in development and in the successful regeneration of damaged axons. In the present study, the difference of neurofilament expression between SNI and SNI+impulse 5 was evident. Namely, five impulsive spinal manipulation conspicuously increased the expression of neurofilaments (Fig. 4). From this result, it can be inferred that the impulsive spinal manipulation may have the beneficial effect on the regeneration of damaged axons.

Crush injury produces a Sunderland type II injury, in which the myelin sheath and the axons are disrupted (Moradzadeh et al., 2010). The myelin sheath, an important structure surrounding the axon of a neuron, is synthesized by glial cells, especially Schwann cells in the peripheral nerve system. Schwann cells play a critical role in successful regeneration after nerve injury because functional regeneration requires axon regrowth and remyelination of the regenerated axons by Schwann cells (Chen et al., 2007b). Moreover, intact axons with abnormal myelination are functionally impaired and are the underlying pathology of many peripheral neuropathies (Chen & Strickland, 2003; Meyer et al., 2006). As a major myelin component, MBP is considered to be specific to Schwann cells and to be involved in intracellular membrane adhesion and regulation

of oligodendrocyte maturation (Brophy et al., 1993; Pedraza et al., 1997). In addition, MBP is important for maintaining integrity and compactness of peripheral nerve in development and after nerve injury (LeBlanc & Poduslo, 1990; Martini & Schachner, 1997). Therefore, many studies have investigated MBP as the Schwann-related remyelination-associated factor. Substantially, peripheral nerve injuries including sciatic nerve injury significantly reduce the expression of MBP, and the elevated MBP expression is considered beneficial to the nerve recovery or repair (Yin et al., 2001; Rustemeyer & Dicke, 2009; Usach et al., 2011). However, there is inconsistent in the aspect of MBP change induced by the sciatic nerve injury. Pan et al. (2010) reported that sciatic nerve injury upregulates the expression of MBP, and further elevated expression of MBP by atorvastatin used as intervention in their study produces a microenvironment favorable to nerve regeneration related to remyelination. Although the aspect of MBP expression induced by the sciatic nerve injury in their research is inconsistent with other studies, it is commonly suggested that further enhanced MBP expression is beneficial to the nerve regeneration or the recovery. In our study, crush nerve injury significantly decreased the expression of MBP, but the impulsive spinal manipulation significantly enhanced the expression of MBP (Fig. 5). Thus, it can be suggested that the impulsive spinal manipulation partially contributes to the nerve regeneration by facilitating Schwann-related remyelination.

GAP-43 is also induced by crush nerve injury (Seo et al., 2006; Pan et al., 2010; Wang et al., 2012). Our study also showed that crush nerve injury significantly increased the expression of GAP-43 in the injured sciatic nerves (Fig. 6). GAP-43 is an axonal growth-associated protein, and is highly localized in the axons. GAP-43 is critical for guiding the growth of axons and modulating the formation of new connections (Aigner et al., 1995; Benowitz & Routtenberg, 1997). Aigner et al. (1995) reported that GAP-43 is considered an intrinsic determinant of the neuron's growth state because it enables neurons to sprout new terminals without additional trophic factor.

Thus, increased synthesis of GAP-43 protein promotes neurite out-growth, and is closely linked with axonal regeneration processes as an intrinsic determinant of axonal elongation in peripheral axons after injury (Benowitz & Routtenberg, 1997; Irwin et al., 2002). In addition, many studies have suggested that the further enhanced expression of GAP-43 reflects the regenerative potential and thus it can promote the nerve regeneration (Seo et al., 2006; Pan et al., 2010; Wang et al., 2012). In the present study, the impulsive spinal manipulation significantly further enhanced the expression of GAP-43 (Fig. 6). Therefore, the impulsive spinal manipulation can be thought to exert the neuroprotective effect by promoting the axonal growth after nerve injury.

In general, spinal manipulation is applied to alleviate neck, low back or pelvic pain (Hurwitz et al., 1996; Koes et al., 1996; Dishman & Burke, 2003). Pickar (2002) suggested that neural inputs induced by the mechanical thrust, such as stimulation or silence of nonnociceptive and mechanosensitive nerve endings in paraspinal tissues, may influence pain producing mechanisms and other physiological systems controlled by the nervous system. Namely, a biomechanical alteration between vertebral segments hypothetically changes the signaling properties of mechanically or chemically sensitive neurons in paraspinal tissues including skin, muscle, and intervertebral disc. These changes in sensory input may modify neural integration either by directly affecting reflex activity and/or by affecting central neural integration within motor, nociceptive and possibly autonomic neuronal pools, resulting in induction of changes in efferent somatomotor and visceromotor activity. Through such a manner, spinal manipulation can improve physiological function, and this explanation is considered one of the most rational neurophysiological bases for the mechanisms underlying the effects of spinal manipulation (Pickar, 2002). Based on Pickar's review (2002), it is plausibly suggested that monthly manipulative therapy may retard the progression of adhesion formation, joint degeneration, neuronal changes, and the changes in muscular strength and recruitment patterns, resulting in improved function, decreased episodes of injuries, and

improved sense of well-being (Taylor, 2011). Taylor (2011) also suggested that manipulation and remobilization of the joint may reverse the neuronal degeneration and muscular weakness on the assumption that He & Dishman's foundings (2010) in guinea pigs could be found to correlate in humans. He & Dishman (2010) reported that knee joint immobilization induces motor neuronal degenerative changes and demyelination, and such neuronal abnormalities are reversible after removal of immobilization, resulting in the restoration of knee joint activity. However, the concrete evidences showing effects of manipulation on recovery from the neuronal degeneration and demyelination have not been presented yet. This study showed that the impulsive spinal manipulation facilitated the functional recovery after sciatic nerve injury by increasing the expression of neurofilaments and enhancing the expressions of proteins related to Schwann-related remyelination and axonal growth (Fig. 3～6). Thus, this is the first study, to the authors' knowledge, to give the molecular- and biological evidences showing the effect of the impulsive spinal manipulation on the sciatic nerve regeneration.

V. Study Limitations

Although it is worth to note that our study first showed the therapeutic potential of the impulsive spinal manipulation by presenting molecular and biological evidences, this study has some limitations. In this study, we did not present various evidences related to nerve regeneration, such as neurotrophic factors, extracellular matrix proteins and hormones. In addition, we did not investigate the effect of the impulsive spinal manipulation under various conditions, such as the velocity, the strength, and direction of the impulse. Finally, we did not discuss the molecular and biological mechanisms of spinal manipulation because there are not many previous studies on it. Therefore, further studies are required to elucidate more exact mechanisms of the impulsive spinal manipulation as therapeutic method and to solve these limitations.

VI. Conclusion

In the present study, sciatic nerve crush injury impaired the function of sciatic nerve by decreasing the expressions of MBP and neurofilaments. On the other hand, impulsive spinal manipulation into the level of L6-S1 significantly improved the function of sciatic nerve through enhancing the synthesis of proteins related to axonal growth and Schwann-related remyelination, which are considered an important mechanism of nerve regeneration. In addition, impulsive spinal manipulation enhanced the expression of neurofilaments, which are intermediate filaments of neurons and responsible for maintaining the calibre of axons. From these results, it can be inferred that impulsive spinal manipulation may facilitate regeneration of the sciatic nerve after injury.

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국문초록

본 연구는 임펄스 척추 교정이 좌골신경 압박 손상을 가진 흰쥐의 재생에 어떤 영향이 있는지 알아보는데 목적을 두고 있다. Sprague-Dawley 암컷 성인 흰쥐 35마리를 무작위로 대조군, 좌골신경 손상 그룹, 좌골신경 손상을 가진 1번 임펄스 교정 그룹, 좌골신경 손상을 가진 5번 임펄스 교정 그룹, 좌골신경을 가진 10번 임펄스 교정 그룹으로 나누었다 (n=7).

좌골신경 압박손상에 검증된 방법을 이용하여 하루에 한번 연속 7일 동안 Impulse Adjusting Instrument® (임펄스)을 요추 제6번과 천추 제1번 레벨에 척추 교정을 실시하였다. 좌골신경의 기능 회복을 측정하기 위해서 좌골신경기능지수 (SFI), 특수 단백질 검출 검사 중 myelin basic protein (MBP), growth associated protein-43 (GAP-43) 및 신경미세섬유에 대한 면역 조직 화학 분석을 하였다.

좌골신경 압박손상에서 손상된 압박신경의 SFI와 MBP의 발현이 상당히 감소하였다 (p<0.05). 게다가 일부 어긋난 섬유와 줄어든 신경미세섬유는 좌골신경 압박손상으로 인정하였다. 반면에, 임펄스 척추 교정에서 SFI는 크게 상승하였고 MBP의 발현은 크게 증가하였다 (p<0.05). 더욱이, 5번의 임펄스 교정 그룹에서 GAP-43의 발현이 강하게 나타났으며 (p<0.05) SNI와 비교했을 때 좌골신경의 신경미세섬유의 발현이 크게 나타났다.

본 연구는 임펄스 척추 교정은 신경미세섬유의 발현을 증가시키고, 축삭 성장과 재수초화와 관련된 Schwann-단백질의 발현은 좌골신경의 기능을 회복시킬 수 있다고 사료된다.

Key Words : 좌골 신경 압박 손상, 좌골신경기능지수, Myelin Basic Protein, Growth Associated Protein-43, 신경미세섬유