Master of Medicine

Tension-induced nerve injury correlates with functional loss and transmission electron microscopic degeneration in the rat model

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English abstract

Tension-induced nerve injury correlates with functional loss and transmission electron microscopic degeneration in the rat model

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Introduction

Postoperative peripheral nerve damage or paralysis usually occurs during oral and maxillofacial surgery. Traction injury is the most common type of peripheral nerve dysfunction in civilian populations. The sciatic nerve has an almost identical capacity for regeneration in rats and subhuman primates. It is the approximate size of the intra-parotid facial nerve of human. The purpose of this study is to evaluate the relation between amount of traction force to nerve and its functional damage.

Material & Methods

Eighteen male Sprague-Dawley rats weighing 250 gram were divided into three groups; A, B and C (n=6 in each group). In the sham operation (group A), after skin incision and complete dissection of the sciatic nerve, no further injury or tension was applied. Group B was given tension injury of 150g for a minute. Group C was subjected to 300g of tension injury for a minute. Functional change of motor action was evaluated by gait analysis with sciatic function index. And histological changes were evaluated by H&E staining and average myelin thickness and relative sheath thickness (G-ratio) were measured by transmission electron microscopic (TEM) examination at 2 and 4 weeks after operation.

Results

The sciatic function index (SFI) was -6.59±5.14 for group A, -20.89±5.26 for group B, -48.85±12.58 for group C respectively at 2 week after surgery. After 4 weeks, the SFI was -6.45±4.47 for group A, -20.60±5.71 for group B, -23.80±7.81 for group C. Transmission electron microscopic results showed partial demyelination and decreased diameter of nerve fiber in both Group B and C at postoperative 2 and 4 weeks. The myelin thicknesses of Group A, B, C were 1298.36±246.45,

1061.06±245.46, 801.64±435.71 nm after 2 weeks and 1293.84±207.81, 735.94±280.48, 493.38±98.45 nm after 4 weeks. The G-ratio of the myelinated fiber was 0.60±0.09 for Group A, 0.64±0.07 for Group B and 0.67±0.10 for Group C after 2 weeks. G-ratio of Group A, B, C was 0.62±0.08, 0.71±0.09 and 0.75±0.06 respectively at 4 weeks.

Conclusion

The functional loss of the rat sciatic nerve had positive relation with the extent of traction injury in the first 2 weeks. However, damage due to the 300g of traction injury was temporary and it tends to recover following next 2 weeks. Both 150g and 300g of traction injury group showed degenerating pattern in the TEM examination during 4 weeks after operation.

Key words: traction injury, rat sciatic nerve, nerve regeneration, sciatic function index, transmission electron microscopy

Contents

Introduction

Postoperative cranial nerve damage or paralysis usually occurs during oral and maxillofacial surgery.[1, 2] In many procedures, it is usually necessary to dissect, mobilize and retract the peripheral nerve to make appropriate surgical exposure because of the compact anatomical feature in the head and neck. Generally, the nerves are intact after operation, but the functional disorder is thought to be resulted from traction injury.[3] Most of these injuries recover over time showing difference in degree. For benign parotid tumor, permanent facial nerve injury occurred in the incidence rate of between 3% and 5% after surgery.[4-6] At the same time, the incidence of temporary functional disorder of facial nerve is ranged from 8.2% to 65%.[4-6] Leipzig et al reported that after radical selective neck dissections, 30% of patients showed shoulder dysfunction which is related to damage of spinal accessory nerve.[7]

It is essential to know about the relevant anatomy, pathology, pathophysiology, and regeneration process of nerve in traumatic peripheral nerve injury patients.[8, 9] Although it is derived from central nervous system, peripheral nerves are able to move extensively without affecting electrophysiological function.[10] The structures about non-linear path axons follow through peripheral nerve sheaths make nerve elongate without damage, therefore axons may straighten with no harm.[11] But, when it is over-elongated beyond physiological limit, peripheral nerve usually get problems about signal conduction and it may lead to disruption of nerve anatomy.[12, 13] Traction injury is the most common type of peripheral nerve dysfunction in civilian populations.[8] These injuries usually occur due to longitudinal trauma like sprains and dislocations.[10] In Seddon classification, peripheral nerve injury is divided into neurapraxia, axonotmesis and neurotmesis.[14] Traction injury is commonly included in neurapraxia or axonotmesis. The prognosis of neurapraxia is fairly better than axonotmesis and the prediction of recovery duration is the important thing in the clinical field.[15] Severe dysfunction originated in nerve damage gives patients a significant adverse effect on their life, so it is necessary to be cautious about nerve injury during surgery. Human study of traction injury is unavailable due to ethical problem. Because of this, a well-established model is required for the study of traction nerve injury.

Numbers of animal models have been investigated to study peripheral nerve regeneration.[16] Among them, rat provides a relatively cheap source of mammalian nervous tissue of equivalent genetic storage which is easy to manage and well-studied.[17-20] The sciatic nerve has an almost identical capacity for regeneration in rats and subhuman primates.[21] It is the approximate size of the intra-parotid facial nerve of human.[3] For these reasons, rat sciatic nerve model is in wide use model for the evaluation of motor nerve function.[22-24] Many investigators have regarded walking track analysis as an assessment of function recovery after the sciatic nerve injuries or repair methods.[25] It is a valid, non-invasive and reproducible method of evaluating the functional status of the sciatic nerve. Sciatic function index(SFI) may be better choice than using electrophysiology of axon growth and muscle innervation, if the research focus to functional outcome.[18] It is easy to be performed and obtained by foot prints. Evaluating the thickness of myelin is the second method to assess nerve regeneration. It is related to regeneration capacity of the Schwann cells which is responsible for myelin formation.

The purpose of this study was to evaluate the functional deficit and recovery of the rat sciatic nerve after given traction injury through sciatic nerve function index and transmission electron microscopic changes during follow-up periods.

Materials and Methods

All of the Animal handling protocols were approved by the ethics committee on experimental use of animals of the Asan Medical Center Animal Research Committee.(K-2015-17229701).

1. Surgical technique

Eighteen male Sprague-Dawley rats weighing 250 gram were divided into three groups; A, B and C (n=6 in each group). The rats were anesthetized and sedated by an intraperitoneal injection of tiletamine (15 mg/kg) and zolazepam (Zoletil 50; Virbac Laboratories, France). After achieving proper

levels of anesthesia, the hind quarter of the operative field of left thigh was shaved and routine povidone iodine (Betadine™, ChoongWae Pharm, Korea) preparation was done. The rats were placed on a mounting board, upper and lower extremities were being fixed in full extension.(Fig. 1) The left sciatic nerves were exposed through incising the fascia between the hamstring muscles. Using blunt dissection, about 15mm length of the sciatic nerve was isolated from the adjacent tissues. And then, two dye marks were placed on the sciatic nerve which notes that the distance between marks was 10mm, measured with digital caliper. In group B and C, the tension was given to the middle-point of two dye marks. (Fig. 2)

In the sham operation group (group A), skin incision and complete dissection of the sciatic nerve was performed with no further injury or tension in the sciatic nerve. Group B was given traction injury of 150g for a minute. Group C was subjected to 300g of traction injury for a minute.(Fig. 3) After experiment, skin was sutured by 4-0 Vicryl™ (Ethicon, UK) with double-layered technique. Antibiotic (Amoxicillin™ 150mg/kg SC, Il Sung Pharm, Korea) and analgesic (Ketoprofen™ 2–5 mg/kg SC, Dong Kook Pharm, Korea) were injected intramuscularly daily after operation. Surgical procedure was identical to all animals in each group.

After tension-induced nerve injury, the occurrence of the nerve rupture or discontinuity was inspected. Evaluation for functional changes were performed at 2 and 4 weeks. Two weeks after operation, six rats of each group were taken functional evaluation via gait analysis and randomly chosen rats were sacrificed to harvest the sciatic nerve for TEM examination and histological examination. Four weeks after operation, five rats of each group were taken functional evaluation and randomly chosen to harvest the sciatic nerve in the same way.

2. Functional analysis

Each animal underwent a clinical evaluation of the motor function of the left sciatic nerve by gait analysis and with sciatic function index(SFI), a method which was described by Bain et al.[18] First of all, autotomy was inspected to rule out the miscalculation of the SFI, and then footprints were obtained with the corridor which is made of paper. Hind paws of rats were painted to get feet

prints.(Fig. 4) The corridor was made tunnel-like shape and the bottom of the walking pathway was opened. White 150cm x 30cm paper was placed at the bottom of the walking pathway in every single experiment to get footprints of rats. Rats were made walk through the tunnels several times in each trial to get enough footprints that made the experiment more accurate.

Several parameters were obtained from the footprints as followed; distance from the heel to the toe of the third toe (Print Length; PL); distance between the first and fifth toe (Toe Spread; TS); distance between the second and fourth toe (Intermediary Toe Spread; ITS or IT). All measurements were taken from the experimental side (EPL, ETS, EIT) and also from the control side (NPL, NTS. and NIT) in each group.(Fig. 5,6,7) SFI was calculated as following formula.

SFI formula

SFI = (-38.3 x PLF) + (109.5 x TSF) + (13.3 x ITF) - 8.8

PLF = (EPL - NPL) / NPL; TSF = (ETS - NTS) / NTS; and ITF = (EIT - NIT) / NIT

If SFI value is 0, it indicates the normal state and an SFI of -100 means total impairment, such as result from a complete deformation of the sciatic nerve. In each group, randomly selected 20 pairs of feet prints which include one of experimental side and one of control side were used to calculate the SFI value as mentioned previously.

3. Transmission electron microscopic (TEM) examination

The sciatic nerves in each group were harvested at 2 and 4 weeks and examined by TEM examination for measuring the diameter of total nerve fiber and axon and length of myelin thickness. The sciatic nerve was washed by phosphate buffer solution 0.1M, and applied to osmium tetroxide for 90 minutes. The sample was dehydrated in ethyl alcohol water solutions of growing concentration (50%, 60%, 70%, 80%, 90%, 95%) for 10 minutes each and then twice for 20 minutes in absolute alcohol. Once dehydrated, the segments were routinely processed and embedded in epoxy resin. Transverse semi-thin (4μm-thick) sections were cut with a microtome, stained with toluidine blue.

The cross-sectional photograph was taken and it was examined with electron microscope (x1000, x2500, JEOL 1200 EX-II, Japan).

In each group, 50 cross-sections of nerve fibers were selected randomly, and then diameter of nerve fiber and axon and length of myelin thickness were measured using image analysis program (ImageJ 1.44p, NIH, USA). Degree of demyelination was also evaluated in TEM examination and G-ratio of the myelinated nerve fiber was calculated by dividing the diameter of axon by the diameter of total nerve fiber. The value of G-ratio is from 0 to 1, in which 1 means unmyelinated axon.

4. H&E staining

The sciatic nerves were harvested from all groups at 2 and 4 weeks for histological examination, respectively. In each group, nerve specimens were selected randomly and fixed in 10% neutral formalin for 24 hours. After fixating the tissue and embedding in the paraffin block, specimens were sectioned about 4μm thickness. And then, they were stained with hematoxylin and eosin (H&E). Cross-sectional photo was taken at magnifying x100 and x200 with light microscope to evaluate the damage of nerve fibers. Longitudinally sectioned slides were stained same as described previously and examined with x200 and x400 magnification.

5. Statistical analysis

Experimental data were presented as mean ± standard deviation. In this study, all of the numerical data taken from measurement were based on independent multiple groups. SFI of three groups were compared by Kruskal-wallis test to determine the statistical significance among them because each group failed to satisfy the test of normality. Myelin thickness and G-ratio were analyzed using one way analysis of variance (ANOVA) test. P value less than 0.05 was considered as significant. All statistical processes were carried out with SPSS software (ver. 23.0, SPSS, Inc., Chicago, IL, USA).

Results

1.SFI

There was no rat which expressed autotomy. The SFI was -6.59±5.14 for group A, -20.89±5.26 for group B, and -48.85±12.58 for group C at 2 weeks after surgery. After 4 weeks, the SFI was -6.45±4.47 for group A, -20.60±5.71 for group B, and -23.80±7.81 for group C.(Table 1, Fig.8) In walking track analysis, there were statistically significant differences($p<0.001$) between each group at 2 weeks. At 4 weeks, between group A and B, A and C, they consistently showed significant difference($p<0.001$), but there was no statistical difference between group B and C.(Table 2)

2. TEM analysis

With TEM examination, partial demyelination of nerve fiber was observed and diameter of total nerve fiber and axon decreased in both Group B and C after 2 and 4 weeks. The myelin thickness of Group A, B, C were 1298.36±246.45, 1061.06±245.46, 801.64±435.71 nm at 2 weeks and 1293.84±207.81, 735.94±280.48, 493.38±98.45 nm at 4 weeks.(Table 3, Fig. 9) In myelin thickness of nerve fibers, between group A and C, and between group B and C show higher deviational means($P<0.001$) as compared with group A and B($P<0.05$) at 2 weeks. Myelin thickness analysis at 4 weeks, statistically significant difference turned out in all compared groups; between group A and B, B and C, C and A.(Table 4)

The G-ratio of the myelinated fiber was 0.60±0.09 for Group A, 0.64±0.74 for Group B and 0.67±0.99 for Group C at 2 weeks. G-ratio of Group A, B, C was 0.62±0.08, 0.71±0.09 and 0.74±0.58 respectively at 4 weeks.(Table 5, Fig. 10) In one-way ANOVA analysis, between group A and C, there was higher different means($P<0.001$) than between group A and B($P<0.05$) at 2 weeks. At the same time, between group B and C showed no significant difference. After 4 weeks, as seen in myelin thickness, there were also statistically significant differences in all compared groups; between group A and B, B and C, C and A.(Table 6)

In TEM photographs, dark bands which meant stained myelinated sheaths of nerve fibers were clearly seen in group A with regular thickness and round shape. Group B showed disrupted

myelinated sheaths with uneven surfaces which stood for damaged axons at post-operative 2 weeks. This pattern went severed in group C.(Fig. 11) At post-operative 4 weeks, only group A showed similar pattern with post-operative 2 weeks, and in group B and C, dark band went thinner and dead spaces were also seen. (Fig. 12)

3. Histologic analysis

Fig. 13,14 showed the histological cross-sections of injured sciatic nerve at 2 and 4weeks after operation. Clearly stained myelin sheaths of group A showed higher density compared with group B and C which meant nerve fibers were relatively intact. Slides of postoperative 2 weeks in group B and C showed decreased diameter of nerve fibers and collapsed masses. (Fig. 13) At postoperative 4 weeks, in group B and C, the nerve fibers showed more severed pattern compared with postoperative 2 weeks.(Fig. 14) Longitudinal sections presented thinner axons in group B and C compared with group A. (Fig. 15) Post-operative 4 weeks, group B and C showed severely exacerbated axons and thinner axons in comparison with 2 weeks before. (Fig. 16)

Discussion

In the rat sciatic nerve study, it is uncertain that how much of traction force is critical to nerve resulting in irreversible damage so far. Spiegel et al. reported that mean amount of 626g traction force results in a loss of continuity of sciatic nerve.[26] Fowler et al. demonstrated that 50g traction injury for 1 or 2 minutes to sciatic nerve of rat did not affect any motor capabilities. However, continuing for 5 minutes, the same amount of traction force resulted in a significant functional deficit.[3] Alant et.al. reported that 50g traction force could also result in additional nerve damage such as neuroma under compression force.[27] In this experiment, 150g or 300g of traction was applied by precise tension device in each group except control group. Even sham operation group showed nerve function damage after 2 weeks. There was meaningful difference between group B and C which meant 300g traction injury is harmful compared with 150g at postoperative 2 weeks. At 4 weeks, the functional recovery between group B and C did not show statistical differences and it means that

group C had recovered their motor function during 2 weeks as approximate level of group B. Considering this, damage due to 300g traction injury was partially temporary.

Myelin thickness gradually decreased as it went from group A to C at postoperative 2weeks showing similar tendency compared with SFI value. Means of myelin thickness of sham group didn't show any difference between 2 weeks and 4 weeks after operation. On the other hand, in 150g and 300g traction injury group, means of myelin thickness had remarkably decreased during following 2 weeks that meant myelin had gone degenerated in both groups. G-ratio analysis is useful tool for demonstrating the structural regeneration of damaged nerve fiber in TEM cross-section. Many studies have researched past decades about normal nerve tissue and concluded that optimal value of G-ratio of myelinated nerve fiber is in the range of 0.55 to 0.65.[28, 29] With mild injury under controlled laboratory environment, inner part of nerve fibers (axons) remain intact and relatively ordinal shape. Outer portion of fiber would be deformed by a traction force beyond the physiological limit and it causes the beginning of elastic recovery immediately. Because of this deformity, diameter value of outer fiber changed the G-ratio unlike before traction force was applied. If G-ratio is larger than normal, that nerve fiber would be expected to get severe injury. As regeneration of nerve fiber induced by nearly located Schwann cells goes on, G-ratio value gradually decrease which approaches to normal value. In this study, At 2 weeks after operation, G-ratio of group B and C were statistically significantly different from group A. They didn't show statistical differences between them. 4 weeks later, the values slightly increased in all groups and it means that the nerve fibers went degenerated during 2 weeks as shown in the analysis of myelin thickness.

Based on the previously mentioned results, it is considered that initial damage of rat sciatic nerve was proportional to the extent of traction injury, especially on the 150g and 300g at 2 weeks. This was shown from the SFI value and in the TEM examination. And then during 2 weeks, the sciatic nerves of 150g and 300g injury groups had gone degenerated anatomically which were verified in the TEM examination. Despite of this observation, functional capacity of nerve had been improved in the group which was subjected to 300g traction injury as the similar level of 150g group and it implied that myelin thickness was not only the index for evaluating the recovery or degeneration of

total nerve fiber. It was the limitation of our study that if the follow-up period was longer than 4 weeks, there would be the possibilities to expect the more functional recovery in the injured group.

During oral and maxillofacial surgery such as extraction of lower third molar, open reduction of mandibular angle fracture, genioplasty, sagittal split osteotomy, total parotidectomy and neck dissection for oral cancer surgery, traction nerve damage could occur unexpectedly.[1, 2] The most common question from patients who was subjected to nerve damage is that when the nerve damage will totally recover. Peripheral nerve goes through pathological changes after traction injury.[30, 31] With minor injuries, repair and regeneration processes begin immediately.[8] In neurapraxia, remyelination can occur pretty rapidly. If severed injury was given, there would be an initial shock phase and take several months to achieve full recovery. In peripheral nervous system(PNS), when this system gets injuries, it starts to repair itself and this is what is essentially distinguished from central nervous system(CNS).[9, 32-34] Repair would progress with three mechanisms: remyelination, collateral sprouting distally from preserved axons, and regeneration from the site of injury.[35] If lesion involves less than 20-30% of axons, nerve recovery would mainly depend on collateral sprouting from surviving axons which continues during 2-6 months. With lesions involving more than 90% of the axons, the primary mechanism of repair would be regeneration from the injury site.[8] Even if motor function totally recovered after nerve damage, sensory deficiency, especially proprioception may impair functional outcome.[8] Regeneration process starts as soon as get injured.[36, 37] After a while, a cascade of events including neurotrophic factors and cell signaling molecules begins.[38] Schwann cells undertake a crucial role in regenerating process by increasing their synthesis of surface cell adhesion molecules, and by exquisite basement membrane containing extracellular matrix proteins including laminin and fibronectin.[9, 39, 40] They produce neutrophic factors which have responsibility for a signal that leads to gene activation.[41] Within 30 minutes after nerve damage, intracellular processes which induce repair and regeneration would already get activated.[42] This was the reason why myelin thickness was chosen to evaluate the state of sciatic nerve in this study which is related to Schwann cells.

If the damaged nerve is only limited to sensory part, patient could bear better than motor

nerve impairment. Damage of the motor nerve such as facial nerve causes difficulty making facial expressions like closing eyes or smiling. Drooling of the saliva and blinking dysfunction are other chief complaints. The prognosis of the nerve damage depends on the initial extent of damage. As mentioned previously, rat sciatic nerve has similarity compared with human nerve branch. It is a commonly used model for studying the nerve function. The sciatic nerve is large enough and easily accessible for surgical approach so that various research model could be planned with this nerve. It is a mixed nerve carrying sensory axons and motor axons for antagonistic muscle groups to the lower extremity.[43] Gait analysis is a valid and non-invasive assessment method as compared with other evaluation method to evaluate the sciatic nerve function. Because gait requires coordination of function involving sensory input, motor response and cortical integration, SFI could be more simple and better indicator than using basic electrophysiology and histomorphometry of axon growth and muscle innervation. However, there are shortcomings of SFI such as the presence of autotomy or the occurrence of flexion contractures after sciatic nerve injury.

To apply the results of this study to the clinical field of oral and maxillofacial surgery, it is important to know the differences of metabolism rate between rat and human being. Because the rat sciatic nerve and human nerve branch are similar in size, it is possibly hypothesized that their nerve regeneration rate would be almost same.[3] Some research reported that humans and primates bring implications. In evolutionary rate in divergence of brain developmental patterns, humans express 3-5 times faster than chimpanzee.[44] In aspects of nerve regeneration, it is hard to demonstrate the correlation between evolutionary rate and nerve regeneration rate. To apply the duration of clinical prognosis about damaged nerve recovery in human, further investigation will be needed.

Conclusion

The functional loss of rat sciatic nerve had positive relation with the extent of traction injury in the first 2 weeks. However, damage due to the 300g of traction injury was temporary and it tends to recover following next 2 weeks. Both 150g and 300g of traction injury group showed degenerating pattern in the TEM examination during 4 weeks after operation.

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List of Tables

Table 1. Sciatic function index (SFI) at post-operative 2 weeks and 4weeks

SFI	Group A	Group B	Group C
2 weeks	-6.6±5.1	-20.9±5.3	-48.8±12.6
4 weeks	-6.4±4.5	-20.6±5.7	-23.8±7.8

Table 2. Statistical analysis of cciatic function index (SFI) at post-operative 2 weeks and 4weeks (Kruskal-Wallis test)

Group	2 weeks	4 weeks
A vs B	p<0.001	p<0.001
A vs C	p<0.001	p<0.001
B vs C	p<0.001	ns p>0.05

Table 3. Mean and standard deviation of myelin thickness at post-operative 2 weeks and 4weeks

Myelin thickness(nm)	Group A	Group B	Group C
2 weeks	1298.36±246.45	1061.53±245.46	801.64±435.71
4 weeks	1293.84±207.81	735.94±280.48	493.38±98.45

Table 4. Statistical analysis of myelin thickness at post-operative 2 weeks and 4weeks (Multiple Comparisons by one-way ANOVA)

Comparison	2 weeks	4 weeks
A vs B	p<0.05	P<0.001
A vs C	p<0.001	P<0.001
B vs C	p<0.001	P<0.001

Table 5. Means of G-ratio at post-operative 2 weeks and 4weeks

G-ratio	Group A	Group B	Group C
2 weeks	0.60±0.09	0.64±0.07	0.67±0.10
4 weeks	0.62±0.08	0.71±0.09	0.75±0.06

Table 6. Statistical analysis of G-ratio at post-operative 2 weeks and 4weeks (Multiple Comparisons by one-way ANOVA)

Comparison	2 weeks	4 weeks
A vs B	$p<0.05$	$P<0.001$
A vs C	$p<0.001$	$P<0.001$
B vs C	ns $p>0.05$	$P<0.05$

List of figures

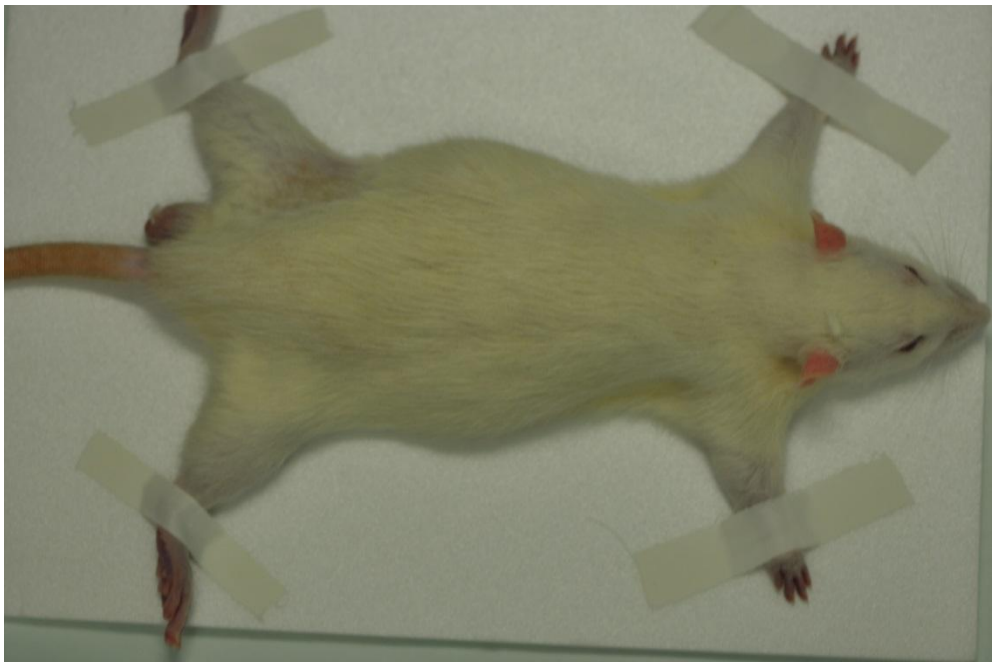
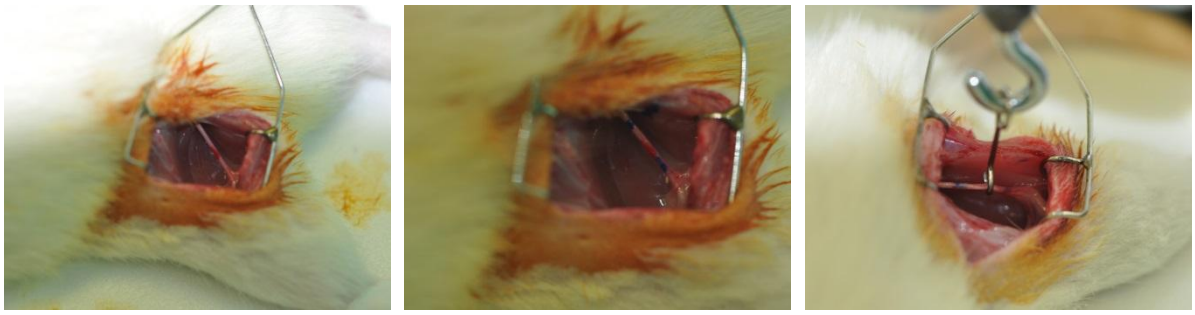


Fig. 1. Sprague-Dawley rat. They were placed on a mounting board with upper and lower extremities being fixed in full extension.



(a) (b) (c)

Fig. 2. Traction injury to rat sciatic nerve. (a) exposure of sciatic nerve. (b) two dye marks with 10mm distance. (c) tension application at the middle-point of two dye marks.

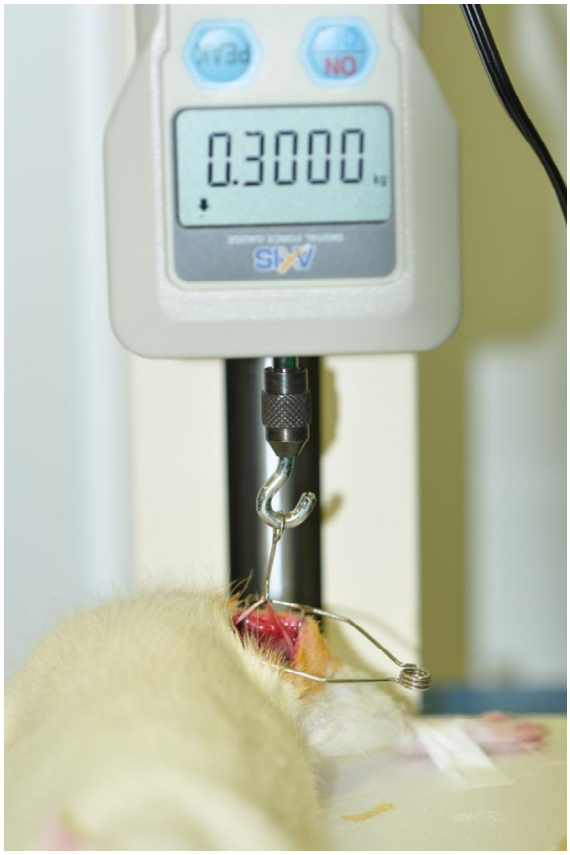
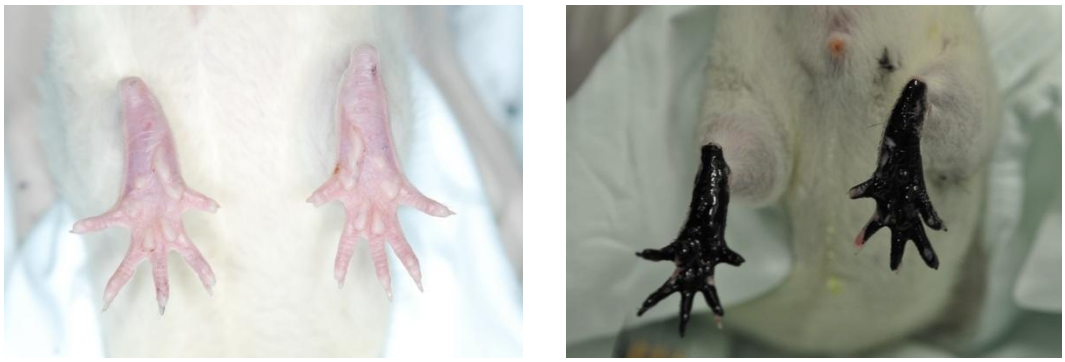


Fig. 3. 150g or 300g of traction injury to the sciatic nerve using precise tension device.



(a) (b)

Fig. 4. Painted hind paws of rats to get foot print. (a) before painting. (b) after painting.

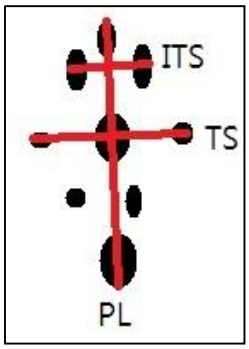
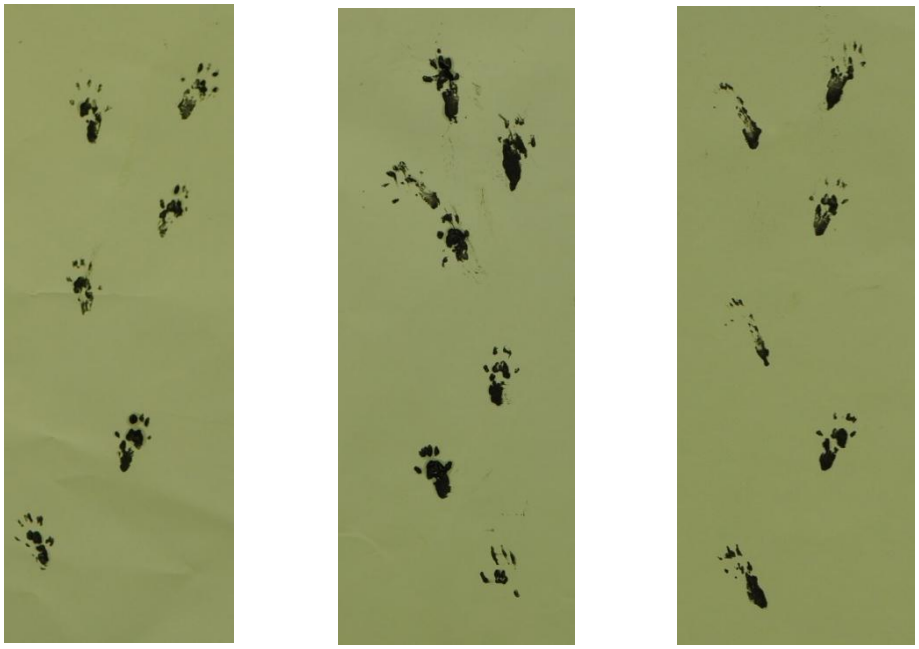


Fig. 5. Measurements for sciatic function index. Distance from the heel to the toe of the third toe (Print Length; PL); distance between the first and fifth toe (Toe Spread; TS); distance between the second and fourth toe (Intermediary Toe Spread; ITS or IT).



(a) group A (b) group B (c) group C

Fig. 6. Footprints of group A, B and C at post-operative 2 weeks.

(a) group A (b) group B (c) group C

Fig. 7. Footprints of group A, B and C at post-operative 4 weeks.

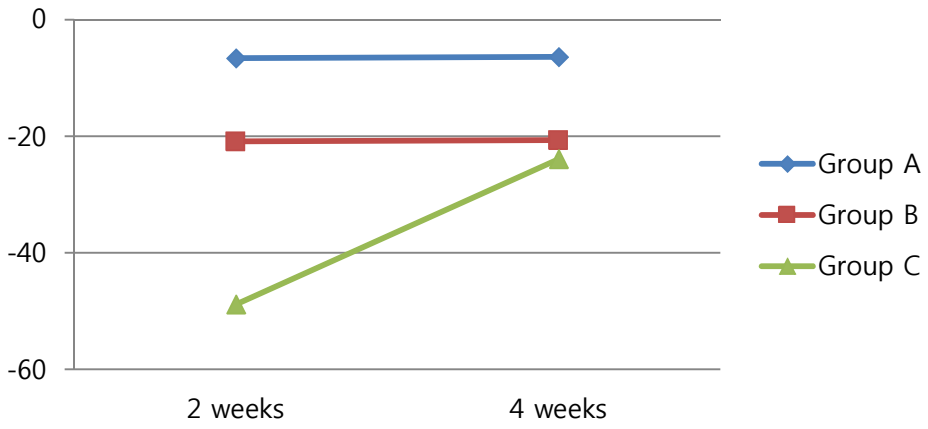


Fig.8. Means of SFI at post-operative 2 weeks and 4weeks. Even sham operation group shows nerve function damage after 2 weeks. There are meaningful differences between group B and C which means 300g traction injury is harmful compared with 150g at postoperative 2 weeks. At 4 weeks, the functional recovery between group B and C does not show statistical differences and it means that group C have recovered their motor function during 2 weeks as approximate level of group B.

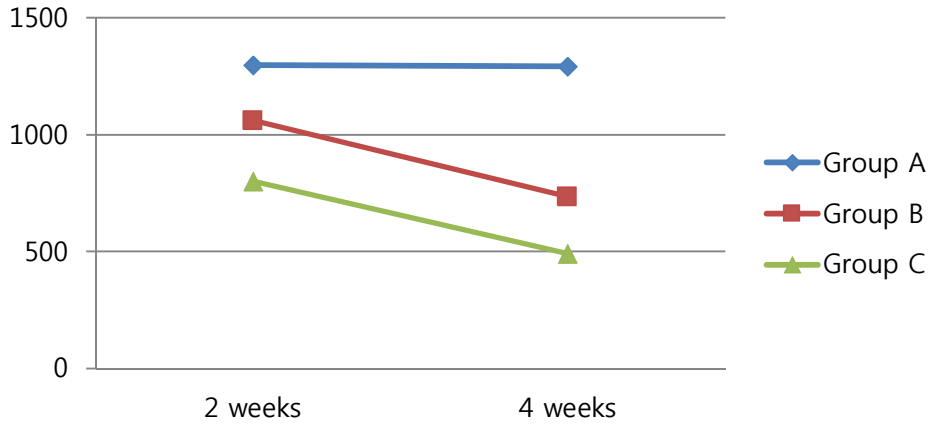


Fig. 9. Means of myelin thickness at post-operative 2 weeks and 4weeks. Myelin thickness gradually decreased as it went from group A to C at postoperative 2 weeks showing similar tendency compared with SFI value. Means of myelin thickness of sham group didn't show any difference between 2 weeks and 4 weeks after operation. On the other hand, in 150g and 300g traction injury group, means of myelin thickness had remarkably decreased during following 2 weeks that meant myelin had gone degenerated in both groups.

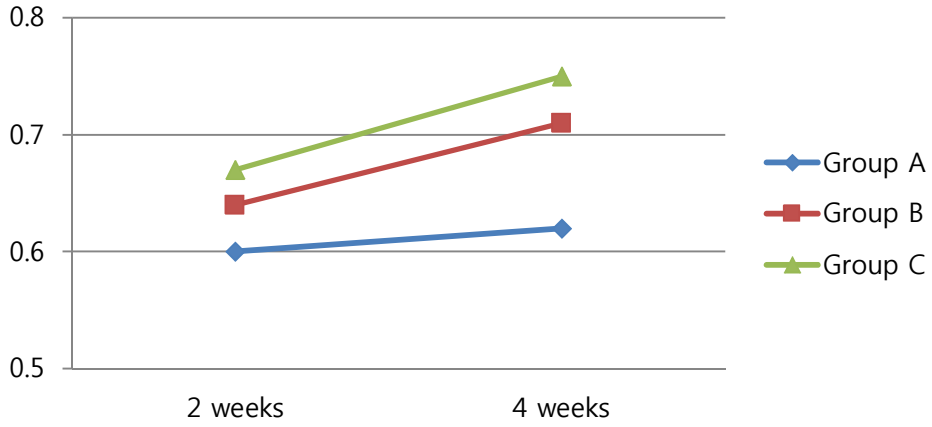
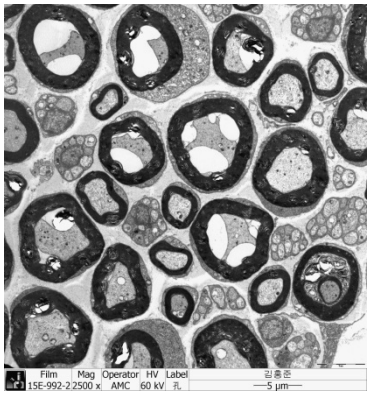
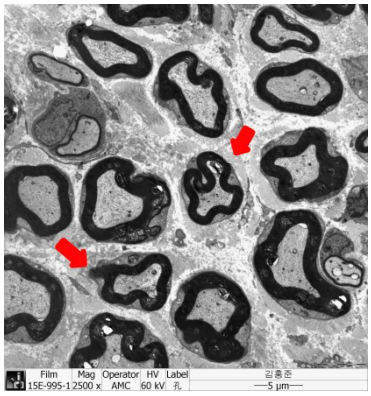


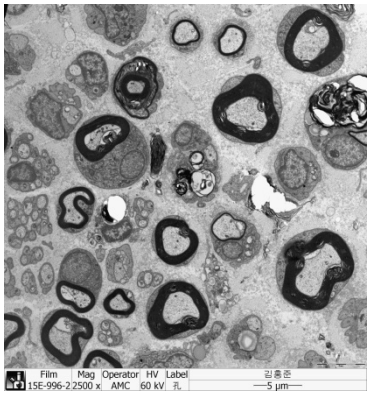
Fig. 10. Means of G-ratio at post-operative 2 weeks and 4weeks. At 2 weeks after operation, G-ratio of group B and C were statistically significantly different from group A. They didn't show statistical differences between them. 4 weeks later, the values slightly increased in all groups and it means that the nerve fibers went degenerated during 2 weeks as shown in the tendency of myelin thickness.



(a) group A

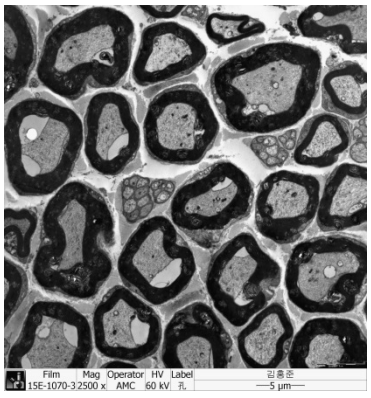


(b) group B

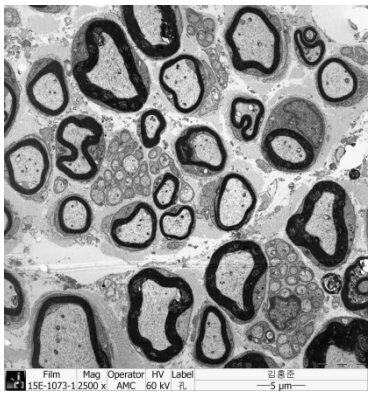


(c) group C

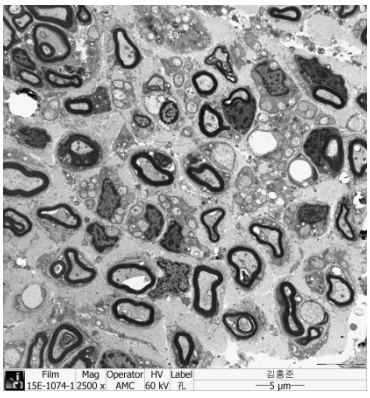
Fig. 11. TEM photographs of group A,B and C at 2 weeks(x2500). In TEM photographs, dark bands which meant stained myelinated sheaths of nerve fibers were clearly seen in group A with regular thickness and round shape. Group B showed disrupted myelin with uneven surfaces (red arrow) which stood for damaged axons at post-operative 2 weeks. This pattern went severed in group C.



(a) group A

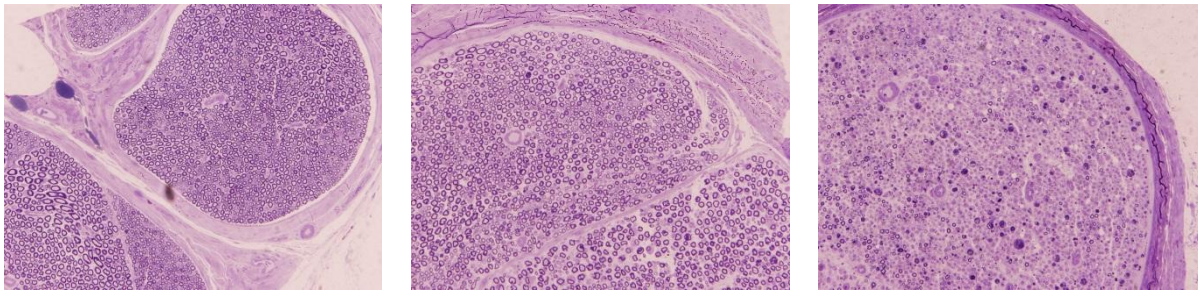


(b) group B



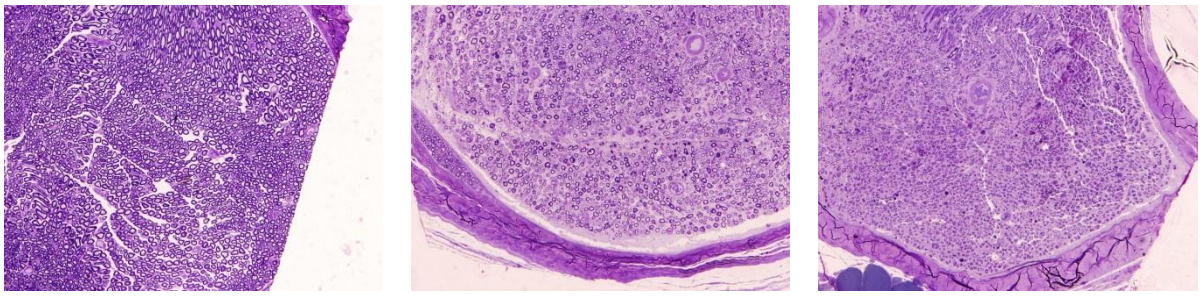
(c) group C

Fig. 12. TEM photographs of group A,B and C at 4 weeks(x2500). Only group A showed similar pattern with post-operative 2 weeks, and in group B and C, dark band went thinner and dead spaces were also seen.



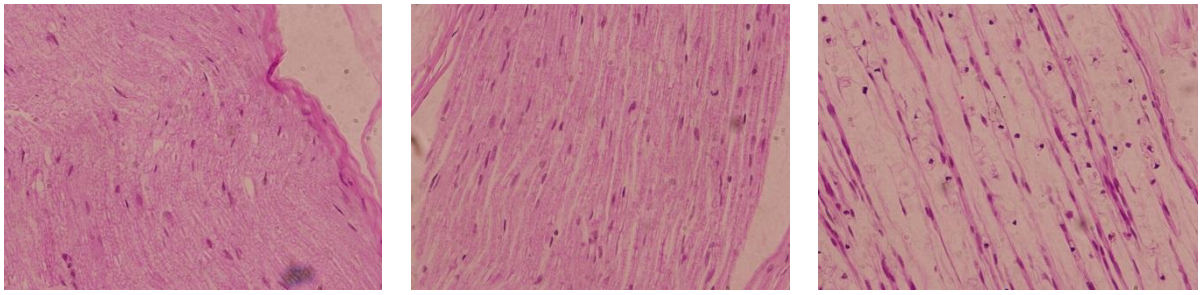
(a) group A at 2 weeks (b) group B at 2 weeks (c) group C at 2 weeks

Fig. 13. Histologic analysis of the sciatic nerve group A,B and C at 2 weeks(x200). Clearly stained myelin sheaths of group A showed higher density compared with group B and C which meant nerve fibers were relatively intact. Slides of postoperative 2 weeks in group B and C showed decreased diameter of nerve fibers and collapsed masses.



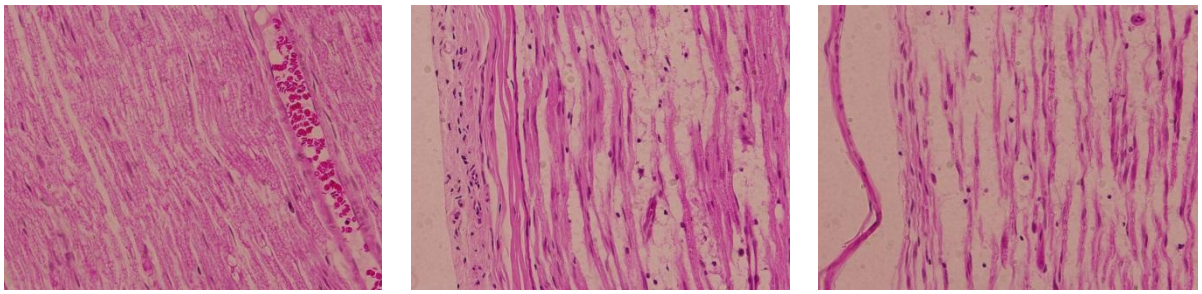
(a) group A at 4 weeks (b) group B at 4 weeks (c) group C at 4 weeks

Fig. 14. Histologic analysis of the sciatic nerve group A,B and C at 4 weeks(x200). In group B and C, the nerve fibers showed more severed pattern compared with postoperative 2 weeks.



(a) group A at 2 weeks (b) group B at 2 weeks (c) group C at 2 weeks

Fig. 15. H&E stained longitudinal section of group A,B and C at 2 weeks(x400). As it went from group A to C, density of nerve fibers gradually decreased and axons got thinner.



(a) group A at 4 weeks (b) group B at 4 weeks (c) group C at 4 weeks

Fig. 16. H&E stained longitudinal section of group A,B and C at 4 weeks(x400). Group B and C showed severely exacerbated axons and thinner axons in comparison with post-operative 2 weeks.

Appendix

1.Sciatic function index after 2 and 4 weeks in sham group (Group A)

2w	EPL	ETS	EIT	NPL	NTS	NIT	PLF	TSF	ITF	SFI
1	36.2	22.0	12.0	37.8	23.0	16.0	0.0	0.0	-0.3	-15.3
2	34.3	22.3	14.8	35.1	21.9	13.4	0.0	0.0	0.1	-4.5
3	37.9	24.5	14.0	36.6	23.0	12.7	0.0	0.1	0.1	-1.7
4	34.1	24.7	13.4	37.4	25.9	13.6	-0.1	0.0	0.0	-10.7
5	37.9	23.9	14.3	37.3	24.7	12.9	0.0	0.0	0.1	-11.5
6	36.8	24.3	13.2	37.9	24.3	13.5	0.0	0.0	0.0	-8.0
7	34.2	22.4	14.1	36.6	21.4	13.3	-0.1	0.0	0.1	-0.4
8	34.0	24.0	13.4	37.2	26.2	13.5	-0.1	-0.1	0.0	-14.8
9	37.1	24.5	15.5	37.1	23.2	14.0	0.0	0.1	0.1	-1.2
10	36.7	22.9	12.6	36.0	23.5	14.0	0.0	0.0	-0.1	-13.7
11	37.0	24.0	12.9	37.8	22.9	15.0	0.0	0.0	-0.1	-4.6
12	37.2	23.6	14.2	38.2	24.4	14.8	0.0	0.0	0.0	-11.9
13	34.2	24.7	13.5	38.6	25.0	16.0	-0.1	0.0	-0.2	-7.8
14	35.4	25.5	14.6	37.4	24.3	14.8	-0.1	0.0	0.0	-1.5
15	33.9	24.5	13.2	38.5	24.2	15.4	-0.1	0.0	-0.1	-4.8
16	37.4	23.5	12.2	37.6	22.7	13.2	0.0	0.0	-0.1	-5.7
17	37.8	22.8	14.7	38.1	23.7	12.5	0.0	0.0	0.2	-10.3
18	35.9	23.7	12.4	37.7	22.4	12.1	0.0	0.1	0.0	-0.3
19	36.5	24.4	14.9	37.6	23.0	14.4	0.0	0.1	0.0	-0.6
20	36.0	22.6	14.3	37.1	21.5	14.8	0.0	0.1	0.0	-2.5
Av	36.0	23.7	13.7	37.4	23.6	14.0	0.0	0.0	0.0	-6.6
SD	1.4	1.0	1.0	0.8	1.3	1.1	0.0	0.0	0.1	5.1

4w	EPL	ETS	EIT	NPL	NTS	NIT	PLF	TSF	ITF	SFI
1	34.9	26.0	15.6	35.1	27.0	16.0	0.0	0.0	0.0	-13.0
2	37.1	26.6	16.0	37.2	26.8	17.3	0.0	0.0	-0.1	-10.5
3	37.6	25.2	17.0	37.7	25.0	14.3	0.0	0.0	0.2	-5.3
4	32.2	26.9	16.4	35.0	27.1	14.7	-0.1	0.0	0.1	-5.0
5	36.8	27.7	17.1	35.8	26.0	16.4	0.0	0.1	0.0	-2.1
6	33.2	25.4	14.3	35.4	26.5	14.9	-0.1	0.0	0.0	-11.5
7	36.9	25.2	14.3	38.2	24.0	15.8	0.0	0.1	-0.1	-3.3
8	35.2	23.4	15.6	36.0	23.6	15.6	0.0	0.0	0.0	-8.9
9	34.7	26.1	15.5	35.6	26.9	17.0	0.0	0.0	-0.1	-12.3
10	37.7	26.6	16.9	37.2	27.1	17.1	0.0	0.0	0.0	-11.5
11	37.9	25.3	17.1	37.1	25.1	14.3	0.0	0.0	0.2	-6.1
12	35.0	27.8	16.5	36.7	27.7	15.5	0.0	0.0	0.1	-5.8
13	37.3	27.7	17.4	37.6	25.9	16.7	0.0	0.1	0.0	-0.3
14	35.7	25.8	13.9	36.9	26.3	14.9	0.0	0.0	-0.1	-10.5
15	36.8	23.5	14.3	37.8	22.3	13.4	0.0	0.1	0.1	-1.0
16	35.8	25.7	15.7	36.3	24.5	15.4	0.0	0.0	0.0	-2.7
17	35.0	25.8	15.4	35.8	26.5	17.0	0.0	0.0	-0.1	-12.1
18	38.2	25.3	17.8	38.0	25.3	14.3	0.0	0.0	0.2	-5.7
19	37.3	27.1	17.0	37.9	25.5	16.5	0.0	0.1	0.0	-0.9
20	37.0	23.8	14.0	38.5	22.0	16.6	0.0	0.1	-0.2	-0.4
Av	36.1	25.8	15.9	36.8	25.6	15.7	0.0	0.0	0.0	-6.4
SD	1.6	1.3	1.2	1.1	1.6	1.1	0.0	0.0	0.1	4.5

(Print Length; PL,Toe Spread; TS, Intermediary Toe Spread; IT, experimental sides: EPL, ETS and EIT, non-operated side :NPL, NTS. and NIT)

2. Sciatic function index after 2 and 4 weeks in Group B

2w	EPL	ETS	EIT	NPL	NTS	NIT	PLF	TSF	ITF	SFI
1	36.7	21.9	11.1	34.4	22.7	12.1	0.1	0.0	-0.1	-16.3
2	34.7	21.3	11.4	33.3	21.7	12.6	0.0	0.0	-0.1	-13.7
3	36.1	21.5	12.6	34.6	24.4	14.1	0.0	-0.1	-0.1	-24.9
4	36.3	22.0	12.3	33.0	24.0	15.2	0.1	-0.1	-0.2	-24.3
5	34.8	21.5	11.5	33.6	22.8	12.5	0.0	-0.1	-0.1	-17.5
6	37.6	22.3	12.3	36.7	23.5	14.6	0.0	-0.1	-0.2	-17.4
7	39.1	22.1	11.1	38.3	23.4	13.0	0.0	-0.1	-0.1	-17.6
8	35.2	21.9	11.2	33.2	22.7	12.6	0.1	0.0	-0.1	-16.4
9	36.5	20.9	12.2	33.1	24.3	15.3	0.1	-0.1	-0.2	-30.7
10	36.8	21.8	12.4	34.7	24.4	14.1	0.1	-0.1	-0.1	-24.4
11	34.9	19.8	11.1	32.7	21.6	11.4	0.1	-0.1	0.0	-20.9
12	35.9	21.9	11.5	34.6	22.2	13.9	0.0	0.0	-0.2	-14.0
13	37.8	23.2	12.4	33.6	26.8	14.8	0.1	-0.1	-0.2	-30.5
14	37.7	22.1	12.8	35.0	24.8	14.0	0.1	-0.1	-0.1	-24.8
15	34.8	21.6	11.3	33.5	23.0	11.6	0.0	-0.1	0.0	-17.3
16	35.8	19.2	10.9	33.6	21.9	11.4	0.1	-0.1	0.0	-25.4
17	39.6	22.0	12.2	39.0	22.9	12.9	0.0	0.0	-0.1	-14.4
18	36.8	21.6	12.6	33.2	24.0	15.3	0.1	-0.1	-0.2	-26.3
19	36.3	21.4	11.1	33.9	22.8	12.0	0.1	-0.1	-0.1	-19.2
20	37.9	21.3	12.2	36.2	23.7	12.3	0.0	-0.1	0.0	-21.8
Av	36.6	21.6	11.8	34.5	23.4	13.3	0.1	-0.1	-0.1	-20.9
SD	1.4	0.9	0.6	1.8	1.2	1.3	0.0	0.0	0.1	5.3

4w	EPL	ETS	EIT	NPL	NTS	NIT	PLF	TSF	ITF	SFI
1	38.9	24.2	13.0	35.4	24.6	15.9	0.1	0.0	-0.2	-16.8
2	37.6	22.2	13.1	35.3	25.2	13.5	0.1	-0.1	0.0	-24.7
3	38.8	23.3	11.6	38.2	24.4	13.2	0.0	0.0	-0.1	-16.0
4	36.9	23.4	14.0	34.8	26.6	15.5	0.1	-0.1	-0.1	-25.6
5	35.5	22.8	14.9	33.2	25.9	15.0	0.1	-0.1	0.0	-24.6
6	38.8	22.9	14.5	35.0	25.3	15.4	0.1	-0.1	-0.1	-24.1
7	37.9	20.5	13.2	35.5	24.9	15.1	0.1	-0.2	-0.1	-32.4
8	37.3	22.0	11.0	36.7	23.4	11.8	0.0	-0.1	-0.1	-16.9
9	39.6	26.1	15.8	39.2	27.4	17.5	0.0	0.0	-0.1	-15.7
10	34.9	25.8	15.3	33.9	27.0	15.7	0.0	0.0	0.0	-15.1
11	39.2	24.2	13.5	35.7	24.4	16.5	0.1	0.0	-0.2	-15.9
12	37.0	22.5	13.4	35.2	25.0	13.9	0.1	-0.1	0.0	-22.2
13	38.4	23.3	12.2	37.5	24.9	13.3	0.0	-0.1	-0.1	-17.9
14	35.3	23.1	14.2	34.4	25.3	15.0	0.0	-0.1	-0.1	-20.0
15	38.1	20.3	13.2	37.3	24.1	14.9	0.0	-0.2	-0.1	-28.4
16	36.5	22.3	11.3	36.4	22.7	12.1	0.0	0.0	-0.1	-11.7
17	38.3	26.2	16.2	38.2	27.4	16.2	0.0	0.0	0.0	-13.7
18	34.8	25.4	15.1	34.0	27.1	15.7	0.0	-0.1	0.0	-17.1
19	38.1	20.9	13.2	37.9	24.6	15.0	0.0	-0.2	-0.1	-27.1
20	37.7	22.2	13.1	35.6	25.6	13.7	0.1	-0.1	0.0	-26.2
Av	37.5	23.2	13.6	36.0	25.3	14.7	0.0	-0.1	-0.1	-20.6
SD	1.4	1.7	1.4	1.6	1.3	1.5	0.0	0.0	0.1	5.7

(Print Length; PL,Toe Spread; TS, Intermediary Toe Spread; IT, experimental sides: EPL, ETS and EIT, non-operated side :NPL, NTS. and NIT)

3. Sciatic function index after 2 and 4 weeks in Group C

2w	EPL	ETS	EIT	NPL	NTS	NIT	PLF	TSF	ITF	SFI
1	39.8	15.1	12.0	35.9	22.6	13.9	0.1	-0.3	-0.1	-51.1
2	38.9	13.0	8.3	35.6	22.2	13.1	0.1	-0.4	-0.4	-62.6
3	40.1	16.1	8.5	38.9	20.1	10.8	0.0	-0.2	-0.2	-34.6
4	41.0	19.5	8.3	33.3	24.0	12.5	0.2	-0.2	-0.3	-42.7
5	40.2	14.2	9.8	33.8	22.2	11.5	0.2	-0.4	-0.1	-57.5
6	39.6	11.7	7.3	37.8	21.9	11.9	0.0	-0.5	-0.4	-66.8
7	39.6	14.8	9.6	34.0	22.4	11.4	0.2	-0.3	-0.2	-54.4
8	38.2	10.8	6.2	36.6	21.6	13.2	0.0	-0.5	-0.5	-72.3
9	40.4	11.1	7.6	33.4	20.9	11.3	0.2	-0.5	-0.3	-72.5
10	37.3	18.3	12.2	32.7	25.3	15.5	0.1	-0.3	-0.2	-47.3
11	40.0	20.2	9.1	32.8	23.7	12.6	0.2	-0.1	-0.3	-37.1
12	40.2	19.4	9.1	34.0	23.7	13.6	0.2	-0.2	-0.3	-40.1
13	40.3	15.0	11.9	36.7	22.3	13.6	0.1	-0.3	-0.1	-50.1
14	39.3	16.3	7.4	35.9	22.8	12.5	0.1	-0.3	-0.4	-49.1
15	41.7	16.8	8.7	39.9	20.0	11.4	0.0	-0.2	-0.2	-31.2
16	41.8	19.6	9.3	34.3	23.5	12.7	0.2	-0.2	-0.3	-38.9
17	37.8	19.5	13.2	33.3	25.6	16.4	0.1	-0.2	-0.2	-42.7
18	40.0	16.9	7.7	35.4	22.0	11.9	0.1	-0.2	-0.4	-43.9
19	40.7	15.1	11.8	36.0	22.6	13.4	0.1	-0.3	-0.1	-51.7
20	42.1	16.4	9.5	39.8	19.6	10.9	0.1	-0.2	-0.1	-30.6
Av	40.0	16.0	9.4	35.5	22.5	12.7	0.1	-0.3	-0.3	-48.8
SD	1.3	2.9	1.9	2.2	1.6	1.5	0.1	0.1	0.1	12.6

4w	EPL	ETS	EIT	NPL	NTS	NIT	PLF	TSF	ITF	SFI
1	36.3	22.3	14.3	35.1	25.1	17.2	0.0	-0.1	-0.2	-24.6
2	38.2	23.3	13.0	30.6	23.7	16.0	0.2	0.0	-0.2	-22.7
3	35.2	22.2	15.2	34.6	24.7	15.5	0.0	-0.1	0.0	-20.8
4	38.8	23.6	14.5	37.7	25.4	15.9	0.0	-0.1	-0.1	-18.8
5	39.9	21.2	12.4	37.3	23.2	13.9	0.1	-0.1	-0.1	-22.3
6	36.0	22.7	15.6	30.4	23.4	15.8	0.2	0.0	0.0	-19.3
7	38.5	22.6	13.3	30.0	23.1	15.2	0.3	0.0	-0.1	-23.7
8	38.5	22.0	13.4	36.3	22.8	14.9	0.1	0.0	-0.1	-16.3
9	35.9	21.7	14.1	35.4	25.1	16.7	0.0	-0.1	-0.2	-26.2
10	37.6	23.8	13.5	34.2	26.6	16.9	0.1	-0.1	-0.2	-26.8
11	35.9	20.4	12.9	32.7	27.6	17.9	0.1	-0.3	-0.3	-44.8
12	38.1	23.7	14.6	29.4	24.8	16.7	0.3	0.0	-0.1	-26.7
13	38.0	23.1	12.6	29.1	23.4	15.2	0.3	0.0	-0.2	-24.2
14	35.5	22.5	14.6	34.6	25.1	14.8	0.0	-0.1	0.0	-21.3
15	39.1	24.5	14.5	37.7	25.9	15.7	0.0	-0.1	-0.1	-17.2
16	39.3	21.4	11.8	37.1	23.7	14.1	0.1	-0.1	-0.2	-23.9
17	35.9	21.6	13.4	31.5	21.7	16.0	0.1	0.0	-0.2	-16.8
18	36.3	21.6	14.1	35.2	23.6	15.1	0.0	-0.1	-0.1	-20.2
19	39.0	25.4	16.6	36.9	26.3	17.9	0.1	0.0	-0.1	-15.7
20	36.6	20.5	12.8	34.2	27.7	18.1	0.1	-0.3	-0.3	-43.8
Av	37.4	22.5	13.9	34.0	24.6	16.0	0.1	-0.1	-0.1	-23.8
SD	1.5	1.3	1.2	2.9	1.6	1.2	0.1	0.1	0.1	7.8

(Print Length; PL,Toe Spread; TS, Intermediary Toe Spread; IT, experimental sides: EPL, ETS and EIT, non-operated side :NPL, NTS. and NIT)

4. The myelin thickness of group A, B and C in 2 weeks

2w	Group A	Group B	Group C
1	1017.71	833.34	736.45
2	1240.82	948.67	1198.99
3	832.32	1022.71	656.84
4	1198.99	1014.53	1224.26
5	973.97	1237.39	979.03
6	1360.63	1201.11	1155.64
7	1700.73	1085.65	1382.46
8	1521.65	885.60	998.30
9	1608.50	1386.89	1363.63
10	1216.45	1020.38	936.58
11	1242.47	1422.25	484.18
12	1601.72	1570.62	796.60
13	1407.83	749.05	1270.63
14	1504.11	1014.70	1096.88
15	1460.50	443.47	476.03
16	1325.67	1014.53	542.50
17	1417.58	1188.01	749.05
18	1344.66	1132.59	1524.33
19	709.87	676.49	1365.00
20	1522.99	1276.51	1365.50
21	1562.80	587.09	1093.92
22	1208.03	1098.27	1517.73
23	1397.15	1240.14	1233.81
24	1544.40	1453.02	800.86
25	1700.63	1291.35	587.09
26	1654.49	1200.26	437.29
27	1295.16	1006.95	247.48
28	1348.07	1028.52	855.90
29	1547.04	917.49	1039.39
30	1551.22	1507.16	808.68
31	1320.01	1048.83	472.45
32	1148.10	1171.86	585.93
33	1370.97	1051.58	298.00
34	809.94	911.16	1683.84
35	1345.04	1263.11	373.50
36	913.03	1160.92	340.13
37	948.13	734.60	240.51
38	1579.26	1365.50	378.48
39	1173.17	1140.97	851.12

40	1535.34	980.59	1748.87
41	1343.56	833.34	240.51
42	886.72	551.47	300.28
43	1017.89	1177.37	209.55
44	1154.21	704.86	253.45
45	1213.61	856.59	234.29
46	1417.75	1062.18	258.38
47	1200.89	1128.61	957.82
48	995.19	1293.89	545.93
49	1287.22	927.60	672.55
50	1239.79	1233.07	511.27
Av	1298.36	1061.06	801.64
SD	246.45	245.46	435.71

5. The myelin thickness of group A, B and C in 4 weeks

4w	Group A	Group B	Group C
1	1055.13	1064.76	420.63
2	1346.55	1034.45	425.86
3	1363.63	610.95	501.78
4	1082.67	562.22	450.32
5	1064.29	455.58	435.34
6	1095.94	501.78	418.20
7	1474.29	1089.41	516.49
8	1187.58	986.64	329.97
9	1620.41	1072.41	421.44
10	1185.72	613.17	527.57
11	1360.51	598.57	536.20
12	1136.64	991.29	380.27
13	1252.42	1270.63	397.34
14	1176.64	1254.32	560.10
15	1287.13	1312.91	358.16
16	1318.98	620.07	438.46
17	1085.02	613.17	446.53
18	1146.77	1032.97	649.81
19	1367.48	503.48	486.99
20	1276.47	603.10	479.59
21	1396.66	1083.30	564.34
22	1064.45	636.05	627.43
23	959.19	686.48	629.60

24	1388.70	604.23	613.17
25	1000.34	676.49	554.91
26	1702.73	829.04	524.66
27	1208.46	1239.18	609.00
28	1294.51	987.50	418.20
29	1312.91	516.82	466.65
30	1164.58	987.50	455.58
31	1209.16	609.00	661.23
32	1179.68	784.77	470.28
33	1284.61	506.51	685.49
34	1023.04	542.51	340.13
35	1538.99	486.99	405.81
36	1430.01	979.41	458.49
37	1798.74	577.11	591.95
38	1426.55	395.24	429.07
39	1618.21	1308.71	365.40
40	1702.73	501.60	490.99
41	1062.53	439.16	729.68
42	1452.08	810.86	449.18
43	1840.31	560.41	338.64
44	1359.37	560.41	378.36
45	1334.97	521.33	524.31
46	1293.20	452.63	652.85
47	1214.40	468.57	463.80
48	1084.84	439.32	597.32
49	1321.57	401.76	517.04
50	1140.12	412.14	474.39
Av	1293.84	735.94	493.38
SD	207.81	280.48	98.45

6. G-ratio of group A in 2 weeks

2 w	Fiber diameter	Axon diameter	G ratio
1	4359.06	1725.36	0.40
2	7173.67	4275.17	0.60
3	4036.34	2414.10	0.60
4	6865.89	4024.09	0.59
5	4036.46	1539.99	0.38
6	6610.24	3978.39	0.60
7	6733.56	3225.04	0.48
8	7909.42	4740.07	0.60
9	7776.34	4610.53	0.59
10	5256.58	2316.86	0.44
11	5390.01	2961.71	0.55
12	9669.92	6283.01	0.65
13	8075.37	4355.90	0.54
14	6824.93	4233.94	0.62
15	9427.74	5997.18	0.64
16	7981.15	4505.12	0.56
17	8294.77	5311.44	0.64
18	8769.36	5480.96	0.63
19	7680.63	3428.22	0.45
20	3834.63	2327.48	0.61
21	6475.03	3074.73	0.47
22	6793.75	4397.92	0.65
23	8856.67	4682.69	0.53
24	8766.20	5591.29	0.64
25	8691.88	5452.08	0.63
26	10378.80	5040.34	0.49
27	6790.37	2806.76	0.41
28	7945.78	5235.05	0.66
29	7048.31	4061.67	0.58
30	10123.79	6222.30	0.61
31	6157.14	3606.79	0.59
32	9496.35	6712.96	0.71
33	9946.07	7008.93	0.70
34	4304.28	2791.75	0.65
35	11138.38	8113.29	0.73
36	5958.07	4021.43	0.67
37	5945.12	4056.52	0.68
38	9832.11	6211.55	0.63
39	8097.02	5332.44	0.66

40	8266.28	5080.18	0.61
41	9641.88	6579.78	0.68
42	6402.19	4286.93	0.67
43	6135.14	4499.43	0.73
44	6030.71	4251.85	0.71
45	6919.53	4567.17	0.66
46	8290.21	5822.50	0.70
47	5981.88	3121.98	0.52
48	8640.23	5975.72	0.69
49	5134.37	2557.94	0.50
50	5959.44	2676.41	0.45
Ave	7337.06	4431.50	0.60
Sd	1810.08	1454.71	0.09

7. G-ratio of group B in 2 weeks

2 w	Fiber diameter	Axon diameter	G ratio
1	4600.89	3000.06	0.65
2	4649.33	2778.24	0.60
3	5192.86	3368.40	0.65
4	6100.59	3828.10	0.63
5	7491.19	5440.15	0.73
6	7947.19	5464.05	0.69
7	6030.25	2508.45	0.42
8	4294.58	3135.00	0.73
9	5096.23	3095.24	0.61
10	6480.44	3707.64	0.57
11	6519.02	3973.38	0.61
12	4275.01	2368.06	0.55
13	5924.45	4056.69	0.68
14	7904.62	4021.26	0.51
15	2835.46	2062.32	0.73
16	4934.73	2501.18	0.51
17	6641.18	3871.59	0.58
18	6380.54	3935.56	0.62
19	5316.34	3200.57	0.60
20	3397.26	2457.12	0.72
21	4434.59	2783.14	0.63
22	5988.46	3594.74	0.60
23	8577.92	5554.56	0.65

24	9329.97	6315.98	0.68
25	8828.08	6367.03	0.72
26	8463.66	5678.51	0.67
27	9096.10	6027.28	0.66
28	8149.56	5253.73	0.64
29	8354.57	5421.64	0.65
30	5772.29	4358.40	0.76
31	6345.52	4154.44	0.65
32	3892.49	2015.68	0.52
33	5415.61	3661.42	0.68
34	8141.04	6055.78	0.74
35	9339.79	6471.35	0.69
36	6040.65	3731.01	0.62
37	5779.95	3590.33	0.62
38	6613.02	3914.24	0.59
39	8566.97	5953.24	0.69
40	7606.98	5400.13	0.71
41	5017.30	3622.79	0.72
42	2830.17	1648.01	0.58
43	5171.01	2562.81	0.50
44	4111.03	2814.53	0.68
45	5564.55	3808.78	0.68
46	6857.51	4533.45	0.66
47	8224.30	6545.64	0.80
48	4446.75	2465.36	0.55
49	6446.63	3922.26	0.61
50	7014.70	4528.14	0.65
Ave	6248.67	4030.55	0.64
Sd	1727.48	1335.34	0.07

8. G-ratio of group C in 2 weeks

2 w	Fiber diameter	Axon diameter	G ratio
1	4004.30	2725.51	0.68
2	8052.35	5329.38	0.66
3	3162.93	1897.78	0.60
4	4299.97	2274.92	0.53
5	7862.15	5413.19	0.69
6	6152.99	3378.23	0.55
7	9444.22	6547.38	0.69

8	5635.65	3327.28	0.59
9	6557.27	4001.41	0.61
10	3593.89	1863.41	0.52
11	4151.00	3278.25	0.79
12	4900.52	3180.26	0.65
13	8526.92	5817.06	0.68
14	2230.21	1281.30	0.57
15	2934.76	1985.06	0.68
16	5274.70	3647.08	0.69
17	3673.71	2313.70	0.63
18	6026.86	2914.52	0.48
19	11389.54	7590.50	0.67
20	7495.87	4695.94	0.63
21	8560.91	5829.04	0.68
22	6409.54	3878.04	0.61
23	7610.16	4619.60	0.61
24	5569.49	3981.81	0.71
25	4042.70	2018.29	0.50
26	3715.02	3093.48	0.83
27	4004.30	2873.78	0.72
28	4184.92	2507.90	0.60
29	3893.67	2904.17	0.75
30	4279.62	2545.61	0.59
31	6496.67	4272.66	0.66
32	2576.37	1443.62	0.56
33	2356.25	1812.03	0.77
34	8521.99	5095.66	0.60
35	3093.48	2384.74	0.77
36	2851.08	2281.64	0.80
37	3193.34	2441.84	0.76
38	3083.07	2606.69	0.85
39	3764.29	2138.86	0.57
40	6661.52	3269.15	0.49
41	2381.81	1920.60	0.81
42	2720.64	1865.23	0.69
43	2136.54	1722.25	0.81
44	2566.40	2175.72	0.85
45	3254.82	2820.42	0.87
46	2066.33	1525.03	0.74
47	4183.86	2925.48	0.70
48	6456.37	4426.23	0.69

49	3764.78	2419.38	0.64
50	3523.05	2547.48	0.72
Ave	4865.86	3196.17	0.67
Sd	2219.94	1418.02	0.10

9. G-ratio of group A in 4 weeks

4 w	Fiber diameter	Axon diameter	G ratio
1	7256.56	4523.02	0.62
2	6382.41	4795.95	0.75
3	7255.34	2582.43	0.36
4	4588.74	2545.61	0.55
5	4258.58	2401.32	0.56
6	6741.46	4219.65	0.63
7	9105.82	5520.55	0.61
8	8988.94	6306.39	0.70
9	7990.25	5414.19	0.68
10	7807.72	5024.79	0.64
11	6778.73	3792.97	0.56
12	5895.24	3342.74	0.57
13	6539.89	3573.85	0.55
14	6884.59	4461.10	0.65
15	5453.58	1901.37	0.35
16	4984.71	2344.38	0.47
17	5347.16	3197.75	0.60
18	6702.76	3862.66	0.58
19	6379.77	3207.90	0.50
20	6683.17	3731.01	0.56
21	8659.88	5893.94	0.68
22	6640.75	4252.42	0.64
23	7379.14	5638.10	0.76
24	5950.93	3144.48	0.53
25	5486.39	3120.86	0.57
26	8182.73	5138.08	0.63
27	9471.76	6321.56	0.67
28	7820.32	4908.50	0.63
29	7657.89	5304.29	0.69
30	7582.25	5214.83	0.69
31	7319.14	4711.38	0.64
32	8125.65	5454.05	0.67

33	6971.35	4487.98	0.64
34	6972.08	5077.60	0.73
35	8953.89	5608.66	0.63
36	9887.87	6696.29	0.68
37	11269.41	7296.82	0.65
38	11204.46	8027.64	0.72
39	11724.74	8241.54	0.70
40	8717.29	5338.02	0.61
41	5453.39	3137.52	0.58
42	10336.54	7497.19	0.73
43	11841.12	7848.54	0.66
44	9856.98	5916.07	0.60
45	11144.05	7182.12	0.64
46	7349.91	4572.63	0.62
47	7371.58	5254.15	0.71
48	6543.66	4341.43	0.66
49	6526.26	3610.97	0.55
50	7355.30	5110.26	0.69
Ave	7635.64	4821.95	0.62
Sd	1852.62	1552.97	0.08

10. G-ratio of group B in 4 weeks

4 w	Fiber diameter	Axon diameter	G ratio
1	5245.35	2613.08	0.50
2	7510.83	5258.39	0.70
3	5190.10	4031.61	0.78
4	4784.33	3731.10	0.78
5	5207.51	4288.67	0.82
6	3737.75	2674.66	0.72
7	8973.91	6648.35	0.74
8	9915.50	7545.09	0.76
9	5376.77	3203.44	0.60
10	3344.62	2239.34	0.67
11	4605.77	3415.29	0.74
12	5997.91	3985.44	0.66
13	8113.08	5827.17	0.72
14	9102.66	6699.36	0.74
15	6076.54	3359.60	0.55
16	5142.29	3963.48	0.77

17	4242.73	3093.87	0.73
18	6892.82	5413.09	0.79
19	10498.02	9092.06	0.87
20	6415.51	5023.94	0.78
21	6037.35	3715.38	0.62
22	6991.24	5224.64	0.75
23	2995.41	1496.97	0.50
24	3397.26	1756.24	0.52
25	4517.49	2952.56	0.65
26	6171.98	4823.68	0.78
27	6617.19	4057.61	0.61
28	5634.48	3038.60	0.54
29	4538.64	3287.42	0.72
30	4850.13	3622.18	0.75
31	5024.65	3047.60	0.61
32	6202.72	4128.68	0.67
33	3663.28	2748.69	0.75
34	5914.80	4793.75	0.81
35	5306.54	3991.24	0.75
36	7458.97	4899.24	0.66
37	3274.04	2502.45	0.76
38	4592.83	3520.00	0.77
39	8127.74	5589.13	0.69
40	6002.22	4401.43	0.73
41	4384.05	3359.21	0.77
42	10463.19	8429.89	0.81
43	4770.84	3439.59	0.72
44	3855.37	2859.84	0.74
45	3917.44	2836.27	0.72
46	2757.36	2164.75	0.79
47	2941.41	2097.82	0.71
48	4729.85	3591.69	0.76
49	3543.21	2644.07	0.75
50	2512.77	1569.09	0.62
Ave	5551.37	3973.93	0.71
Sd	1979.49	1648.14	0.09

11. G-ratio of group C in 4 weeks

4 w	Fiber diameter	Axon diameter	G ratio

1	2537.58	1730.48	0.68
2	3104.73	2048.17	0.66
3	4331.66	3326.67	0.77
4	3391.65	2296.95	0.68
5	3798.12	3063.64	0.81
6	5018.96	4062.30	0.81
7	4769.91	3819.07	0.80
8	2765.17	2045.84	0.74
9	5671.40	4767.09	0.84
10	4779.60	3783.58	0.79
11	3757.59	2848.09	0.76
12	3166.10	2250.48	0.71
13	2844.51	2005.95	0.71
14	4475.38	3370.11	0.75
15	4395.95	3679.03	0.84
16	3383.06	2371.22	0.70
17	4700.93	3509.58	0.75
18	6278.48	4948.37	0.79
19	4050.93	2943.10	0.73
20	6390.53	4781.88	0.75
21	5258.00	3463.77	0.66
22	5887.15	4205.88	0.71
23	7501.88	6077.24	0.81
24	6797.15	5210.16	0.77
25	6315.42	4531.44	0.72
26	6264.68	4751.79	0.76
27	5559.06	4451.44	0.80
28	3448.89	2593.54	0.75
29	5795.94	4827.70	0.83
30	4619.97	3576.04	0.77
31	4977.06	3042.02	0.61
32	5227.63	4383.16	0.84
33	7180.62	4206.32	0.59
34	2958.32	2292.05	0.77
35	2999.50	2295.09	0.77
36	3415.70	2407.36	0.70
37	4584.36	3118.32	0.68
38	5608.16	4467.14	0.80
39	4129.19	3136.51	0.76
40	3694.13	2616.86	0.71
41	5623.97	4564.65	0.81

42	4410.92	3161.10	0.72
43	4351.05	3348.26	0.77
44	3628.33	3019.85	0.83
45	4930.18	3590.70	0.73
46	5546.87	3820.17	0.69
47	4596.06	3268.69	0.71
48	5269.72	3759.99	0.71
49	4267.97	3070.76	0.72
50	5454.20	3977.34	0.73
Ave	4678.29	3497.74	0.75
Sd	1210.97	975.65	0.06

국문요약

백서 모델에서 신경 견인 손상에 의한 기능적 회복 및 전자현미경적 변화에 대한 고찰

김홍준

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개요: 구강악안면외과 영역의 수술에 있어서 신경 손상에 의해 술후 나타나는 일시적 또는 영구적 기능 저하는 종종 발생하는 일이다. 수술 과정에서 의도치 않게 신경을 과도하게 신장시키거나 견인함으로써 발생하는 신경 손상은 가장 대표적인 예라고 할 수 있다. 쥐의 좌골 신경은 인간의 안면 신경과 비슷한 직경을 가지고 있는 것으로 알려져 있다. 좌골 신경에서의 연구를 통해 실제 수술에서 신경에 견인력을 가했을 때 일어날 수 있는 변화 및 회복에 필요한 시간에 대해 알아보고 적용하고자 하였다.

재료 및 방법: 18 마리의 수컷 백서를 3 그룹으로 분류하였고, 각 군의 개체수는 6 마리였다. A 군에서는 백서를 마취하고 좌측 하지를 절개한 후에 좌골 신경을 노출시키고 어떠한 외력도 가하지 않았으며, B 군과 C 군에서는 좌골 신경을 노출시킨 후에 각각 150g, 300g 의 견인력을 1 분 동안 적용하였다. 수술 후 2 주, 4 주에 보행 분석을 통해 운동 능력을 평가하였고, 전자 주사 현미경적 관찰을 통해 신경초와 슈반세포의 회복 정도를 분석하였다.

결과: 수술 2 주 후, A 군의 좌골신경 기능지수(SFI)는 -6.59±5.14 였고, B 군은-20.89±5.26, C 군은 -48.85±12.58 이었다. 4 주 후에는 SFI 가 A 군은 -6.45±4.47, B 군은 -20.65±5.71, C 군은-23.80±7.81 이었다. 전자 주사 현미경 결과에서 2 주와 4 주의그룹 B 와 C 모두 축색돌기의 직경감소와 수초탈락이 관찰되었다. 수술 후 2 주에서는 그룹 A, B, C 의 평균 수초 두께는 각각 1298.36±246.45, 1061.06±245.46, 801.64±435.71 nm 이었으며, 4 주 후에는 1293.84±207.81, 735.94±280.48, 493.38±98.45 이었다. 2 주 후, 전자주사 현미경 검사에서 G-ratio 는 A 군에서 0.60±0.09, B 군은 0.64±0.07, C 군은 0.67±0.10 이었으며, 4 주 후에는 각각 0.62±0.08, 0.71±0.09 그리고 0.75±0.06 으로 계산되었다.

결론: 처음 2 주 동안에는 백서의 좌골 신경에서의 기능적 소실의 정도는 가해진 견인력의 크기에 비례한 것으로 분석되었다. 하지만, 300g 의 견인력에 의한 손상은 일시적이었으며, 술후 2 주에서 4 주 사이에 기능이 회복되어서, 술후 4 주 째에는 150g 의 견인력을 가한 군과 비슷한 정도까지 운동 기능을 회복한 것이 관찰되었다. 150g 과 300g 의 견인력을 가한 두 군 모두에서 전자주사 현미경 검사 상에서 해부학적으로 퇴행성 변화가 진행되는 양상이 관찰되었다.

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중심어: 신경 견인 손상, 좌골신경, 백서, 신경 재생, 기능회복, 좌골신경 기능지수, 전자주사 현미경