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Original Article

Influence of the Combination of Single Fascicle Grafting and Vascular Epithelial Growth Factor on the Nerve Reconstruction in the Rat Sciatic Nerve Injury Model: Experimental Study

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ABSTRACT

Background and Aim: The purpose of this study was to evaluate the regeneration effects after sciatic nerve reconstruction with single fascicle grafting + vascular endothelial growth factor (VEGF) compared with traditional epineural suture. **Methods**: Sixty-four Wistar rats were divided into 2 groups randomly and created 10-25 mm sciatic nerve defects. In control group, nerve was reconstructed using traditional epineural suture and the rats of the study group were treated with single fascicle grafting + VEGF. At the point of 6 and 12 weeks, testing consisted of sciatic nerve function index (SFI), gastrocnemius muscle weight, toe spread test, pin-prick test was undergone. **Results**: The rats of 25mm defect subgroup repaired with single fascicle grafting + VEGF demonstrated significantly higher SFI score, toe spread test score, re- innervated muscle weight at the points of 6, 12 weeks. And in the 20, 25 mm defect rats of study group, pin-prick test score were significantly higher than the control group. **Conclusion:** Single fascicle grafting + VEGF was better to reconstruct long nerve defect compared to epineural suture.

Key words: Single fascicle grafting, Nerve reconstruction, Sciatic nerve injury model, Vascular endothelial growth factor.

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INTRODUCTION

The development of the operating microscope, improved microsurgical techniques, and a greater understanding of the internal topography of peripheral nerves has greatly improved functional outcomes. The best treatment after peripheral nerve injury consists of a primary, tension-free suture with matching of intraneural topography. In cases where a tension-free primary end-to-end neurorrhaphy is not possible, several alternatives exist. To bridge nerve defects, at present, autologous nerve graft offers the best outcome. Several synthetic substances and biogenic conduits are also applied but none of these material seem to be as effective as autologous nerve tissue.[1] Peripheral nerve allografting offers an unlimited supply of nerve for reconstruction but requires long-term immunosuppression. Although single fascicle grafting usually has not better outcome compared to end-to-end epineural suture, in some cases, we suggest its indication that traditional technique could not be applied.

Vascular endothelial growth factor (VEGF) represents one of the main factors involved not only in angiogenesis and vasculogenesis but also in neurogenesis. Since the vascular and the nervous system show similar anatomical features, an increasing number of studies focus the attention on VEGF activity on different neural cell types and recent evidence shows a role for VEGF as a neurotrophic and neuroprotective factor for neurons and glial cells. In fact, VEGF stimulates the proliferation of neuronal precursors in vitro and in vivo models of neurogenesis.[2] Furthermore it has been reported that VEGF administration enhances axonal outgrowth from dorsal root ganglia adult mice explants promoting the survival of neurons and satellite glial cells.[3-5]

Evidence has also been provided that VEGF administration increases the functional recovery after peripheral nerve injury since it was shown that after end-to-end neurorraphy and end-to-side neurorraphy of transected musculocutaneous rats nerves, plasmid VEGF transfection in the distal stumps resulted in a better axon regeneration in terms of fiber density, axon diameter and myelin sheath thickness of regenerated axons.[6]

In this study, we compared the regeneration effect after the sciatic nerve reconstruction by single fascicle grafting+ VEGF treatment in 10, 15, 20 and 25 mm nerve defect models.

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MATERIALS AND METHODS

Animals

Mature Wistar rats (200-250g) were provided by Laboratory Animal Centre of the Pyongyang University of Medical Sciences and adapted in a lab environment before experiments for a week. Sixty-four rats were randomly chosen and divided into two groups (study and control). During the experiment, feed and water were available to rats at any time. The temperature was maintained at 20±2°C and the humidity was 55%. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Basic Medicine, Pyongyang University of Medical Sciences.

Surgical Model

Anesthesia was induced by intraperitoneal injection of 50mg/kg thiopental sodium in rats. The rats were fixed on dorsal position and shaved the left femoral region. Using aseptic technique, following a mid-thigh incision in all animals, a standard biceps femoris semitendinosis muscle splitting approach was used to expose the sciatic nerve and major branches. And then 10, 15, 20 and 25mm gaps were created between the proximal and distal stumps. In the study group, the epineurium was dissected from the graft and split the largest fascicle. Utilizing microsurgical technique, microscope visualization, and with 10-0 nylon suture, the gap were reconstructed with a single fascicle. The suture was passed through the stump's center zone and fascicle's perineurium. And it was rotated in a 180-degree arc and underwent one more suture. And then VEGF was injected in the epineurium of the proximal stump.

In the control group, the direct nerve repair with 6 epineural sutures was performed on the proximal and distal stump.

Sciatic Nerve Function Index (SFI)

After 6 and 12 weeks, functional recovery was assessed by calculating SFI on walking-track testing.[2] From the footprints, 6 parameters were obtained: print length (PL) is the distance from the heel to the top of the third toe, intermediary toe (IT) spread is the distance from the second to the fourth toe, and toe spread (TS) is the distance between the first and the fifth toe. The SFI was calculated according to the equation:

$$SFI = -38.3 \times (EPL - NPL)/NPL + 109.5 \times (ETS - NTS)/NTS + 13.3 \times (EIT - NIT)/NIT - 8.8$$

where E is for the experimental foot and N is for the non-operated foot.[7]

Gastrocnemius Muscle Weight

Recovery of muscle re-innervation assessment was also indexed using the weight ratio of the gastrocnemius muscles 6, 12 weeks after surgery. Immediately after sacrificing of animals, gastrocnemius muscles were dissected and harvested carefully from intact and injured sides and weighed while still wet using an electronic balance. All measurements were made by two blinded observers unaware of the analyzed group.

Toe Spread Test

At periods of 6 weeks, toe spread test was performed to evaluate motor recovery. The rats were lifted by the tail to note the rat's response. The absence of any movement was scored "0", the presence of any sign of toe-spread was scored "1", toe abduction as "2" and both abduction and extension of the toes were accepted as score "3".

Pin-Prick Test

Another way to evaluate the functional recovery was pin-prick test. It was performed by applying pinching stimuli to the hind-limb skin from the knee to the toes until a withdrawal reflex of the extremity was obtained in the response to the pain stimulus. The reflex was graded as 1 when obtained above the knee, as 2 when obtained distal to ankle and proximal plantar region and 3 when obtained at the toe level. The grade was accepted as 0 in the absence of withdrawal reflex.

Statistical Analysis of Data

Results were expressed as the mean and SD. Data were analysed by one-way analysis of variance (ANOVA) using SPSS 16.0. P value of < 0.05 was taken as the level of statistical significance.

RESULTS

Sciatic Nerve Function Index (SFI)

After operation, the sciatic nerve function index closed to -100, showing total impairment. The values of SFI increased in the study group of the combination of single fascicle grafting and VEGF than the control group as the defect size lengthened. As shown in Table 1, the value of SFI was higher significantly than the control group 6, 12 weeks later in the 25mm defect rats of the study group.

Gastrocnemius Muscle Weight

Six weeks after operation, dystrophy of the surgical limb were noticed macroscopically. Changes of the gastrocnemius muscle weight were showed in Table 2. The gastrocnemius muscle weight increased significantly compared to the control group 6, 12 weeks later in the 25mm defect rats of the study group.

Toe Spread Test

After 3 weeks, motor function recovery were not observed in both study and control group. Since 6 weeks, motor function started to recover and toe spread reflex appeared with abduction. Since 12 weeks, the motor function didn't improve anymore. 12 weeks later, in the 25mm defect animals of the study group the score increased significantly than the same size defect ones of control group (Table 3).

Pin Prick Test

Three weeks later, the average skin sensory was noticed above the knee. Since 6 weeks later, the test had positive results at the region of toes and feet. After 6 and 12 weeks, the score increased in the 20, 25mm defect animals of study group significantly compared to same size defect ones of control group (Table 4).

DISCUSSION

Peripheral nerve injury represents a very complex process that involves different morphological and molecular changes occurring to both

Table 1: Sciatic functional index values during the experimental periods.

Defect size (mm)	Post-operative week	
	6	12
Control 10	-45.3±1.59	-32.7±2.45
15	-51.8±2.83	-43.2±1.38
20	-59.6±2.72	-45.5±2.62
25	-64.4±2.28	-58.6±2.64
Study 10	-52.3±2.21	-44.2±1.46
15	-53.7±1.76	-48.3±2.43
20	-54.6±2.38	-44.3±1.96
25	-55.4±1.83*	-47.6±2.42*

Each value represents the mean ± SD of 8 rats per group. *P<0.05 as compared with same size defect rats of control group.

Table 2: Gastrocnemius muscle weight during the experimental period.

Defect size (mm)	Post-operative week	
	6	12
Control 10	3.23±0.13	3.42±0.09
15	3.05±0.12	3.28±0.13
20	2.64±0.14	2.96±0.11
25	2.42±0.08	2.69±0.12
Study 10	3.02±0.11	3.55±0.08
15	2.87±0.09	3.04±0.12
20	2.81±0.12	2.98±0.11
25	2.78±0.09*	2.88±0.12*

Each value represents the mean ± SD of 8 rats per group. *P<0.05 as compared with same size defect rats of control group.

Table 3: The value of the toe spread test.

Defect size (mm)	Post-operative week	
	6	12
Control 10	2.78±0.08	2.82±0.12
15	2.63±0.11	2.71±0.09
20	2.42±0.12	2.48±0.11
25	2.15±0.08	2.22±0.12
Study 10	2.84±0.11	2.86±0.08
15	2.71±0.09	2.74±0.12
20	2.41±0.12	2.52±0.11
25	2.36±0.09*	2.46±0.12*

Each value represents the mean ± SD of 8 rats per group. *P<0.05 as compared with same size defect rats control group.

Table 4: The value of the pin-prick test.

Defect size (mm)	Post-operative week	
	6	12
Control 10	2.42±0.08	2.52±0.12
15	2.15±0.11	2.28±0.09
20	2.12±0.12	2.18±0.11
25	2.05±0.08	2.12±0.12
Study 10	2.34±0.11	2.56±0.08
15	2.21±0.09	2.34±0.12
20	2.31±0.12*	2.46±0.11*
25	2.26±0.09*	2.14±0.12*

Each value represents the mean ± SD of 8 rats per group. *P<0.05 as compared with same size defect rats of control group.

proximal and distal stumps.[8,9,10] Any break or defect in the axonal or neuronal bilayer lipid membrane unless rapidly repaired results in an irreversible cascade of programmed cell death.[11] Axonal degeneration follows a sequence of events within the zone of trauma extending both proximally and distally. Disconnected axons and cell bodies (in proximal axon injuries) degenerate via chromatolysis.[12] Reconstruction of peripheral nerve remains a challenging work. Today, reconstruction with autologous nerve grafts still represents to gold standard for clinical reconstruction of nerve defects. However nerve regeneration through autografts, as well as natural or engineered conduits remains suboptimal

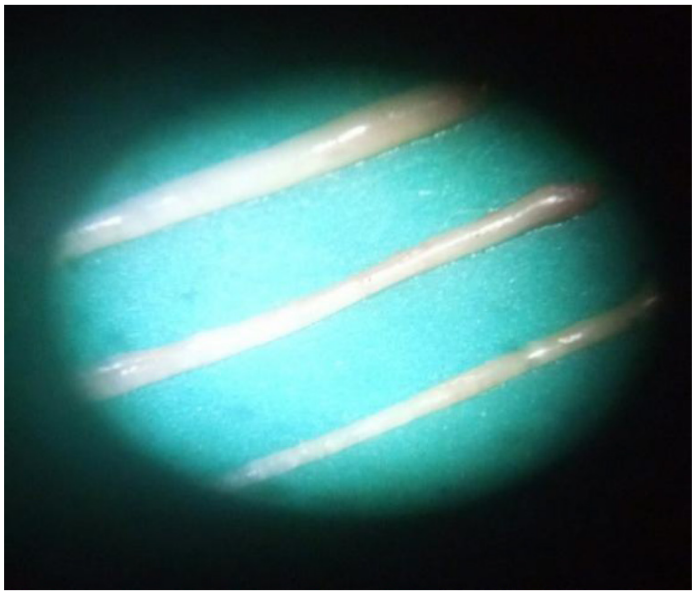

Figure 1: Split fascicles.

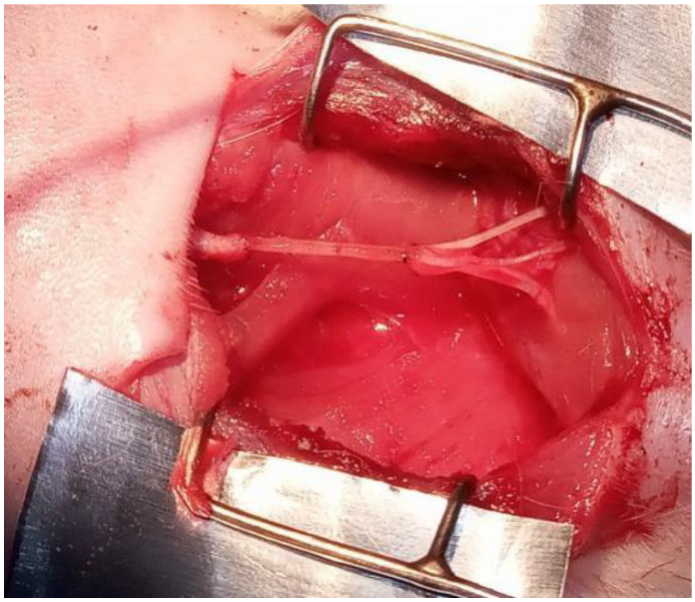

Figure 2: Single-fascicle grafting.

due to neuronal death, fibrosis, and delayed ingrowth of axons into the distal stump. Several studies have focused on enhancing nerve regeneration and overcoming these problems with the use of growth factors. Vascular and nervous systems share common molecular pathways during development and regeneration; furthermore the anatomical parallelism between vessel and nerve patterning is well documented. Furthermore evidences show that axon guidance and vessel navigation are regulated by similar classes of molecules.[13,14] Single fascicle grafting is one of the alternative reconstruction and has not widely applied. But in cases of combination with some growth factors, this method might result in higher outcome of nerve recovery. And VEGF has positive effect also when single fascicle grafting is applied. This combination treatment could take another chance of great functional recovery after wide nerve injury.

CONCLUSION

Our results suggest that the combination of new reconstruction method and vascular epithelial growth factor used in the present study have potential for enhancing of peripheral nerve regeneration and nerve function recovery. The longer nerve defect, the better this combination of single fascicle grafting and VEGF has a reconstruction effect. It is assumed that single fascicle grafting +VEGF might be more beneficial for the treatment of long nerve injury. Further study is required to determine the more effective dose and administration stage of VEGF.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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ABBREVIATIONS

VEGF: Vascular Endothelial Growth Factor; **SFI:** Sciatic Nerve Function Index; **PL:** Print Length; **IT:** Intermediary Toe; **TS:** Toe Spread.

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