Electric stimulation and decimeter wave therapy improve the recovery of injured sciatic nerves

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Research Highlights
(1) This study concluded that decimeter wave therapy promoted the proliferation of Schwann cells and elevated S-100 protein expression in injured sciatic nerves, and contributed to neural regeneration and functional recovery at cellular and molecular levels.
(2) Decimeter wave therapy promoted axonal regeneration and remyelination, delayed myatrophic and facilitated neural regeneration and functional recovery.
(3) Decimeter wave therapy inhibited the inflammatory reaction and anticoagulation, improved the local blood circulation, reduced the formation of scar and effectively prevented nerve adhesion and re-entrapment after repairing.

Abstract
Drug treatment, electric stimulation and decimeter wave therapy have been shown to promote the repair and regeneration of the peripheral nerves at the injured site. This study prepared a Mackinnon’s model of rat sciatic nerve compression. Electric stimulation was given immediately after neurolysis, and decimeter wave radiation was performed at 1 and 12 weeks post-operation. Histological observation revealed that intraoperative electric stimulation and decimeter wave therapy could improve the local blood circulation of repaired sites, alleviate hypoxia of compressed nerves, and lessen adhesion of compressed nerves, thereby decreasing the formation of new entrapments and enhancing compressed nerve regeneration through an improved microenvironment for regeneration. Immunohistochemical staining results revealed that intraoperative electric stimulation and decimeter wave could promote the expression of S-100 protein. Motor nerve conduction velocity and amplitude, the number and diameter of myelinated nerve fibers, and sciatic functional index were significantly increased in the treated rats. These results verified that intraoperative electric stimulation and decimeter wave therapy contributed to the regeneration and the recovery of the functions in the compressed nerves.

Key Words
neural regeneration; peripheral nerve injury; physical therapy; electric stimulation; sciatic nerve compression; Schwann cells; functional recovery; neuroregeneration
INTRODUCTION

Nerve compression syndromes have long been a common clinical problem in hand surgery. Sophisticated microsurgery skills have much improved neurorrhaphy and a number of biologically active factors and gene transfer have been used to promote nerve regeneration. However, the functional recovery of an injured nerve is often unsatisfactory. Peripheral nerve regeneration involves a complex process of biochemical and cellular events, and the microenvironment of the regenerating nerve very much influences its repair. How to protect the nerve to avoid the secondary damage is currently an unresolved problem. Moreover, mechanisms underlying neural repair and regeneration are very complicated. Some scholars treated patients with flexor tendons injury after repairing and patients with peripheral nerve diseases of diabetes by decimeter wave, they found that decimeter wave could accelerate tendon intrinsic healing and inhibit extrinsic healing after tendon repairing and then reduce tendon adhesion\cite{1-13}. They also deduced that decimeter wave could improve local blood circulation of the repairing site, alleviate hypoxia of regenerating nerves and increase the expression of immunologic reaction to S-100 protein in Schwann cells\cite{14-21}. Some bioactive substances and relevant drugs that are effective in promoting neural regeneration have been reported in recent studies\cite{22-33}. Decimeter wave, a long-wavelength electromagnetic radiation with effects of improving local blood circulation, accelerating metabolism, reducing local inflammation and alleviating pain, has attracted the attention of many researchers and clinical workers. Some scholars\cite{7, 12-13} gave decimeter treatment to patients recovering from flexor tendons injury and patients with peripheral nerve diseases of diabetes, and found that the excellent and good rate of tendon function recovery was 97% according to TAM standard and the whole effective rate of treatment to the pathological nerves was 83%. He deduced that decimeter waves could accelerate intrinsic tendon healing and inhibit extrinsic healing after tendon repairing and then reduce tendon adhesion. This could alleviate pain and be advantageous for patients’ earlier rehabilitation exercises.

Physical therapy is a type of natural therapy, has no side effects and is relatively cheap. Previous studies have shown that physical therapy promotes nerve regeneration; however, mechanisms underlying the improvement have not been investigated.

This study aims to investigate the mechanism of the effects of electric stimulation and decimeter waves on peripheral nerve regeneration, and then to provide a theoretical basis for clinical applications. It was investigated by anatomical observation, light microscopy, electron microscopy, immunohistochemistry, and morphometric analysis in a Mackinnon’s model of sciatic nerve compression in adult Sprague-Dawley rats.

RESULTS

Quantitative analysis of experimental animals

Under anesthesia, the right sciatic nerves of the 90 healthy adult Sprague-Dawley rats were each compressed by a silicone tube similar to the model of Mackinnon. A total of 90 healthy adult Sprague-Dawley rats were randomly divided into two groups as follows ($n = 45$ in each group): the intraoperative electric stimulation and decimeter wave group and the control group. At 4 weeks post-operation, three rats in each group were randomly sacrificed; the original wounds were reopened and simple decompression was applied, such that the silicone tube was simply moved and the epineurium of the entrapped segment was not decompressed. The animals in the intraoperative electric stimulation and decimeter wave group were treated with electric stimulation. After thorough hemostasis, the intermuscular space was closed and the skin was sutured. A DXZ-1 polymorphism wave therapy instrument was used in the present study. The rats of the intraoperative electric stimulation and decimeter wave group were fixed on a table prostrated and the right posterior thigh was exposed to decimeter waves.
every day from 1 day post-operation to the sacrifice day. The rats of the control group were also fixed on a table prostrated at the same time, but were not exposed to decimeter waves. At 1, 2, 3, 4 and 12 weeks post-operation, the samples were observed by anatomical, light and electron microscope observation, morphometric analysis and S-100 protein immunochemical staining. At 12 weeks post-operation, the latency, nerve conduction velocity and amplitude of compound muscle action potentials were measured electrophysiologically. At 4, 8 and 12 weeks post-operation, the sciatic functional indices were analyzed. All data were analyzed statistically using two-sample t-test. Ninety rats were involved in the final analysis.

**Effects of electric stimulation and decimeter wave on general morphology of sciatic nerves in rats with sciatic nerve compression**

Stage I healing was observed in each group, without infection, plantar ulceration or autophagocytic phenomena. At each time point, hyperemia and edema were observed in the subcutaneous tissues and tissues surrounding the nerves 1 week post-operation. Signs of entrapment were also clearly visible. The entrapped segment became thin with an enlarged neuroma-like appearance at both ends. These changes were smaller in the intraoperative electric stimulation and decimeter wave group than those in the control group. Hyperemia and edema of the tissues surrounding the neurons abated gradually and the entrapped neural segments thickened in both groups. By the 8th week post-operation, neither hyperemia nor edema was observed in the tissues surrounding the neurons in both groups, whereas the entrapped segments were significantly thickened in both groups. Neuroma-like changes in both ends of the entrapped segment had significantly abated in both groups. At 12 weeks post-operation, the surface of the entrapped nerve was smooth in the intraoperative electric stimulation and decimeter wave group, and there were mostly thin-membrane adhesions. The neural tissue surrounding adhesions, which could be readily blunt-separated, was looser in the intraoperative electric stimulation and decimeter wave group than in the control group. The entrapped nerves in both groups were almost normal.

**Effects of electric stimulation and decimeter wave on the structure of the sciatic nerves in rats with sciatic nerve compression**

Examination by light microscope, 2 weeks post-operation, showed that the inflammatory cells had infiltrated tissues surrounding the nerve in both groups. In the intraoperative electric stimulation and decimeter wave group, the inflammatory cells were limited to the superficial layer of the epineurium, while in the control group, many inflammatory cells and interspersed multinucleated giant cells had infiltrated around the nerves (Figure 1A, B). The epineuria in both groups were intact; however, the myelin sheaths were uneven in thickness and irregular in shape, and different degrees of demyelination were noticeable. By the 4th week post-operation, the inflammatory responses were significantly reduced and swelling of the myelin sheaths had lessened in both groups. In the intraoperative electric stimulation and decimeter wave group, the inflammatory cells were rarely observed, whereas they were still accumulating in the control group (Figure 2A, B).

At 8 weeks post-operation, the demyelination of the two groups had significantly been alleviated. In the intra-
operative electric stimulation and decimeter wave group, the swelling of the axons had almost disappeared, detached myelin sheaths were gradually decreasing, and regenerated neural fibers were observed in the entrapped segment and at the distal end. However, the regenerated axons were of fine diameter and their myelin sheaths were thin. In the control group, axon swelling had abated despite the increased demyelination. There were few regenerated nerve fibers in both the entrapped segment and its distal end. The development of these fibers was still immature (Figure 3A, B).

At 12 weeks post-operation, the surfaces of the nerves in the intraoperative electric stimulation and decimeter wave group were smooth and showed no adhesion to their surrounding tissues, or only had slight filamentous adhesions. In the control group, the surfaces of the nerves were rough, and many adhesions were observed. In both groups, the myelin sheath fragments had significantly decreased, neovascularization was noted, and regenerated axons and myelin sheaths had formed. The damaged neural fibers, with fine diameters and thin myelin sheaths, were mostly adhering together and arranged orderly. In conclusion, the intraoperative electric stimulation and decimeter wave group recovered better than the control group (Figure 4A, B).

**Effects of electric stimulation and decimeter wave on ultrastructure of the sciatic nerves in rats with sciatic nerve compression**

At 2 weeks post-operation, a novel axon bud, encapsulated by the epineurium, was present in the proximal end of the entrapped segment in a rat of the intraoperative electric stimulation and decimeter wave group. Organelle such as mitochondria, microfilament, microtubule and vesicle within the neonatal axon bud was few and sparsely distributed. In the control group, the cellular membrane of the myelin sheath epineurium was detached from the axon, the myelin sheath was swollen unevenly, and the mitochondria had necrotic vacuoles and many were disintegrating (Figure 5A, B).

In the intraoperative electric stimulation and decimeter wave group, 4 weeks post-operation, the myelin sheaths were relatively thick and the regenerated axons appeared normal. A few nearly normal organelles were noted in sparse but arranged axons. In the control group, there were a few myelinated fibers that had fine axons and thin myelin sheaths but they were arranged irregularly. In addition, parts of the sheaths were empty, and oval-like bodies associated with myelin sheath degeneration were occasionally noticed (Figure 6A, B).
However, by the 8th week post-operation, there was a large quantity of myelinated fibers seen in the control group. They had small diameters and were arranged orderly. Furthermore, the myelin sheath was relatively thick and the regenerated axon had a normal appearance. (Figure 7A). In the intraoperative electric stimulation and decimeter wave group, the regenerated myelinated fibers were orderly arranged, the fasciculated structure was fine and the endoneurium was well developed. Connective tissue hyperplasia was not visible (Figure 7B).

**Effects of electric stimulation and decimeter waves on S-100 protein expression in rats with sciatic nerve compression**

There were fewer S-100-positive particles in the proximal end of the entrapped segment in both groups 2 weeks post-operation. In contrast, the positive particles in the Schwann cells increased with time, and were approaching normal both in quantity and distribution by the 4th week post-operation (Figure 8A, B).

**Effects of electric stimulation and decimeter wave on morphometric analysis and electrophysiological assessment of the sciatic nerves**

At 12 weeks post-operation, distal nerve segments in the intraoperative electric stimulation and decimeter wave group had more myelinated axon counts and a larger mean axon diameter than those in the control group. The parameters (latency, nerve conduction velocity and amplitude of compound muscle action potentials) revealed a better recovery in the intraoperative electric stimulation and decimeter wave group compared with that in the control group ($P < 0.01$; Table 1).

**Effects of electric stimulation and decimeter wave on the recovery of sciatic nerve function**

Sciatic functional index recovery rate in the intraoperative electric stimulation and decimeter wave group was significantly higher than that in the control group ($P < 0.01$; Table 2).

**DISCUSSION**

Chronic peripheral nerve compression is a functional disorder of the peripheral nerves that results from chronic entrapment of specific parts of the nerve.
Decimeter wave therapy has been shown to inhibit inflammatory reaction and improve local circulation of the affected site; thus, the treatment enhances the metabolism of the affected area and reduces scar formation and adhesion[5,6,56]. In the present study, in the rats without decimeter wave therapy, extensive infiltration of inflammatory cells around the nerves and dense adhesion around tissues surrounding the nerve were observed. In contrast, at 1 week post-operation, decimeter wave therapy significantly reduced the inflammation and adhesion, and the inflammation was hardly observed by the 4th week after the operation. These findings demonstrate that decimeter waves could effectively inhibit the inflammatory reaction after injury, as well as improve the local circulation of the injured nerve and consequently reduce adhesion between the injured nerves. Furthermore, the treatment facilitated neural degeneration[57-61].

Here, we used S-100 protein as a Schwann cell marker to compare the effect of decimeter wave therapy on the proliferation of Schwann cells after nerve injury[62-64]. Previous immunohistochemical and immunocytochemical studies have shown that S-100 protein expression was limited in Schwann cells in the peripheral nervous system, and its expression was absent in axons[65-69]. However, S-100 protein expression increases when Schwann cells proliferate. Therefore, high levels of S-100 protein indicate active proliferation of Schwann cells, which has been shown to promote nerve regeneration[25,52-55]. Thus, treatment strategies that up-regulate S-100 protein expression may be beneficial for the enhancement of neural regeneration[70,71]. Results of the present study showed that S-100 protein expression during surgery is advantageous because of its accurate stimulation of the selected site. Moreover, it does not induce complications, such as infection and poor compliance, and patients suffer less pain, as compared with transcutaneous electric stimulation[62-65].

There are three basic pathological changes for chronic peripheral nerve compression: chronic ischemia; blood-neuron barrier changes; and severe Wallerian degeneration[34-39]. In the initial phase of entrapment, endoneurial fluid pressure increases, leading to edema in the endoneurium and perineurium, followed by progressive thickening of the epineurium, and finally, segmental demyelination in local nerve fibers[40-46]. Moreover, there are antegrade and retrograde axoplasmic flows in neural fibers, both of which can be blocked by entrapment[5,39,41]. Decompression, in releasing entrapment, eliminates the above-mentioned adverse factors, reduces neural compression, improves neural microcirculation, facilitates myelin sheath regeneration and modifies electrolyte concentration and distribution, all of which contribute to restoring neural functions[42-48].

It has been shown that electric stimulation promotes the regeneration of capillaries and leakage of certain elements, and then provides a good microenvironment for peripheral nerve repair and regeneration[47-51]. Electric stimulation could accelerate the Waller degeneration of distal nerve injury to promote the functional recovery of nerve cells. Studies have shown that electric stimulation begins shortly after nerve injury to promote nerve generation[47-51]. Technically, intraoperative electric stimulation during surgery is advantageous because of its accurate stimulation of the selected site. Moreover, it does not induce complications, such as infection and poor compliance, and patients suffer less pain, as compared with transcutaneous electric stimulation[62-65].
levels in the compressed nerve from the rats with decimeter wave therapy were significantly higher than those in the rats without the treatment in various phases (data not shown), suggesting that decimeter waves induced S-100 protein expression in the regenerated nerves. In conclusion, the decimeter wave therapy was effective in promoting the proliferation of Schwann cells and increasing S-100 protein expression levels, which might have been beneficial for nerve regeneration and neural repair.

The process of repair and regeneration of the peripheral nerves is characterized by the growth of the proximal end into the distal end. Therefore, regeneration status can be reflected by the quantity of regenerated axons and the degree of maturation. Our results from transmission electron microscopy showed that at 2 weeks post-operation, in the nerves from the rats treated with decimeter wave, many neonatal axon buds containing different types of organelles extended from the proximal end of the entrapped nerve. With time, regenerated myelin sheaths became thick, regenerated axons looked normal, and organelle structure was nearly normal at the proximal end of the axon. In contrast, there were no neonatal axon buds in the nerves from rats without the treatment. Quantitative analysis results further revealed that the number of myelinated nerve fibers and the diameter and thickness of myelin sheath in the decimeter wave therapy group were significantly greater than those in the group without treatment. These results indicated that the regenerated nerves in the treatment group were more mature than those in the rats without treatment. The nerves in the treatment group were characterized by a shorter latent period, faster conductivity and higher wave amplitude, suggesting that nerve function recovered better in the treatment group than in the non-treatment group.

This study illustrates the mechanism underlying electrical stimulation and decimeter wave therapy in the improvement of peripheral nerve regeneration. Our findings will provide a theoretical basis for clinical application.

**MATERIALS AND METHODS**

**Design**
A randomized, controlled animal experiment.

**Time and setting**
Experiments were performed at the Experimental Center of the First Hospital of Hebei Medical University, China, from January 2009 to February 2012.

**Materials**
A total of 90 healthy adult Sprague-Dawley rats weighing 200–250 g were purchased from the Animal Experiment Center of Hebei Medical University, China (license No. DK0408-0089). The protocols were conducted in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China.

**Methods**

**Preparation of chronically compressed nerve model of Mackinnon**
Rats were fasted for 8 hours before operation, and then anesthetized with 1% pentobarbital sodium (30 mg/kg) (the Experimental Center of the First Hospital of Hebei Medical University, China) by intra-abdominal injection. The posterior median line of the left thigh was cut and the biceps femoris and semitendinosus muscle were separated. The sciatic nerves were exposed in the intermuscular space of the semi-membranous muscle. The diameter of the nerve was measured with a vernier caliper to be 1.0 mm. A silicone tube with a diameter of 1.0 mm and length of 6.0 mm was cut longitudinally and fixed to the nerve in the lower margin (10 mm) of the piriformis. The silicone tube was sutured with three stitches of non-invasive suture with an operating microscope. Thorough hemostasis and prophylactic therapy with 0.4 mL gentamicin was completed. Finally, the intermuscular space was closed and the skin was sutured (Figure 9A, B).

**Figure 9** Morphology of sciatic nerves of normal rats (A) and model of Mackinnon (B).

Three experimental animals in each group were randomly selected at the 4th week post-operation. The original wound was opened and the silicone tube was observed to be wrapped with connective tissue without adhesion. Both ends of the entrapped segment became thickened with a fibroepithelial-like appearance, and the surface of the entrapped segment appeared pale. Conductivity was decreased below 1/6 of the normal value by electrophysiological recording. Moderate-to-severe de-
myelination was considered as pathological phenomenon of the entrapped segment, indicating a successful operation\(^{[79]}\) (Figure 10A, B).

Figure 10  Electrophysiological indexes of normal rats (A) and model of Mackinnon (B).

Postoperative treatment of decimeter wave

Male and female animals were fed separately, and free movement was allowed. Rats of the intraoperative electric stimulation and decimeter wave group were treated with electric stimulation. After thorough hemostasis, the intermuscular space was closed and the skin was sutured. A DXZ-1 polymorphism wave therapy instrument (Medical Equipment Technology Development Co., Ltd., Tianjin, China) was used in the present study. Parameters of frequency, intensity and treatment time were set to 2 Hz, 2 mA and 30 seconds, respectively.

Morphological observation of the sciatic nerves

Postoperative observations of wound healing included ulceration of the plantar and/or auto-phagocytic phenomenon. At the 1\(^{\text{st}}\), 2\(^{\text{nd}}\), 4\(^{\text{th}}\), 8\(^{\text{th}}\) and 12\(^{\text{th}}\) weeks post-operation, the neural segments in three animals from each group were observed. The entrapped segment of the sciatic nerve was exposed, and the lesions of the sciatic nerves and surrounding tissues and neuronal adhesion were grossly observed. Neural segments (2 mm) were obtained from the proximal and distal end of the entrapped segment, followed by 10% formalin fixation and hematoxylin-eosin staining. The nerve structure was observed under a light microscope (Shanghai Optical Instrument Factory, Shanghai, China).

Ultrastructural observation of the sciatic nerves under an electron microscope

At 2, 4 and 8 weeks post-operation, samples of the sciatic nerve from three animals from each group were selected. The samples were then fixed with 3% glutaraldehyde and embedded with resin. Thereafter, ultrathin sections were obtained to observe the neural regeneration conditions under an electron microscope (Japanese Electronics Co., Ltd., Tokyo, Japan).

S-100 expression in regenerating tissues as detected by immunohistochemistry

At the 3\(^{\text{rd}}\) day and 1\(^{\text{st}}\), 2\(^{\text{nd}}\), 4\(^{\text{th}}\) and 8\(^{\text{th}}\) weeks post-operation, the samples from three animals in each group were selected to be cut into 5 µm-thick paraffin sections and stained by streptavidin peroxidase method. Paraffin sections were de-waxed, and then incubated in 3% H\(_2\)O\(_2\) for 10 minutes to eliminate endogenous peroxidase. The sections were rinsed with water and soaked in PBS for 5 minutes. They were then blocked in 10% normal goat serum (diluted in PBS), rinsed, and incubated for 10 minutes at room temperature in a rabbit anti-rat S-100 antibody (Boster, Wuhan, Hubei Province, China) 1:100 for 1 hour at 37°C. The sections were washed in PBS three times for 5 minutes each, followed by incubation in biotinylated-secondary goat anti-rabbit antibody (1:100; Boster; diluted in 1% bovine serum albumin-PBS solution) at 37°C for 30 minutes. After washing in PBS three times, the sections were incubated in streptavidin-horseradish peroxidase at room temperature for 30 minutes. After washing in PBS, they were stained with 3,3’-diaminobenzidine and counterstained with hematoxylin. S-100 expression in regenerating tissues was observed under a light microscope (Shanghai Optical Instrument Factory, Shanghai, China).

Morphometric analysis of the sciatic nerves

At the 12\(^{\text{th}}\) week post-operation, three animals were selected from each group to analyze the osmium tetroxide-stained distal end of the entrapped nerve; the number of axons, axon diameter, and myelin thickness were analyzed by image analyzer (Quantimet 970, Cambridge, England).
Electrophysiological assessment of the sciatic nerves

At 12 weeks post-operation, six animals were selected from each group to analyze muscle action potentials (EMG evoked instrument DISA-1500, Aalborg, Denmark). The maximum stimulation was 18 V, the stimulation phase was 0.1 ms, and the frequency was 1 Hz. Profiles of six animals were recorded and the latency, nerve conduction velocity and amplitude were measured.

Determination of sciatic functional index

A trough that opened at both ends was made; it was 60 cm long, 10 cm wide and 15 cm high. A sheet of white paper was spread into the bottom of trough. At the 4th, 8th and 12th weeks post-operation, the posterior limbs of rats were pigmented, and then the rats were put in at one end of the trough, induced to walk to the other end of the trough to a food reward. Each side of the posterior limbs left a 4–5 podogram, and a clear podogram was selected to measure three indicators of the normal foot (N) and the exceptional foot (E): (1) print length (PL): the greatest distance from the point of foot to the calcar pedis; (2) toes spread (TS): the distance from the first toe to the fifth toe; (3) intermediate toe (IT): the distance from the second toe to the fourth toe. Results were accurate to 0.1 mm. The data were put into the Bain formula to calculate sciatic functional index. Sciatic functional index = 0 for normal, −100 for complete injury. Bain formula is[79]:

\[
\text{Sciatic functional index} = -38.3\times\frac{\text{EPL}-\text{NPL}}{\text{NPL}} + 109.5\times\frac{\text{ETS}-\text{NTS}}{\text{NTS}} + 13.3\times\frac{\text{EIT}-\text{NIT}}{\text{NIT}} - 8.8.
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Statistical analysis

All data were analyzed using SPSS 16.0 software (SPSS, Chicago, IL, USA). Measurement data were expressed as mean ± SD and a two-sample t-test was used for intergroup comparison. A value of \( P < 0.05 \) was considered statistically significant.

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