

The Effect of Ultrasound on Acceleration of Peripheral Nerve Injury

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Abstract

Background: Many people suffer from nerve injury. The time consuming nature of the treatments makes the condition worse. Therefore, finding a way to accelerate the process of nerve repair is very important.

Objective: To determine the effect of ultrasound on the acceleration of crushed peripheral nerve regeneration.

Methods: The experimental model included crushed rat's sciatic nerve. 40 rats were categorized into control and test groups. We used functional and electrophysiological tests to measure the amount of nerve regeneration. We extracted SFI from rats paw print for functional test and measured CMAP wave amplitude for electrophysiological test. After testing 3 types of ultrasound radiation parameters, we selected the best parameters. Ultrasound radiation in test group had the intensity of 0.2 W/cm² and frequency of 3 MHz. It was applied 3 days per week for 2 min each time starting 72 h after the injury. Control group just received sham expose.

Results: During the treatment period, both SFI and CMAP amplitude was significantly higher in test group than in control groups ($p < 0.05$).

Conclusion: We found that ultrasound accelerates injured peripheral nerve regeneration. In addition, type of injury can be assessed according to changes in CMAP amplitude during the treatment period.

Keywords

Sciatic nerve; Crush injury; Nerve regeneration; SFI; CMAP

Introduction

Some types of peripheral nerve injury can be repaired spontaneously; some of them need surgical treatment. When nerve repair is spontaneous, the low rate of repair would be problematic. Researchers are trying to find a solution for acceleration in nerve repair. Use of laser, electromagnetic field or some substances have been proved to be effective in stimulating nerve regeneration [1,2]. One of safe non-invasive therapeutic methods is application of low intensity ultrasound.

Ultrasound therapy is used in clinical applications to treat blood clot in vessels [3], kidney stones [4], and tumors [5]. Research studies about the effect of ultrasound on nerve regeneration are being reported [6-9]. Most of these studies use functional index as the principal factor for the measurement of nerve regeneration. Although use of this index is simple and reliable to some extent, we cannot evaluate the condition of nerve regeneration accurately. Functional index can show us the effect of treatment on the functional recovery but the condition of nerve fibers will be

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unclear. Histological and electrophysiological studies can give us information about the status of nerve fibers. Histological experiments are expensive and need to extract the tissue so that the animals are useless after tissue extraction. But electrophysiological experiments do not need tissue extraction.

Compound muscle action potential (CMAP) is a group of almost simultaneous action potentials originated from several muscle fibers in the same area usually evoked by stimulation of the supplying motor nerve and are recorded as one multiphased summated action potential. Decrease in CMAP amplitude demonstrates decrease in the quantity of nerve fibers. This index can estimate the amount of axons that are destroyed or regenerated.

We conducted this study to determine the effect of ultrasound on the rate of regeneration in injured peripheral nerve. Since most of the reported experiments on nerve regeneration are based on functional evaluation of nerve repair and this index cannot give us enough information about nerve fibers condition, we used electrophysiological tests in addition to functional test in our research.

Material and Methods

Experimental animal

We used Wistar rats weighing 300–350 g obtained from Pasteur Institute of Iran. The rats lived in standard situation in clean cages and had access to food and water *ad libitum*.

Experimental groups

At first, 20 rats were categorized into a control and three test groups to find out the best condition for ultrasound radiation. After finding the best radiation parameters, 40 rats were categorized into two groups—20 rats were placed in control group and 20 rats in test group. Immediately after injury and at the end of each week, five rats were selected from each study group and their CMAP wave was recorded. These five rats were then scari-

fied. Paw prints were recorded after each time of ultrasound radiation. The results of sciatic functional index (SFI) reported in this paper were according to paw prints of the five rats that were kept alive until the fourth week.

Surgical procedure

At first, the rats were anesthetized by peritoneal injection of ketamine (50 mg/kg) and xylazine (50 mg/kg). Then, their left sciatic nerves were exposed through a 2-cm long incision on the lateral side of the left thigh skin. The sciatic nerve can be exposed from the natural incision between *gluteus maximus* and quadriceps muscles. The nerve was pulled out to be crushed. By means of a hemostatic forceps, a stable force of 95 N was applied on 2 mm of sciatic nerve approximately 5 mm below the sciatic notch for 30 sec. The site of injury was marked with a 7-0 nylon suture. This injury reduced SFI and the amplitude of CMAP significantly. So that SFI reduced to about 90 and CMAP amplitude reduced to 10 mv.

Ultrasound radiation

Starting 72 h after injury, the injury site was irradiated with ultrasound for three days per week. We had three test and one control groups to find the effective ultrasound radiation parameters. Control group received sham exposure; the first test group received ultrasound at an intensity of 0.5 W/cm², frequency of 1 MHz, and a duty cycle of 20%. Radiation was applied for 5 min each time. In the second group, the duty cycle was increased to 100% and the ultrasound was irradiated for 2 min each time. In pulsed-wave radiation, the dominant effects are non-thermal. When the duty cycle increases to 100%, we have a continuous wave, in which thermal effects are dominant. Compared with the second group, in the third test group, the intensity of ultrasound was changed to 0.2 W/cm² and the frequency was 3 MHz. The intensity is directly proportionate to the heat produced. Therefore, reduction in intensity leads to heat reduction. The third

group was selected as the best group. Then, we placed 40 rats into two control and test groups. The test group received the radiation with an intensity of 0.2 W/cm² operated at 3.0 MHz for 2 min every day in continues mode.

For the experiment, we used a portable ultrasound equipment (EMS, England and Novin, Iran) with a therapeutic applicator (7 cm² crystal area) with a frequency range of 1.1 MHz±5% to 3.3 MHz±5%, and a pulse frequency of 100 MHz.

Sciatic functional index (SFI)

Analysis of paw print was done before each session of ultrasound radiation. SFI can be calculated by the following equation:

$$SFI = -38.8\left(\frac{EPL - NPL}{NPL}\right) + 109.5\left(\frac{ETS - NTS}{NTS}\right) + 13.3\left(\frac{EIT - NIT}{NIT}\right) - 8.8$$

In this equation, *PL* is print length or the maximal distance between the tip of the longest toe and the heel. *TS* is toe spread or the distance between the first and fifth toe, and *IT* represents intermediate toes or the distance between the second and fourth toes. *E* refers to experimental paw and *N* refers to normal paw (Fig. 1).¹⁰

To record the paw print, we used a 70-cm long box ending with a black curtain. The rat's

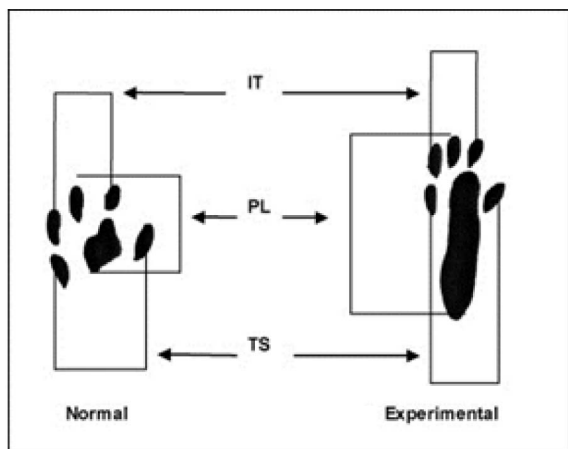


Figure 1: Sciatic functional index (SFI) parameters

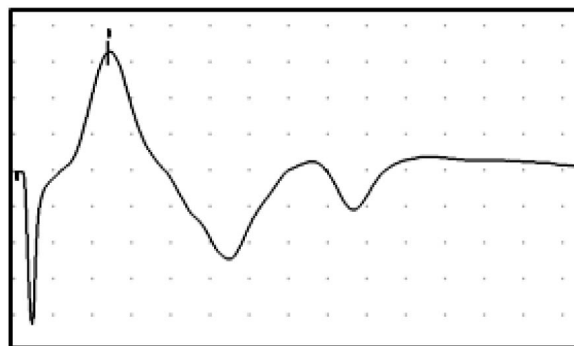


Figure 2: CMAP wave recorded from a rat's sciatic nerve

paw colored with blue ink and then the rat moved on the surface of the box covered with a white paper. The dimensions were measured with an accuracy of 0.5 mm, and analyzed.

Electrophysiological assessment

For electrophysiological assessment, we used a stimulus electrode on the proximal and then distal segment of the nerve to the injury site. The recording electrode was placed on the gastrocnemius muscle at the back side of the ankles of rats. After applying a stimulus with enough amplitude, compound muscle action potential wave was recorded by electrophysiograph equipment. A sample of CMAP wave recorded in our experiment is shown in Figure 2.

Results

Sciatic functional index (SFI)

SFI curves are shown in Figure 3 for the control and three test groups that were used to select the best radiation parameters at first.

As it is clear, among the SFI curves, only that of the third test group is always above the curve of the control group (Fig. 3).

Functional assessments showed that ultrasound radiation increased the rate of functional recovery. At the second week, the test group showed a significant improvement ($p < 0.05$) compared to the control group. A negative

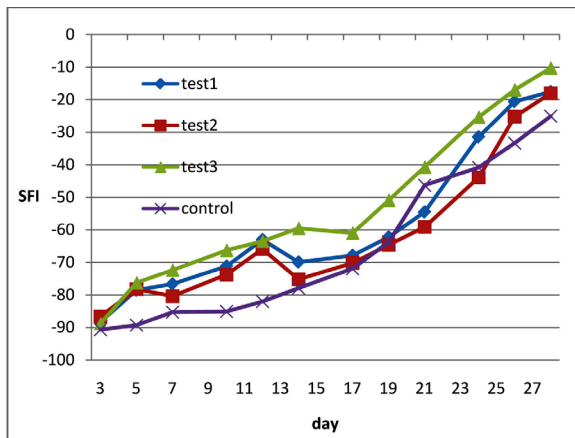


Figure 3: SFI from the control and three test groups to select the best radiation parameters

slope caused the two curves become close to each other. This negative slope is compensated so that at the fourth week, there was a significant difference between the control and test groups.

Electrophysiological result

We can estimate type of injury by comparing the amplitude of proximal and distal wave in the control or the test group (Figure 4).

The CMAP amplitude recorded from the distal segment is high immediately after the injury and decreases at the end of the first week (Fig. 4). However, the amplitude of proximal wave decreases immediately after the injury. This information makes it possible to evaluate

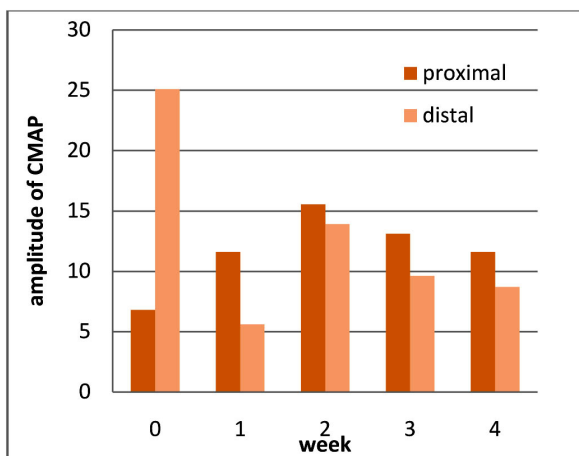


Figure 4: CMAP amplitude in control group

the type of injury.

To evaluate the effect of ultrasound on the electrophysiological results, it is better to compare the results of the proximal wave in the control and test groups Figure 5 demonstrates that the amplitude of this wave is higher in the test group compared to the control group during all but the second weeks ($p < 0.05$). Like SFI results, at the end of the second week, the control and test curves become close to each other.

Discussion

After analyzing the amplitude of the CMAP wave recorded in the control group from proximal and distal segments of the nerve (Figure 5), we could define the type of nerve injury. Immediately after the injury, the amplitude of wave is high in the distal segment (Fig. 5). The amplitude decreases significantly at the end of the first week. We know that in Wallerian degeneration, the distal segment of injured nerve starts to degenerate 24 to 36 h after the injury. This process followed by myelin clearance. It seems that in our injury model some axons are damaged and some are not (Fig. 5).

SFI results proved that ultrasound can accelerate functional recovery of injured nerves. Ultrasound caused all test group curves to be higher than those in the control group (Fig. 4). Nevertheless, in all test groups, there was a negative slope at the end of the second week of ultrasound therapy. This negative slope caused the control and test curves become close to each other. Although, it is compensated after the third week, eliminating this phenomenon would help us to have better results. It is therefore suggested to evaluate the effect of disruption of ultrasound radiation during this period of negative slope. To study the effect of ultrasound radiation on electrophysiological results, CMAP recorded from the proximal stump is more suitable (Fig. 5). The SFI results and electrophysiological findings are well correlated. Therefore, electrophysiological experiments can be conducted as a sur-

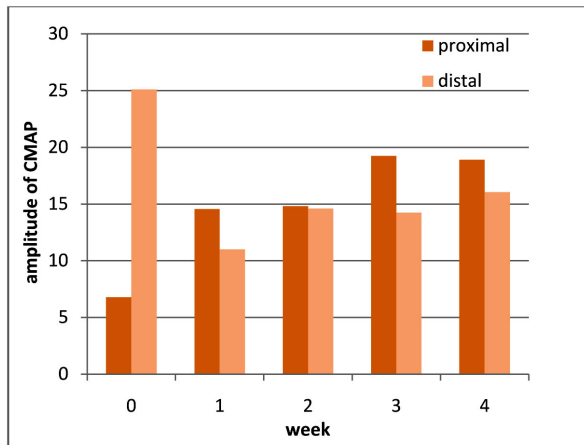


Figure 5: CMAP recorded from proximal stump

rogate for functional experiments. In conclusion, both functional and electrophysiological experiments showed that ultrasound radiation accelerates regeneration of injured nerves.

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