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The Effects of Altered Ultrasound Parameters on the Recovery of Sciatic Nerve Injury

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ABSTRACT

Background: Initial studies have shown that low-energy ultrasound stimulates living tissue cells to reduce regeneration or speed up their recovery. The purpose of this study was to examine the effects of various ultrasound parameters on the speed of recovery in injured sciatic nerves. **Methods:** NMRI mice (n = 200) with injured left paw, caused by crushing their sciatic nerves, were randomly selected. The animals were exposed to ultrasound radiation with various frequencies, intensities, and exposure time. They were allocated into 20 groups (19 treatment and 1 control groups). Sciatic functional index (SFI) test was used to evaluate the difference between the groups with respect to functional efficiency of the sciatic nerve and its recovery. **Results:** The results of SFI test obtained from the 14th day showed a significant difference among the groups ($P<0.05$). On the 14th day after treatment, one of the groups (US11) recovered up to 90%. **Conclusion:** Altered ultrasound exposure parameters had more favorable outcomes compared with our previous work. *Iran. Biomed. J. 16 (2): 107-112, 2012*

Keywords: Sciatic nerve, Ultrasonic therapy, Regeneration

INTRODUCTION

Peripheral nerve injury is a widespread neurological problem, which usually takes many months to regenerate [1, 2]. If the nerve is left to recover naturally during the regeneration period, the innervated muscles are inflected with atrophy leading to complete malformation. Different modalities have been used to address this issue, including operation, physiotherapy by using electric shock, and using magnetic fields or ultrasound [3, 4]. Moreover, the treatment may have positive effects on the therapeutic process of different tissues, such as the skin, muscle, tendons, and nerves [3-7].

Regarding the treatment with ultrasound, the first evaluations were done on conduction velocity of peripheral sensory nerves of Ulna and Radius [7], because the changes in conduction velocity of neurons due to different intensity and period of ultrasound implication are related to mechanical or thermal effects of the ultrasound [8-13].

Lowdon and colleagues [11] have studied the therapeutic effects of ultrasound on the recovery of stress-induced injury of rat tibial nerve. They applied continuous, 1-minute radiation (1 MHz, 0.5 and 1 W/cm^2 intensity) for 2-3 weeks. They found that neural conduction speed was improved with 0.5 W/cm^2 and more improvement was achieved with 1 W/cm^2 compared with non-radiated nerve. They concluded that ultrasound radiations sped up the recovery of stress-induced injury of peripheral nerve, although intense radiation resulted in delayed recovery. In another study, similar results were obtained by applying ultrasound radiation to crushed sciatic nerve of rat. The nerve was recovered using ultrasound radiation of 0.25 W/cm^2 and 2.25 MHz repeated three times a week for one month [12].

Although previous studies showed the role of ultrasound in speeding up the recovery of injured sciatic nerve, only 1 or 2 parameters of ultrasound were examined in those studies to analyze the efficacy of such a modality [14-19]. Therefore, we aimed to examine the effects of various parameters of ultrasound (intensity, frequency, duty cycle, radiation time, and radiation mode [continuous/pulsed]) in various groups of rats to assess the performance of sciatic functional index (SFI) test, to obtain the optimal parameters of therapeutic ultrasound in healing sciatic nerve injury.

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Table 1. Different parameters of ultrasound used in various therapeutic groups.

Groups	Intensity (W/cm^2)	Frequency (MHz)	Pulse/ continuous	Duty cycle (%)	Time (minute)
US1	0.2	1	C	-	2
US2	0.5	1	C	-	2
US3	1.0	1	C	-	2
US4	2.0	1	C	-	2
US5	1.0	1	C	-	5
US6	0.2	3	C	-	2
US7	0.5	3	C	-	2
US8	1.0	3	C	-	2
US9	0.2	1	P	20	5
US10	0.2	1	P	20	2
US11	0.5	1	P	20	2
US12	1.0	1	P	20	2
US13	0.5	1	P	5	2
US14	0.5	1	P	5	5
US15	0.5	3	P	5	2
US16	0.5	3	P	20	2
US17	0.2	3	P	5	2
US18	0.2	3	P	20	2
US19	1.0	3	P	5	2

MATERIALS AND METHODS

Animals. NMRI mice, weighing 27-34 g, were supplied by Pasteur Institute of Iran (Tehran) and were given adequate food and water. The animals were housed in a controlled colony room (temperature 21 ± 3°C), which was maintained under a 12:12 h light/dark cycle. The study was approved by the Ethics Committee of Pasteur Institute of Iran (Tehran).

Preparation. The mice were anesthetized with xylazine (20 mg/kg) and ketamine (50 mg/kg). Their hair was cut and the skin was disinfected for the operations.

Sciatic nerve damage. Firstly, the mice femur was cut 5 mm cross-sectionally and the sciatic muscles were cut with surgical scissors to expose the sciatic nerve. The nerve was pressed with special forceps for 20 seconds under a force of 50 Newton (N).

Groups. Mice (n = 200) with injured sciatic nerve were randomly assigned into 20 equal groups. The first group was treated with false ultrasound (control group, n = 10) and the other 19 groups were treated with ultrasound radiation 2 days after the injury. The groups are shown in Table 1 as US1~19 according to receiving different parameters of ultrasound (intensity, frequency, duty cycle, radiation time, radiation mode [continuous/pulsed]).

Ultrasound radiation. We used the EMS 215A sonotrophy device, which is a therapeutic device used in physiotherapy (EMS Co., UK). Two modes of 1 and 3 MHz frequencies with maximum 2 W/cm^2 intensities, and two duty cycles (5% and 20%) were used. The daily duration of exposure was 2 and 5 minutes [20], and the area of the applied probe was 5 cm^2. Sciatic nerves of the mice in 19 therapeutic groups were exposed to ultrasonic waves using different ultrasound parameters shown in Table 1 two days after the operation (Table 1). Radiation was continued for 14 intermittent days and coupling gel was used all over the injured area exposed to ultrasound.

Sciatic functional index test. Sciatic nerve recovery rate of each mouse was assessed by SFI test. It was done by analyzing the back paw traces of the animals [13, 14]. A laboratory animal treadmill was used to process the images of mice paws. SFI was obtained by NI Vision Assistant 8.6 (National Instruments Co.) under LabVIEW 8.6 software (Fig. 1). SFI was calculated by the following formula 2, 4, 6, 8, 10, 12, 14 days after operation:

$$(1) \quad SFI = -38.3\left(\frac{EPL\text{-}NPL}{NPL}\right) + 109.5\left(\frac{ETS\text{-}NTS}{NTS}\right) + 13.3\left(\frac{EIT\text{-}NIT}{NIT}\right) - 8.8$$

Where PL is print length or maximum distance between the tip of the longest paw to heel, TS is the toe space or spaces of the 1^{st} and 5^{th} toes and IT is middle toes or distance between the 2^{nd} and 4^{th} toes. N is the normal value and E is the experimental value. All the mice were tested for running for a few minutes before operation.

By using SFI values, we can calculate the following values to evaluate the improvement of each group; how every group is recovered, how much is the improvement and how much is the median percentile of each treatment days.

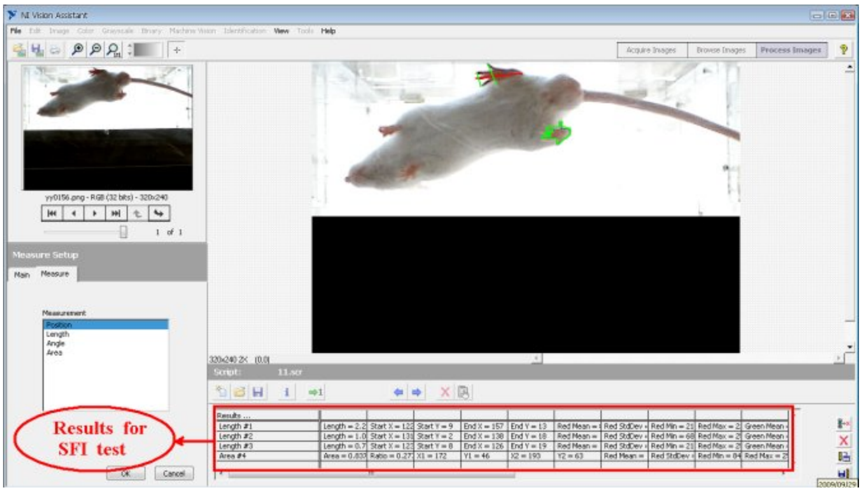

Fig. 1. Image processing software on animal treadmill for imaging and image analysis of animal foot parameter (sciatic function index).

The percent of recovery at the end of treatment days was calculated using the following formula:

$$(2)\quad =100\times\left(1-\frac{(SFI.at.14th.day - SFI.at.befor.surgery)}{SFI.at.second.day - SFI.at.befor.surgery}\right)$$

The percent of recovery at the end of all treatment days was calculated using the following formula:

$$(3)\quad =100\times\left(1-\frac{(Integral.of.total.days)}{6\times(SFI.at.second.day)}\right)$$

Analysis of data. Data were analyzed using SPSS software, version 16.0. The results were shown as mean ± SD. One way analysis of variance (ANOVA) and Post-hoc Tukey test were used as appropriated and P<0.05 was considered as statistically significant.

RESULTS

The toe of the left paw of all the mice in the 20 groups was strained and twitched after the operation. They were almost unable to stand on the left paw in the early days, and it took many days to be recovered. On the 14th day, they were almost returned to the normal condition, and especially the mice in the 11th group (US11) achieved 90%.

Table 2. Mean and standard deviation of sciatic functional index before and after the injury for different ultrasound therapeutic groups.

Groups	Days							
	0	2	4	6	8	10	12	14
control	-3.4 ± 6.19	-101.5 ± 4.86	-97.7 ± 3.77	-88.7 ± 3.79	-90.2 ± 4.06	-78.1 ± 3.23	-62.0 ± 6.18	-43.6 ± 4.85
US1	-4.3 ± 3.51	-100.2 ± 4.02	-100.1 ± 4.01	-96.3 ± 2.23	-83.1 ± 3.81	-68.3 ± 5.20	-49.6 ± 4.90	-40.2 ± 2.02
US2	-4.0 ± 2.32	-99.3 ± 5.61	-99.5 ± 5.08	-93.1 ± 6.20	-84.2 ± 4.19	-71.2 ± 3.87	-52.3 ± 3.91	-43.9 ± 4.01
US3	-4.1 ± 7.72	-97.5 ± 6.13	-100.6 ± 4.79	-94.9 ± 5.02	-87.4 ± 4.46	-74.3 ± 4.87	-56.4 ± 5.06	-45.1 ± 5.24
US4	-4.6 ± 5.06	-81.1 ± 5.93	-99.2 ± 4.43	-101.0 ± 0.00	-101.0 ± 0.00	-101.0 ± 0.00	-101.0 ± 0.00	-101.0 ± 0.00
US5	-5.0 ± 2.78	-96.3 ± 3.01	-100.2 ± 2.71	-93.1 ± 5.63	-89.2 ± 3.71	-99.3 ± 5.01	-102.1 ± 0.00	-102.1 ± 0.00
US6	-4.2 ± 3.42	-98.4 ± 3.46	-99.3 ± 4.52	-98.2 ± 5.91	-85.3 ± 2.95	-70.2 ± 3.08	-53.2 ± 5.12	-45.3 ± 5.61
US7	-5.1 ± 2.91	-101.1 ± 4.51	-99.0 ± 3.91	-95.2 ± 4.88	-87.6 ± 3.65	-75.8 ± 2.99	-56.2 ± 3.18	-47.9 ± 2.43
US8	-4.2 ± 2.56	-99.3 ± 2.23	-98.5 ± 5.21	-96.3 ± 3.43	-90.7 ± 4.36	-77.3 ± 4.83	-60.3 ± 4.31	-50.7 ± 2.53
US9	-4.7 ± 5.70	-103.0 ± 8.88	-99.5 ± 4.36	-76.6 ± 5.89	-80.3 ± 5.60	-50.1 ± 4.18	-41.4 ± 5.80	-30.7 ± 6.69
US10	-4.3 ± 2.49	-99.6 ± 3.09	-95.8 ± 3.96	-86.5 ± 2.59	-81.6 ± 4.38	-51.5 ± 3.61	-39.0 ± 4.97	-27.2 ± 4.51
US11	-6.6 ± 2.94	-98.8 ± 2.50	-97.0 ± 3.69	-78.8 ± 2.79	-60.6 ± 5.42	-43.9 ± 4.61	-25.2 ± 4.03	-15.7 ± 3.55
US12	-5.5 ± 2.69	-95.9 ± 3.59	-92.3 ± 3.27	-86.9 ± 2.93	-69.7 ± 4.47	-46.9 ± 5.01	-37.9 ± 4.10	-28.0 ± 4.38
US13	-4.5 ± 2.84	-93.4 ± 4.16	-89.4 ± 3.04	-76.6 ± 3.38	-74.6 ± 5.30	-53.7 ± 4.93	-39.0 ± 3.31	-32.7 ± 3.26
US14	-3.5 ± 2.44	-89.8 ± 3.11	-90.0 ± 2.51	-81.6 ± 2.73	-75.1 ± 4.02	-64.8 ± 5.09	-50.7 ± 4.76	-34.9 ± 5.30
US15	-4.9 ± 2.85	-95.0 ± 3.56	-102.0 ± 2.55	-93.5 ± 4.58	-84.4 ± 4.01	-79.3 ± 6.80	-59.5 ± 7.36	-40.6 ± 6.48
US16	-4.1 ± 3.01	-96.1 ± 4.56	-99.7 ± 5.12	-94.3 ± 3.47	-82.1 ± 3.49	-78.1 ± 5.12	-57.3 ± 6.13	-39.7 ± 3.14
US17	-3.8 ± 2.86	-101.0 ± 3.03	-100.5 ± 2.95	-96.4 ± 2.90	-91.2 ± 3.30	-77.9 ± 6.52	-57.9 ± 6.27	-37.9 ± 6.34
US18	-3.2 ± 2.16	-101.1 ± 2.34	-98.6 ± 2.73	-96.6 ± 2.06	-83.7 ± 4.08	-67.5 ± 3.24	-57.3 ± 3.47	-41.7 ± 2.96
US19	-5.2 ± 3.22	-99.3 ± 4.03	-98.4 ± 3.69	-95.2 ± 4.58	-87.3 ± 5.01	-84.2 ± 3.19	-63.2 ± 6.12	-45.7 ± 2.18

Statistically, parameters of ultrasound used in US11 group resulted in a better improvement both for the primary and final days of the treatment. Moreover, only US11 group in the final days (from 8 to 14 days) showed a significant difference compared with the other groups (P<0.05).

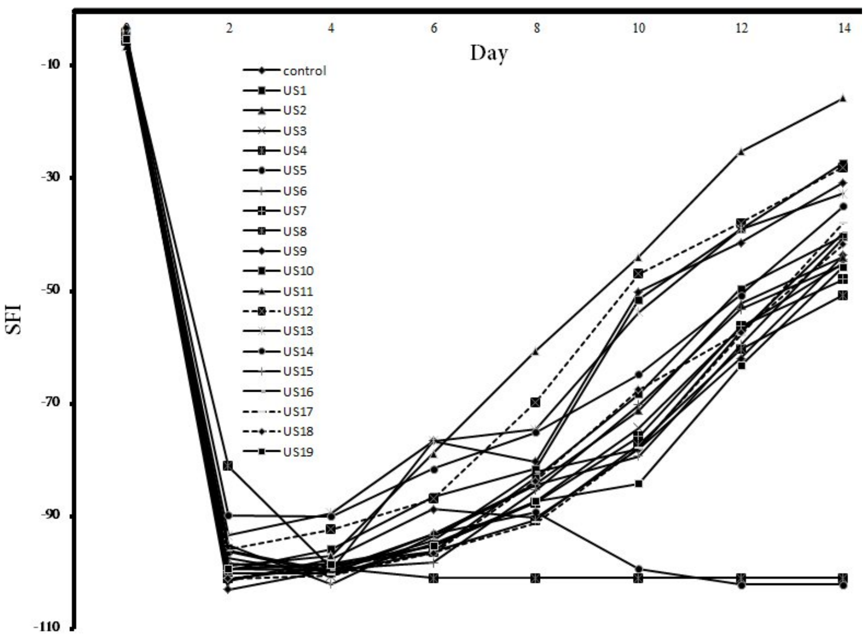

Fig. 2. Sciatic function index of the 20 groups compared with continuous treatment days and pre operation day.

Sciatic functional index. All back paw images of 200 mice were recorded by treadmill system. They were processed before operation and analyzed in 2, 4, 6, 8, 10, 12, 14 days after operation. Mean and standard deviations of SFI are shown in Table 2. Parameters of ultrasound used in US11 group resulted in a better improvement both for the primary and final days of the treatment compared with the other groups (Table 2). We observed that ultrasound of lower intensities had more desirable results in the final days of the treatment, but increased intensities (i.e. I = 0.5 W/cm^2) had more desirable results in the primary days of the treatment.

Generally, ultrasound frequency of 3 MHz was less effective than 1 MHz low duty cycle does not result in very effective recovery and by using 20% duty cycle, the recovery was improved.

It seems that 2-minute radiation had more desirable results, but continuous mode of radiation produced higher heat leading to less effective results compared with the pulse mode. Figure 2 shows the graph drawn according to the data of Table 1, which can be used to analyze the trend of recovery among the mice in various groups.

Table 3 shows the total recovery in the last day of treatment and mean total recovery days in various groups of mice. These values are very important to compare the improvement among groups, because we can compute the amount of the recovery of each group with regard to changes in ultrasound exposure parameters. Hence, the best ultrasound exposure parameters among groups can be obtained.

The most improved sciatic nerves are related to the US11 group with 90% recovery (P<0.05). As shown in Table 4, the best procedures for treatment belonged to US13 group on the 4th day after operation, US9 on the 6th day, and US11 on the final day.

Statistically, only US11 group in the final days (from 8 to 14 days) showed significant difference compared with the other groups (P<0.05). But the other groups did not have significant difference with the other groups (P>0.05).

Table 3. Total recovery in the last day of treatment and mean total recovery days in various groups of mice.

Groups	average total recovery days (%)	last recovery day (%)
control	19.67	59.03
US1	25.74	62.56
US2	21.60	58.13
US3	19.67	56.11
US4	1.94	-26.01
US5	5.34	-15.43
US6	22.01	56.36
US7	20.22	55.41
US8	21.89	51.10
US9	32.89	73.56
US10	30.09	75.98
US11	38.81	90.14
US12	31.24	75.12
US13	29.28	68.28
US14	21.21	63.62
US15	15.61	60.38
US16	19.02	61.30
US17	18.59	64.92
US18	21.68	60.68
US19	20.48	56.96

Table 4. The best treatment procedure in different days of experiment.

Rank	Days					
	4	**6**	**8**	**10**	**12**	**14**
1	US13	US9	US11	US11	US11	US11
2	US10	US11	US12	US9	US9	US10
3	control	US13	US9	US12	US10	US9
4	US12	US10	US13	US10	US12	US12
5	US9	control	US10	US13	US13	US17
6	US18	US12	US18	US18	US1	US13
7	US7	US14	US1	US1	US2	US1
8	US11	US2	US2	US6	US6	US18
9	US19	US7	US14	US2	US7	control
10	US8	US17	US16	US7	US18	US16
11	US17	US18	US7	US14	US17	US2
12	US1	US19	US6	control	US3	US14
13	US14	US1	US19	US3	control	US15
14	US2	US5	control	US17	US14	US19
15	US6	US8	US15	US8	US8	US7
16	US3	US3	US3	US16	US16	US6
17	US16	US16	US17	US15	US19	US3
18	US5	US15	US8	US19	US15	US8
19	US15	US6	US5	US5	US5	US5
20	US4	US4	US4	US4	US4	US4

DISCUSSION

We found that the groups were not different before the operation and 2, 4, and 6 days after the operation (*P*>0.05), but for US11 group and 8, 10, 12 and 14 days after operation, a significant difference was found (*P*<0.05). The trend had fully been effective and there was a significant difference among the groups, which was remarkable in the final days. According to Figures 1 and 2 and Table 3, we conclude that changes in ultrasound parameters and their increase or decrease may help us to interpret and study the improvement trend. Therefore, it is anticipated that by using more groups and selecting precise parameters of ultrasound radiation, the improvement process may be interpreted more precisely [21-25].

As shown in Table 4, the US11 group had the best improvement percentile compared with the other groups. For the US11 group, the parameters of ultrasound were as follows: intensity = 0.5 W/cm², frequency = 1 MHz, pulse mode, duty cycle = 20%, duration = 2 min, and 14 treatment days. The recovery in the last day of treatment was 90% and total recovery was 39% (Table 3). Therefore, it is concluded that the experimented mice had more improvement which is remarkable compared with the previous studies [1-5].

According to Table 4, the best procedure for treatment was that used for the US13 group on the 4th day after operation, US9 on the 6th day, and US11 at the final day. It may be a general instruction for physiotherapists and researchers to recover nerve and speed up the improvement process. As our results were achieved by experimenting on mice, the future instruction of our findings for human beings should be performed with caution. Selecting the continuous mode could produce more heat for NMRI mice, which can cause reverse effects on the recovery. Thus, it can be considered that non-thermal effects of ultrasound are superior to its thermal effects [15-17, 26-30].

In this study, we examined the effects of ultrasound on the recovery of peripheral nerve injury by altering parameters of ultrasound with 20 groups. Based on the tests, we obtained some optimal combination of values in ultrasound parameters compared with previous studies [5-8]. Those desirable parameters could be obtained just for one or two groups. It is considered that these tests are not enough to precisely interpret the improvement of nerve recovery. Therefore, this is the first study to systematically examine and compare the effects of ultrasound parameters on the recovery of injured peripheral nerve.

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