Effects of Laser Therapy (680 nm) on Recovery of the Sciatic Nerve in Rabbits

Mohammad Ashrafzadeh Takhtfooladi, Davood Sharifi, Iraj Sohrabi Haghdost, Gholamreza Abedi

Department of Surgery, Faculty of Specialized Veterinary Science, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Abstract

Peripheral nerve injuries are common, and there is no easily available formula for successful treatment. Incomplete injuries are most frequent. After complete axonal transection, the neuron undergoes a number of degenerative processes, followed by attempts at regeneration. Unfortunately, results of nerve repair to date have been no better than fair. Many evidence lines have shown that peripheral nerves regeneration may be accelerated by physical agents. Laser irradiation is one of the therapeutic methods for the recovery of degenerated peripheral nerves. The objective of this study was to investigate whether laser therapy (680 nm) could stimulate the healing process in the sciatic nerve regeneration in rabbits submitted to complete transection through histological analysis. Twenty adult white New Zealand male rabbits were used, where the injury of the type neurotmesis of the right sciatic nerve under general anesthesia (intramuscular ketamine/xylazine) was approximated using prolene 6-0. The rabbits were randomly distributed in 2 groups with 10 rabbits each. In group I, Arsenate of Gallium Laser with the extension of wave of 680 nm, 10 mW power with 1.0 J/cm² irradiation with 10 seconds for each Cm², in the pulsed form. The laser therapy in group I was initiated on the post-surgical first day, where all the rabbits with application once a day for 14 days or 10 minutes each rabbit. Rabbits in group II not had given treatment (control group). The samples of transected nerves were collected and prepared of histological analysis on the 30 days. The histological aspect showed that in the injury site, blood vessels were more prevalent and thicker for group I than group II. Thick fibers with very thin myelin sheaths were prevalent in the injury site in both groups. Schwann cells with reactive appearance nuclei, characteristic of synthesis activity, as well as typical images of axonal sprouting were more prevalent on group I, while Wallerian degeneration was more evident on group II. Small-gauge fibers and thin myelin sheaths were prevalent, although thick fibers and thick myelin sheaths were also frequent on group II next to a large number of Schwann cells with reactive-appearance nucleus and images of axonal sprouting. In this site, Wallerian degeneration was not so evident for group I. This study suggests that postoperative laser irradiation (680 nm) was able to accelerate and potentialize the peripheral nerve regeneration process of rabbits.

Key words: Laser Therapy, Nerve Regeneration, Sciatic.

Introduction

The regeneration and repair phase following nerve injury may last for many months. The human peripheral neuron’s capacity to initiate a regenerative response appears to persist for at least 12 months after injury, and a robust response can be elicited even after repeated injuries [1]. Many evidence lines have shown that peripheral nerves regeneration may be accelerated by physical agents. Laser therapy seems to be an efficient technique in peripheral nerve recovery, and its effectiveness has been shown over the years. A critical review of the literature [2] found that more than 80% of the experimental studies carried out so far on the use of laser therapy for promoting peripheral nerve repair led to a positive outcome in posttraumatic/ post-operative nerve recovery, thus pointing to this physical therapy tool as a very promising clinical approach for patients who suffered a nerve lesion. In contrast, there are conflicting results on the use of laser therapy. These results may be due to the lists of technical variables (in particular wavelength, doses, and types of radiation) that, if not properly adjusted, can reduce the success of any therapeutic procedure [2, 3]. Following the need to establish clinical protocols for
the application of laser therapy, the present study intends to further the investigation into the effect of this therapy on the peripheral nerve, focusing particular attention on the choice the laser therapy wavelength (680 nm) and the parameters of irradiation (10 mW power, 1.0 J/cm²). The results of the present study can reveal important aspects of the prospects for laser therapy application in patients in the future.

Materials and Methods

All rabbits of the present research were cared according to the norms of the Islamic Azad University Faculty of Specialized Veterinary Sciences Tehran laboratory of animal experimentations; this investigation was approved by the Committee of Ethics in Research with animals in Islamic Azad University. The study was designed so as to minimize the number of animals required for the experiments.

In this experimental study, 20 male adult white New Zealand rabbits were utilized (weight: 2.5 to 3 kg), purchased from Pasteur Institute of Iran (Tehran, Iran). All rabbits were kept at a constant room temperature (20 °c) under standard conditions with food and water ad libitum in individual stainless cages. Animals were divided randomly into two experimental groups of ten rabbits each: group treatment (group I) and group control (group II). Anesthesia was induced using intramuscular ketamine hydrochloride 10% (50 mg/kg), xylazine hydrochloride 2% (5 mg/kg). Rabbits were maintained in the left lateral recumbency. The site of the surgery was prepared on the caudal region of the right femoral area which was clipped and followed by local antisepsis. The 5 cm incision was given longitudinally to that of semi-membrane and semi-tendinous muscles. The sciatic nerve was exposed by longitudinal intramuscular separation semi-membranous and semi-tendineous and quadriceps muscles were carried out allowing the complete vision of the nerve. With the help of scalpel blade, nerve was cut in to 2 pieces and was re-anastomosed using prolene 6-0 threads. Then, the surgical closure of the region was down with nylon 3-0 threads.

In group I Arsenate of Gallium Laser with the extension of wave of 680 nm, 10 mW power, 1.0 J/cm² with irradiation of 10 seconds for each Cm², in the pulsed form. The laser therapy was initiated on the post-surgical first day, where all the rabbits with application once a day for 14 days. On the 30 days, all rabbits were submitted to euthanasia by an anesthetic overdoses, after that a sciatic nerve near the exact site of injury was removed from the right hind-leg of each rabbit, totaling 20 samples. The samples were stained with H&E with an objective lens with 40X; 5 macroscopic fields from those were analyzed and quantified the following histological findings.

Statistical analyses were carried out using SPSS statistical software (version 16). Results were expressed as the mean +/- SD. Distribution of the groups was analyzed with one sample Kolmogorov–Smirnov test. The ANOVAs with the Tukey’s post-test were employed to analyze two groups consecutively. Values less than 0.05 were considered as statistically significant.

Results:

A total of 20 rabbits were operated. All the rabbits tolerated operation and survived until the final study period. Figures 1 and 2 illustrate representative photomicrograph of histological slices of injury site of sciatic nerves on group I and group II. The histological aspect showed that in the injury site, blood vessels were more prevalent and thicker for group I than group II. Thick fibers with very thin myelin sheaths were prevalent in the injury site in both groups. Schwann cells with reactive appearance nuclei, characteristic of synthesis activity, as well as typical images of axonal sprouting were more prevalent on group I, while Wallerian degeneration was more evident on group II. Small-gauge fibers and thin myelin sheaths were prevalent, although thick fibers and thick myelin sheaths were also frequent on group II next to a large number of Schwann cells with reactive-appearance nucleus and images of axonal sprouting. In this site, Wallerian degeneration was not so evident for group I.

Fig. 1: Cross-section treated area in group I. The myelinated axons show no degenerative signs.
Fig. 2: Section from group II, there was myelin sheath but with severe degenerative and inflammatory reactions.

Fig. 3: Neurons in histological findings. *group LLLG is significantly different from CG group (P<0.05).

Fig. 4: Presence of Schwann cells in the histological findings. *group LLLG is significantly different from CG group (P<0.05).

Discussion:

The irradiation with laser interfere somehow in the nerve function, according to some demonstrations that evidence that there is a reduction of latency time and an increase in the speed of the nerve conduction. There are still some experimental evidences that a laser has a positive effect in the regeneration of injured nerves [4]. Animal models have been employed to study laser therapy effects in nerve repair [5, 6]. The He-Ne laser (632.8 nm) in the red emission region of the electromagnetic spectrum was the most studied wavelength on the biomodulation of biological response in the repair process [7,8]. Lately, others wavelengths are being developed and researched, like the lasers that emit radiation in the
bands of 650-830 nm (GaAlAs) [9,10] and that of 904 nm (GaAs) [11,12]. It was adopted the 660 nm GaAlAs laser because of its low intensity and for having a wavelength often used in clinical practice, besides that, there are no significant number of previous studies over this wave length and its effects on peripheral nerve regeneration [13]. In clinical practice, the low intensity laser therapy employs doses of 1 to 4J/cm², associated to an output power between 10 and 90 mW, being widely used in various musculoskeletal injuries, in addition to the algic and inflammatory processes [14]. Based on this fact the use of a density of 41/cm² in this study was justified. It is important to highlight that this parameter is widely variable in the researches using laser therapy on nerve regeneration. The use of low intensity laser therapy as a therapeutic method still has contradictions and its biomodulator effect over the peripheral nerves is obscure yet, since some studies show positive results [15, 12, 13] while others indicate that the laser has no influence over the peripheral nerves regeneration [10]. Byrnes et al used 1,600 J/cm² of 810 nm diode laser to improve healing and functionality in a T9 dorsal hemisection of the spinal cord in rats [16]. Anders et al [17] studied laser therapy for regenerating crushed rat facial nerves; by comparing 361, 457, 514, 633, 720, and 1064 nm and found best response with 162.4 J/cm² of 633 nm HeNe laser.

In the present research, we observed a significant difference among the groups for the proliferation of neurons and Schwann cells over 30 days of treatment through laser therapy, making the treatment efficiency through this resource, even in a short period of time, statistically proved. This study suggests that postoperative laser therapy (680 nm) enhances the regenerative processes of peripheral nerves after complete transection and anastomosis.

Conclusion:

In conclusion, the data presented here show that laser therapy (680 nm) influenced positively the regeneration of the sciatic nerve in rabbits after anastomosis, becoming the nerve recovery more rapid and efficient. Finding the optimal conditions for laser irradiation is essential in the application of laser in medicine, because laser affects tissues differently according to the wave-length, pulse duration, pulse/energy, energy density and delivery system.

Acknowledgment

The authors wish to acknowledge the Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science and Research Branch, Tehran-Iran, Research Council for approval and financial support of this research work.

References


