Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2012, 3 (8):3941-3944
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Effect of 780 nm low level laser therapy on the regeneration of injured sciatic nerves after end to end anastomosis in the rabbit: Histological analysis

Mohammad Ashrafzadeh Takhtfooladi[*1], Davood Sharifi[2], Soheila Mokmeli[3], Gholamreza Abedi[1], Iraj Sohrabi Haghdost[4], Pezhman Mortazavi[4], Hamidreza Fattahian[1], Ahmad Asghari[1], Mohammad Abedi[4], Mojtaba Ziaee[5]

[1]*Department of Surgery, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.*
[2]*Department of Surgery & Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*
[3]*Anesthesiologist and Pain Specialized, Tehran, Iran.*
[4]*Department of Pathology, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.*
[5]*Department of Pharmacology, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran.*

ABSTRACT

Peripheral nerves are often exposed to injuries from traumatic origins, such as crushing impact and total transections, resulting in decreases or complete loss of sensory and motor capabilities in corresponding area of innervations. Laser irradiation is one of the therapeutic methods for the recovery of degenerated peripheral nerves. The present study focuses on the effect of 780 nm laser irradiation on the regeneration of injured sciatic nerves after end to end anastomosis in the rabbit. Twenty adult white New Zealand male rabbits were used, where the injury of the type axontmesis of the right sciatic nerve under general anesthesia was approximated using proline 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 780 nm, 10 mW power with 1.0 J/cm^2 irradiation with 10 seconds for each Cm^2 with penetration of 4cm, 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 15 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 90 days, they were analyzed and quantitifid for Schwann cells, mylenic axons with larger diameter and neurons. The histological aspect was essentially normal on proximal segments of the right sciatic nerve on both experimental groups, with a regular distribution of narrow and wide nervous fibers and an apparently normal proportion between myelin sheaths thickness and fibers diameter, numerous non-myelinated fibers, intraneural blood vessels, and fibroblasts were also observed. In the proximal and intermediate segments (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 intermediate segment in both groups I and II. 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 segment, Wallerian degeneration was not so evident for group I and even II. This study suggests that postoperative low-power Ga-As-Al laser irradiation was able to accelerate and potentialize the peripheral nerve regeneration process of rabbits within 15 days of irradiation.

Key words: Low Level Laser Therapy, Nerve Regeneration, Sciatic, Rabbit.

INTRODUCTION

Peripheral nerve transections represent a very common clinical occurrence [1]. There are two main requirements for a good clinical outcome after this type of lesions. The first is surgical treatment so as to make possible the regeneration of the transected nerve fibers toward their original periphery. The second important requirement for successful recovery after a nerve lesion is postoperative rehabilitation treatment. In fact, although the surgical techniques have proved to lead almost always to the regeneration of nerve fibers, the degree of recovery can be highly variable among patients [2, 3]. It is thus very important to seek effective postoperative physiotherapeutic protocols to improve the final degree of functional recovery in patients.

Many evidence lines have shown that peripheral nerves regeneration may be accelerated by physical agents, such as electric power, magnetic fields, and ultrasound. Laser therapy has also been studied regarding a potential positive role in this particular area, with first investigations focusing changes on nervous stimulus conveyance, with an electrophysiological demonstration of a reduced latency time, and increased conveyance speed in normal nerves, both in animals [4] and in human beings [5, 6, 7]. However, despite advances in the medicinal and surgical management of peripheral nerve injuries, recovery is often incomplete. Therefore it would be clinically beneficial to develop new treatments to accelerate and improve the recovery process. Moreover, despite extensive studies on how axonal growth and myelin regeneration are regulated [8]. Thus, the present study aimed to determine whether Ga-As (Gallium-Arsenide, wave-length: 780 nm) infrared laser irradiation could stimulate the healing process of experimentally injured rabbit sciatic nerves.

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 Iran laboratory of animal experimentations; this investigation was approved by the Committee of Ethics in Research with animals in Islamic Azad University (L1335). Therefore 20 male adult white New Zealand rabbits were utilized, with body weight varying between 2.5 to 3 kg. The rabbits were maintained in individual stainless cages in adequate sanitary conditions, and kept them in the temperature-controlled environment (20 c), provided with water and standardized food ad libitum. Animals were divided randomly into two experimental groups of ten rabbits each: group treatment (group I) and group control (group II). The rabbits were anesthetized intramuscular whit solutions of ketamine hydrochloride 10% (50 mg/kg), xylazine hydrochloride 2% (5 mg/kg) and atropine sulfate (0.05 mg/kg). They 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 anti-sepsis. 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 proline 6-0 threads. Then, the surgical closure of the region was down with nylon 3-0 threads. In group I Arsenate of Gallium Laser (Ga-As Mustang 2000) with the extension of wave of 780 nm, 10 mW power, 1.0 J/cm^2 with irradiation of 10 seconds for each Cm^2 with penetration of 4 cm, 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 15 days. On the 90 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; 15 macroscopic fields from those were analyzed and quantified the following histological findings: Schwann cells, myelinic axons with large diameter and neurons.

Statistical analyses were carried out using SPSS statistical software (version 11.2). Results were expressed as the mean +/- standard deviation. The ANOVAs with the Tukey's post-test were employed to analyze two groups consecutively. Values of $P<0.05$ were considered as statistically significant.

RESULTS

In this study investigation was done to evaluated regenerative capacity of motor nerve repaired by end to end method of neuropathy after being exposed to low level laser for 15 days. All of the sciatic nerve which was

transected immediately after recovery showed serious deformity of the right rear paw with flexed toes and they were unable to apply their load on it until the end of the second post- operative weeks.

When they started to place partial load on the operative limb, the left rear limb obviously overloaded throughout the follow up period during few weeks of recovery. Both the appearance and support improved slowly on right rear paws throughout to consecutive weeks, but had not retained to normal level until levels of 4th weeks. The edges of skin wound were flattened in group I before second weeks.

The histological aspect was essentially normal on proximal segments of the right sciatic nerve on both experimental groups, with a regular distribution of narrow and wide nervous fibers and an apparently normal proportion between myelin sheaths thickness and fibers diameter, numerous non-myelinated fibers, intraneural blood vessels, and fibroblasts were also observed. In the proximal and intermediate segments (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 intermediate segment in both groups I and II. 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 segment, Wallerian degeneration was not so evident for group I and even II (Fig. 1).

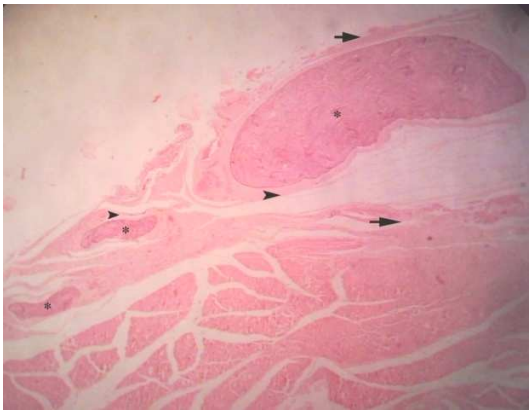

Figure 1: Cross-section treated area in group I. The myelinated axons show no degenerative signs.

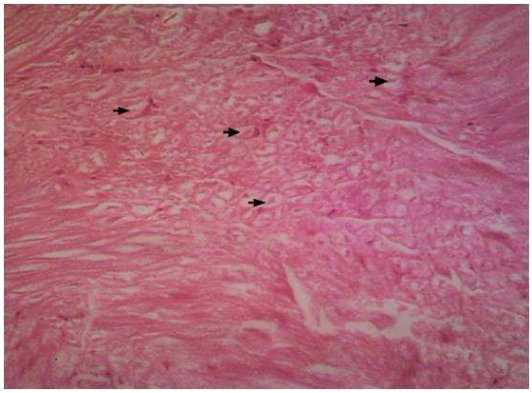

Figure 2: Section from group II, there was myelin sheath but with severe degenerative and inflammatory reactions.

DISCUSSION

Injury of a peripheral nerve frequently results in considerable disability. In an extremity, such lesions may be associated with loss of sensory and motor functions, which leads to severe occupational and social consequences.

Surgical repair is the preferred modality of treatment for the complete or severe peripheral nerve injury [9]. For most patients who suffered from peripheral nerve injuries, the continuation of rehabilitation therapy was recommended. Unfortunately, spontaneous recovery of severe peripheral nerve injury is often unsatisfactory. The usual results after such an injury are degeneration of the axons and retrograde degeneration of the corresponding neurons of the spinal cord, followed by a very slow regeneration. Recovery may eventually occur, but it is slow and frequently incomplete. Understandably, therefore, numerous attempts have been made to enhance and/or accelerate the recovery of injured peripheral nerves. One of the methods studied is the use of different wavelengths of low-power laser irradiation to enhance the recovery of peripheral nerve injuries [9]. Studies that evaluated the effects of laser irradiation on Schwann [10] and nerve cell [11] cultures and injured peripheral nerves of animals [12, 13, 14] showed positive results. Laser phototherapy significantly improves recovery of the injured peripheral nerve [15,16,17,18] and in addition decreases post-traumatic retrograde degeneration of the neurons in the corresponding segments of the spinal cord [19] studies the effects of low-power laser irradiation on injured peripheral nerves of rats by Rochkind have demonstrated: a. Protective immediate effects, which increase the functional activity of the injured peripheral nerve [20]. b. Maintenance of functional activity of the injured nerve over time [16]. c. Influence of the low-power laser irradiation on scar tissue formation at the injured site [17]. d. Prevention or decreased degeneration in corresponding motor neurons of the spinal cord [19] e. Influence on axonal growth and myelinization [16, 18]. In the present research, we observed a significant difference among the groups for the proliferation of neurons and Schwann cells over 90 days of treatment through laser therapy, making the treatment efficiency through this resource, even in a short period of time, statistically proved. Histological findings in the laser-treated group showed an increased total number of axons and better quality of the regeneration process, due to an increased number of large-diameter axons, compared to the non-irradiated control group. The study suggests that postoperative low level laser therapy enhances the regenerative processes of peripheral nerves after complete transaction and end to end anastomosis.

CONCLUSION

Laser affects tissues differently according to the wave-length, pulse duration, pulse/energy, energy density and delivery system. Thus, finding the optimal conditions for laser irradiation is essential in the application of laser in medicine. The irradiation with low level laser (780 nm) influenced positively the regeneration of the sciatic nerve in rabbits after being injured and end to end anastomosis, becoming the nerve recovery more rapid and efficient.

REFERENCES

[1] Evans GR. *Anat Rec*, **2001**, *263*, 396-404.
[2] M Sami; GA Carvalho; G Nikkhah; Penkert G. *J Neurosurg*, **1997**, *87*, 881-886.
[3] Lundborg G. *Peripher Nerv Syst*, **2002**, *7*, 139-140.
[4] M Nissan; S Rochkind; N Razon; Bartal A. *Lasers Surg Med*, **1986**, *6*, 435-438.
[5] JR Basford; JR Daube; HO Hallman; TL Millard; Moyer SK. *Laser Surg Med*, **1990**, *10*, 35-39.
[6] JR Basford; HO Hallman; JY Matsumoto; SK Moyer; JM Buss; Baxter GD. *Lasers Surg Med*, **1993**, *13*, 597-604.
[7] AS Lowe; GD Baxter; DM Walsh; Allen JM. *Lasers Surg Med*, **1994**, *14*, 40-46.
[8] Terenghi G. *J Anat*, **1999**, *194*, 1-14.
[9] Rochkind S. *Photomedicine and Laser Surgery*, **2006**, *24*, 151-157.
[10] HH Van Breugel; Bar PR. *J Neurocytol*, **1993**, *22*, 185-190.
[11] Y Wollman; S Rochkind; Simantov R. *Neurol Res*, **1996**, *18*, 467-470.
[12] D Gigo-Benato; S Geuna; Rochkind S. *Muscle Nerve*, **2005**, *31*, 694-701.
[13] MH Shamir; S Rochkind; Sandbank J. *J Reconstruct Microsurg*, **2001**, *17*, 133-138.
[14] D Gigo-Benato; S Geuna; De castro Rodrigues A. *Laser Med Sci*, **2004**, *19*, 57-65.
[15] JJ Andres; RC Borke; Woolery SK. *Lasers Surg Med*, **1993**, *13*, 72-82.
[16] S Rochkind; L Barr-Nea; Razon N. *Neurosurgery*, **1987**, *20*, 843-847.
[17] S Rochkind; M Nissan; Barr-Nea L. *Lasers Surg Med*, **1987**, *7*, 441-443.
[18] MH Shamir; Rochkind S. *J Reconstruct Microsurg*, **2001**, *17*, 133-138.
[19] S Rochkind; L Barr-Nea; Volger I. *Spine*, **1990**, *15*, 6-10.
[20] S Rochkind; M Nissan; Lubart K. *Acta Neurochir*, **1988**, *94*, 74-77.