Efficacy of 780-nm Laser Phototherapy on Peripheral Nerve Regeneration after Neurotube Reconstruction Procedure (Double-Blind Randomized Study)

SHIMON ROCHKIND, M.D., LEONOR LEIDER-TREJO, M.D., MOSHE NISSAN, Ph.D., MERAV H. SHAMIR, D.V.M., OLEG KHARENKO, M.D., and MALVINA ALON, M.D.

ABSTRACT

Objective: This pilot double-blind randomized study evaluated the efficacy of 780-nm laser phototherapy on the acceleration of axonal growth and regeneration after peripheral nerve reconstruction by polyglycolic acid (PGA) neurotube. Background Data: The use of a guiding tube for the reconstruction of segmental loss of injured peripheral nerve has some advantages over the regular nerve grafting procedure. Experimental studies have shown that laser phototherapy is effective in influencing nerve regeneration. Methods: The right sciatic nerve was transected, and a 0.5-cm nerve segment was removed in 20 rats. A neurotube was placed between the proximal and the distal parts of the nerve for reconnection of nerve defect. Ten of 20 rats received post-operative, transcutaneous, 200-mW, 780-nm laser irradiation for 14 consecutive days to the corresponding segments of the spinal cord (15 min) and to the reconstructed nerve (15 min). Results: At 3 months after surgery, positive somato-sensory evoked responses were found in 70% of the irradiated rats (p < 0.015), compared to 30% of the non-irradiated rats. The Sciatic Functional Index in the irradiated group was higher than in the non-irradiated group (p < 0.05). Morphologically, the nerves were completely reconnected in both groups, but the laser-treated group showed an increased total number of myelinated axons. Conclusion: The results of this study suggest that postoperative 780-nm laser phototherapy enhances the regenerative process of the peripheral nerve after reconnection of the nerve defect using a PGA neurotube.

INTRODUCTION

In cases where a peripheral nerve is injured and complete segmental loss exists, the treatment of choice is nerve reconstruction using an autogenous nerve graft. The use of a regenerating guiding tube for the reconstruction of massive segmental loss of a peripheral nerve has some advantages over the regular nerve grafting procedure. It is simple and time saving, and does not require a donor of an autologous nerve. The use of both biodegradable and non-biodegradable artificial nerve tubes has been extensively investigated in vivo. Using nerve tubes composed of silicone, the nerve recovery was jeopardized due to a late foreign body response and chronic nerve compression. Collagen, polyglycolic acid (PGA), and copolymers of lactide and ε-caprolactone are among the in vivo degradable materials used as alternatives to autologous nerve grafts. The correction of large nerve defects with functional recovery using a degradable graft still remains a challenge. However, for the treatment of complete peripheral nerve injury where the nerve defect is significant, an innovative biodegradable composite co-polymer guiding neurotube, based on tissue engineering technology, was recently described.

After nerve reconstruction, sprouting of regenerated fibers from the proximal stump begins and is followed by the elongation of the fibers along the sheaths of the distal nerve towards the target organ. The number of regenerated fibers and their reconnection with the target organ determines the quality of functional recovery. Functional return and regeneration, in general,
are decreased by excessive delays in reinnervation in humans.\textsuperscript{6–8} and the same response is found in animals.\textsuperscript{9–11} Means of enhancing regeneration are essential, since degeneration is always inevitable in severely damaged peripheral nerves. Therefore, posttraumatic nerve repair still represents a major challenge of restorative medicine, and the need exists to find effective methods for enhancing nerve regeneration, especially after surgical nerve repair.\textsuperscript{12–14} Among the various proposed methods for enhancing nerve repair, phototherapy has received increasing attention over the past two decades. The extensive review article, suggesting a potential mechanism of action of phototherapy, which was published in \textit{Muscle and Nerve} in 2005,\textsuperscript{15} revealed that all experimental studies, but two\textsuperscript{16,17} showed phototherapy to promote the recovery of the severely injured peripheral nerve.\textsuperscript{18–27} Recent experimental studies have shown that phototherapy is effective in influencing nerve regeneration, not only in cases of axonotmesis, but also with the more severe neurotmesis lesions repaired by means of microsurgical techniques, such as end-to-end neurorrhaphy\textsuperscript{26} and end-to-side neurorrhaphy.\textsuperscript{20}

This study evaluated the effects of 780-nm laser phototherapy on the regeneration of the completely injured sciatic nerve in rats after the reconstruction of a 5-mm nerve defect by PGA neurtube.

\section*{METHODS}

The study was conducted on 20 3-month-old male Wister rats, weighing 250–300 g. All rats were anesthetized with 15 mg xylazine and 50 mg ketamine intraperitoneally for surgery, laser treatment, and electrophysiological recording. The study was performed in a double-blind randomized manner. All our animals were treated according to the guidelines listed in the \textit{NIH Guide for the Care and Use of Laboratory Animals} under the supervision and permission of the Tel-Aviv Sourasky Medical Center animal testing committee.

\subsection*{Surgical procedure}

The procedure was performed using a surgical microscope. The right thigh of each rat was prepared for an aseptic procedure. All rats underwent an identical surgical procedure. The operation was carried out by exposing the sciatic nerve on the right side, and separating it from the biceps femoris and semimembranosis muscles. The sciatic nerve was completely transected at the third femur level, using microsurgical seizers, and a 0.5-cm nerve segment was removed. The 0.5-cm distance between the proximal and distal part of the nerve was microsurgically reconnected using a bioabsorbable PGA tube (Neurotube\textsuperscript{TM}, Neuroregen\textsuperscript{TM}). In all rats, the biodegradable neurtube was placed between the proximal and the distal parts of the transected nerve for reconnection of a 0.5-cm nerve defect. The 1-mm proximal and 1-mm distal parts of the nerve were fixed into the neurtube using 10-0 sutures placed in the epineurium (Fig. 1). The muscular, subcutaneous, and skin layers were closed.

\subsection*{Rat assignments}

After surgery, each rat was assigned an identification number. Randomization was performed in two groups: group I, laser treated (10 rats), or group II, non-laser treated (10 rats). None of the investigators were aware of the identity of the treatment given to each group.

\subsection*{Laser phototherapy}

Low-power laser irradiation was applied transcutaneously (in a partially darkened room) under general anesthesia, each day for 14 consecutive days using a continuous wave, 780-nm, 200-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig_1.png}
\caption{A neurtube (NT) placed between the proximal (P) and the distal (D) parts of the nerve for the reconnection of 0.5-cm nerve defect (arrows).}
\end{figure}
mW laser device (Medi-Robot). The irradiation was applied daily over two areas: 15 min for the corresponding segments of the spinal cord (L3-L6) at the level of the L2 lumbar vertebra, and 15 min on the reconstructed sciatic nerve. Laser irradiation was performed directly above the projection of the sciatic nerve, which was divided into three parts: proximal part; tube reconstructed area; and distal part. Each section was irradiated for 5 min per day (totaling in 15 min per day). The irradiating spot size was 3 × 2 mm. The distance between laser and skin was approximately 20 cm. In order to maintain similar treatment protocol, both the irradiated and non-irradiated rats were anesthetized.

Electrophysiological evaluation

Somatosensory evoked potentials (SSEP) were recorded in the experimental and control groups in a blinded manner directly postoperatively and 3 months after surgery. Conductivity of the spinal cord was studied by stimulation of the sciatic nerve and recording from two disc-recording electrodes, active and reference, placed on the scalp of the rats. These electrodes with conductive jelly were attached to the scalp (active over the somatosensory cortex in the midline and reference between the two eyes). The ground electrode was placed on the thigh on the side of stimulation. The sciatic nerve was stimulated by a bipolar stimulating electrode. A total of 256 stimulation pulses of 0.1 msec in duration were generated at a rate of 3/sec. The stimulus intensity was increased gradually, until slight twitching of the limb appeared. The appearance of evoked potentials as a response to stimulation in two consecutive tests, were considered positive. Latency and amplitude (positive [P] wave peak) were measured. The rats were anesthetized intraoperatively. The test was performed by the Medelec/Teca Sapphire 4 ME electromyography apparatus (20 Hz to 2 KHz band pass filter and calibration: sensitivity 10–20 mcV/div and time base 5 msec/div).

Functional assessment

Walking track analysis, sciatic functional index (SFI) based on actual measurements of the geometric parameters, was shown to be reliable and reproducible. Its value changed between 0 for healthy non-operated rats to −100 one fully plegic foot. This sciatic functional index was used to assess gait performance 10 weeks after surgery.

A special walking track (9 cm wide, 45 cm long, and 9 cm high) leading to a darkened box at one end was constructed. White paper was cut into the appropriate dimensions and placed on the track bottom. The hind feet of each rat were covered with ink and the rat was allowed to walk along the walking track. The test was repeated until several steps were identified on each paper that carried the rat number and date. The following parameters were measured from each test using a ruler: TOF, the distance from toes of one foot to toes of opposite foot; PL (print length), the distance from heel to toe in the same foot; TS (toe spread), the distance from first to fifth toe in the same foot; and IT (intermediary toe spread), the distance from second to forth toe in the same foot. All parameters were measured in the experimental (E) and normal (N) foot. Maximal test values were used in all cases. All abbreviations and symbols are identical with those presented by Bain et al. 1989. The following parameters were calculated:

TOFF (distance to opposite foot factor)
= (ETOF − NTOF)/NTOF; PLF (print length factor)
= (EPL − NPL)/NPL; TSF (toe spread factor)
= (ETS − NTS)/NTS; ITF (intermediary toe spread factor)
= (EIT − NIT)/NIT

Using the above values, the SFI was calculated using the De Medinaceli equation:

\[ SFI = \left[ TOFF - PLF + TSF + ITF \right]^{*} \]

Histological study: luxol fast blue staining

Three months after surgery, the rats were sacrificed (anesthetized and transfused with 10% formalin through the left heart ventricle). The sciatic nerve of the operated limb was harvested and was fixed in 10% neutral formalin. The tube reconstructed area was easily identified. Each nerve was dissected into three parts: proximal (P), 0.5-cm proximal to the reconstructed sites; nerve tube reconstructed area (NT); and distal (D), 0.5 cm distal to the reconstructed sites. Tissues were processed by exposure to formalin, escalating concentrations of ethanol and ending with xylene. Sections were then embedded in paraffin, cut into sections of 5 μm, and layered on slides. Tissue sections underwent deparaffinization using xylene, and deescalating concentrations of ethanol with water. Sections were then incubated for 1 h at room temperature with luxol fast blue (LFB) solution. The LFB solution was prepared by mixing 0.1 g of LFB powder in 100 mL of methanol and 0.5 mL of 1.5 M HCl. Tissue sections were washed in running water, followed by incubation for 20 sec in 0.05% lithium carbonate. After rinsing the tissue sections in running water, 1% of the neutral red solution was added for 5 min as counterstain. Blind examination of the microsections was performed by two of the authors. Presence and arrangement of myelinated axons was scored as follows:

1. Isolated axons
2. Mild amount of axons
3. Moderate amount of axons
4. Good amount of axons
5. Normal amount of axons

RESULTS

Electrophysiological study

SSEP were recorded 3 months after complete transection and tube reconstruction of the 0.5-cm nerve defect in the right sciatic nerve of the rats. Seven (70%) out of the 10 rats in the irradiated group had positive SSEP responses, and three (30%) had no response. At the same time, in the non-irradiated group, four (40%) out of 10 rats had a positive response and six (60%) had no response (Fig. 2).

Statistical analysis. Fisher exact test was done to evaluate the significance of the results and indicated a statistically sig-
significant improvement ($p = 0.015$) in the laser-treated group, compared to the non-irradiated group.

Functional assessment

Sciatic functional index (SFI) is presented according to the DeMedinaceli equation. The pre-operative values of SFI were $0 \pm 10$. Ten weeks post-operation the SFI in the non-irradiated group was $-88$ (SD at $\pm 6$), indicating practically total loss of function in all tested rats. In the irradiated group, the SFI level was $-53$ with SD of $\pm 30$, indicating minimal to no effect in five of the treated rats. However, in the other five rats, the effect was significant enough to lead to an overall significant difference between the groups ($p < 0.05$). The irradiated group overall walked better than the non-irradiated group, but both were moving significantly worse on the walking track compared to the non-operated rats.

Morphology

The biodegradable polyglycolic acid neurotube re-created the anatomical connection of the previously transected and divided nerve, and a distance of 0.5 cm was re-created. The neurotube had dissolved at this time (Fig. 3).

Three months after surgery the growth of myelinated axons, which crossed through the composite neurotube was found and the continuation of axonal sprouting through the area of the tube to the distal part of the nerve was recognized. The laser-treated group showed more intensive axonal growth compared to the non-irradiated control group (Fig. 4a,b). Presence and arrange-
ment of axons from 10 rats in the laser-irradiated group were compared with 10 rats in the non-irradiated group (Fig. 5). In the neurotube reconstructed areas, the amount of myelinated axons in the laser-treated group and received a higher score of $3.7 \pm 0.2$ in comparison to $2.6 \pm 0.2$ in the non-laser-treated group. In the distal parts, the laser-treated group received a higher score of $3.4 \pm 0.4$ in comparison to $2.0 \pm 0.3$ score in the non-laser-treated group (Fig. 5).

DISCUSSION

This pilot study evaluated the efficacy of postoperative 780-nm laser phototherapy in peripheral nerve reconstructive surgery. In a clinical situation, where nerve defects frequently have to be corrected, it would be preferable to use a slowly degrading material, which does not swell extensively during degradation. Biodegradable nerve guides provide a successful alternative. Dellon and Mackinnon\textsuperscript{2,31} in their pioneer works showed that PGA conduit may be used as an alternative to a short interfascicular nerve graft.

Rochkind et al.,\textsuperscript{5} using a biodegradable composite co-polymer guiding neurotube, based on tissue engineering technology, suggested that an injury where the nerve defect is significant can also be repaired. In previous studies, we evaluated the effects of low-power laser irradiation on injured peripheral nerves of rats and have found protective immediate effects which increase the functional activity of the injured peripheral nerve\textsuperscript{32}; maintenance of functional activity of the injured nerve over time\textsuperscript{24}; a decrease or prevention of scar tissue formation at the injured site\textsuperscript{25}; prevention or decreased degeneration in corresponding motor neurons of the spinal cord\textsuperscript{33}; and an increase in the rate of axon growth and myelinization,\textsuperscript{24,26} thus accelerating and improving the regeneration of the injured nerve.\textsuperscript{34} The similar results on peripheral nerve regeneration were published by Anders et al.\textsuperscript{18,19} and the Geuna group.\textsuperscript{20}

In the present study, the sciatic nerve defect was reconstructed using a synthetic biodegradable PGA neurotube. This neurotube was used as a reparative conduit for reconstruction of a complete 0.5-cm segment loss of the peripheral nerve. The use of neurotube provides guidance for the regenerating axons, and re-creates anatomical reconnection of the previously transected and divided nerve. Nerve conductivity and growth of myelinated axons, which crossed through the neurotube followed by axonal sprouting in the distal part of the nerve, was found. Comparing the rats treated by neurotube alone, and those

FIG. 5. Mean score of the axons divided into proximal (P), neurotube (NT), and distal (D) parts of the sciatic nerve.
that received 780-nm laser phototherapy postoperatively 3 months after surgery, indicates better motor functions (resulting in less negative SFI value), higher electrophysiological responses, and better morphological results in the laser-treated group.

CONCLUSION

The results of this pilot study in which a 0.5-cm peripheral nerve defect was reconnected by biodegradable PGA neurotube suggest that the use of post-operative 780-nm low-power laser treatment improves regeneration after a peripheral nerve reconstruction procedure.

A long-term study and further investigation are needed. An additional step of this study will be the investigation of nerve recovery using a composite neurotube based on tissue engineering technology followed by postoperative 780-nm laser phototherapy.

ACKNOWLEDGMENTS

We wish to express our sincere thanks to Orit Ben-David and Elena Rasin-Poisik for their assistance in the preparation of this manuscript.

REFERENCES


Address reprint requests to:
Shimon Rochkind, M.D.
Division of Peripheral Nerve Reconstruction
Tel Aviv Sourasky Medical Center
6 Weizmann St.
Tel Aviv 64239, Israel

E-mail: rochkind@zahav.net.il