Original Article

The Efficacy of Low-Level Laser Therapy and Low Intensity Pulsed Ultrasound on Functional Recovery among Experimentally Induced Peripheral Nerve Injury in Wistar Rats

Dhivya S[1], Ramana K[2], Prathap Suganthira Babu[3], Lavanya Prathap[4], Mydhili Govindarasu[5], Kumaresan A[6], Vignesh Srinivasan[7]

[1]Post graduate, [2,7]Assistant Professor, [3,6]Professor, Saveetha College of Physiotherapy, SIMATS, [4]Associate Professor, Department of Anatomy, [5]Assistant Professor, BRULAC, Saveetha Dental College and Hospital, Chennai.

How to cite this article: Dhivya S, Ramana K, Prathap Suganthira Babu et. al. The Efficacy of Low-Level Laser Therapy and Low Intensity Pulsed Ultrasound on Functional Recovery among Experimentally Induced Peripheral Nerve Injury in Wistar Rats. Indian Journal of Physiotherapy and Occupational Therapy / Volume 18 Special Issue 2024;

Abstract

Background: A variety of etiological factors that lead to peripheral nerve injury include crushing, traction, long fractures in bones, cutting injuries, entrapment neuropathies, infection, inflammation, and tumours of varying degrees. LIPUS has a variety of biological effects on tissues, including speeding up the regeneration of soft tissues, and lowering inflammatory reactions. In LLLT, low-level laser light with a wavelength of 808 nm is employed to trigger a biological reaction.

Purpose: The objective of this study was to evaluate the functional recovery of low-level laser therapy (LLLT) and low-intensity pulsed ultrasound (LIPUS) treating the sciatic nerve in rats.

Methods: Using simple random sampling method and the inclusion and exclusion criteria, 18 rats in total were chosen. A mechanical crush will be performed for 30 seconds using haemostatic forceps. The study's rats were divided into 3 groups at random: Group A received ultrasound for 21 days; Group B received laser for 21 days; and Group C received no treatment. The toe spread assay was used to assess functional recovery 3 weeks after surgery. **Result:** All three groups are statistically significant ($p<0.005$) when analysed using one-way ANOVA during intervention period (7^{th}, 24^{th}, 21^{st} day) but LLLT & UST has shown better improvement in their functional index than the control group.

Conclusion: According to the study's findings, LLLT significantly outperforms LIPUS in improving the functional recovery of an experimentally induced rat model.

Keywords: animal study, physiotherapy intervention, nerve regeneration, functional recovery and sciatic nerve injury.

Introduction

Common peripheral nerve injuries frequently prevent peripheral nerve axons from regenerating considerably, and only around 10% of patients are able to fully recover their function.[1] The reason for the poor functional results is typically attributed to

Corresponding Author: Prathap Suganthirababu, Professor, Saveetha College of Physiotherapy, SIMATS, Chennai.

E-mail: emailprathap@gmail.com

the sluggish axon regeneration process.[2] The severity of this problem was brought home by a research done in the US, which found that 50,000 or so people experience severe peripheral nerve injuries annually, making it a major public health issue.[3] Axonotmesis, neurotmesis, and neuropraxia were the three primary forms of nerve injuries discovered by a recent analysis in Brazil that looked at 456 cases of nerve damage.[4] Wallerian degeneration manifests in the distal stump when nerve fibre continuity is broken.

Since nerve innervation is essential to the structure and appropriate operation of muscles, peripheral nerve injury has a significant impact on skeletal muscle.[5] Therefore, enhancing axon regeneration is a viable strategy for treating peripheral nerve injury. After such injuries, a number of rehabilitation strategies have been proposed to aid functional recovery. For instance, a herniated disc might directly compress and irritate the spinal canal's nerve roots. Pain, numbness, tingling, and muscle weakness can all be symptoms of nerve compression and irritation. Despite the fact that recovery is mostly effective, it frequently occurs slowly and insufficiently, which leaves the patient in despair and results in large expenses for the community as a whole.[6]

A damaged peripheral nerve can lead to functional limitations or a lack of sensation in the afflicted location. Glial cells, also known as Schwann cells, play a crucial role in the regeneration of peripheral nerves. They are a part of the peripheral nervous system and are in charge of assisting and directing nerve regeneration. It might discuss topics like how neurotrophic factors are secreted by Schwann cells, how they affect nerve cell survival and proliferation, and how they affect the entire process of nerve healing.

Wistar rats were used in this investigation because they are simple to handle, are reasonably priced, and have human-like anatomy, physiology, and peripheral nerve regeneration. This therapeutic technique, called low-level laser therapy (LLLT), has mostly been utilized to promote regeneration and speed up the functional recovery of peripheral nerves. Crush, compression, stretching, avulsion, and division are the most frequent causes of peripheral nerve harm. Despite the use of advanced and contemporary techniques for treatment and reconstruction, morphologic and functional regeneration is rarely fully achieved because of the influence of variables like the type and degree of damage, the length of denervation, the type and diameter of the damaged nerve fibres, age, and other specific variables.

Even while some degree of healing is possible, it frequently happens slowly and insufficiently, leaving the patient feeling hopeless and placing a heavy financial burden on the community as a whole.[7] The severity or extent of a peripheral nerve damage has a significant impact on the recovery process.[8] Even with microsurgical restoration, functional recovery following peripheral nerve damage is typically inadequate, especially in cases when significant nerve trunks are injured. Low-Level Laser Therapy (LLLT) induces a photochemical reaction in cells known as "biostimulation" or "photobiomodulation."

Peripheral neuropathies have so far not been successfully treated with medication, including several neurotrophic drugs.[9] Even with improvements in surgical and medical procedures, peripheral nerve injury frequently does not completely recover. The poor reinnervation of motor and sensory target areas is one of the major obstacles to obtaining maximum recovery.[10] In order to address this; novel treatment approaches that can quicken and improve the healing process can show therapeutic promise. In this study, the effectiveness of laser therapy and ultrasound in hastening functional recovery in rats with right sciatic nerve injury is being evaluated.

Aim

The aim of this study is to evaluate and compare the efficacy of low-level laser therapy and low-intensity pulsed ultrasound in promoting functional recovery in rats with peripheral nerve injury.

Materials and Methods

This study was conducted from July 2022 to November 2022. It was an experimental study conducted on 18 male adult Wistar rats with experimentally induced peripheral nerve injury, aged 3 months and weighing 150-300g, was chosen for the study. They were kept in cages with one other animal apiece, fed on pellets, and given water. After being weighed, the rats were divided into three groups of

six at random. Every animal underwent the exact identical surgical treatment. Groups 1 and 2 received ultrasound and laser treatment, whereas Group 3 received a control group.

Inclusion criteria

Wistar rats between the ages of 6 months (0.5) and 48 months (4.0) were included.

Only male wistar rats were included.

Rats that are physically active were included.

Exclusion Criteria

Rats with physical abnormalities and skin issues are excluded.

Rats that exhibit psychological distress or aggression are prohibited.

Outcome measure

Assessment was performed at baseline preoperative, 7th, 14th, and 21st postoperative days. Toe Spread Assay was used as an outcome measure.

Procedure

All 18 animals were given intramuscular and intraperitoneal injections of 5% ketamine hydrochloride (70 mg/kg body weight) and 2% xylazine hydrochloride (10 mg/kg body weight) in a 1:4 ratio to induce anaesthesia. Meloxicam was administered subcutaneously in a dose of 1 mg/kg to treat the pain. By carefully dissecting between the gluteus maximus and quadriceps muscles, a 3 cm long postero-lateral longitudinal thigh incision was made, exposing the right sciatic nerve. The skin hair was shaved to make the sciatic nerve area visible, and 54 N of crush injury was then caused for 30 seconds using hemostatic forceps.

Ultrasound group (Group 1)

In this investigation, the crush site was the only target for the pulsed-wave ultrasound, which covered a range of frequencies, durations, and spatial peaks with time-averaged intensities. With a finger probe and a pulsed intensity of 0.4 W/cm2 in a 1:1 ratio, the ultrasound used had a frequency of 1 MHz and was applied transcutaneously. The treatment area over the crush injury site was treated for 4 minutes. The transducer was placed around 2 to 3 cm distant from the sutured area over the glove water bed in order to enable effective transmission of ultrasound into the animal's lower extremities. The treatment focused on the site of the crush injury and started on the second post - operative day (postoperative day 2). It lasted for 21 days in total. On days 7, 14, and 21 following surgery, the toe spread assay was carried out and compared to the pre-injury assessment to gauge functional recovery.

Laser group (Group 2)

For low-level laser irradiation in this study, a portable Aluminium Gallium Arsenide Laser Diode was employed. The laser met the following requirements: an energy density of 3 J/cm2, an area length, width, and tissue depth of 1 cm, a wavelength of 808 nm (infrared light), a power of 200 mW, and continuous mode. Each session's exposure period was set to 60 seconds. Patients in the experimental group 2 had laser irradiation with a focus on the nerve damage location that had been surgically identified. The contact point approach was used to apply a laser pen at a 90-degree angle to the skin during the first 21 days following surgery. According to Monte-Raso, this method proved quantitative, trustworthy, and reproducible in rat sciatic nerve operating settings.

Control group (Group 3)

Control group also underwent the same surgical procedure and no treatment was provided to the animals in the control group.

Assessment of nerve functional recovery
Toe spread assay

On the seventh, fourteenth, and twenty-first days after the right sciatic nerve lesion, the toe spread measurement was performed to gauge the degree of functional recovery. Both the experimental and control animals had water-soluble black ink painted on their hind limbs, and they were both free to travel on a white paper track, leaving their prints in their behind. At first, the measurement, application of the ultrasound, and toe spread assay were all completed on the same day. Since the study was coded by animal number without mentioning the groups they belonged to, one of the authors measured the walking track using an objective manner to ensure impartial evaluation. The distance between the first and fifth

toes was used to determine the toe spread index for each experimental group, and the results were compared to those of the laser and control groups. ANOVA analyses were performed on the toe spread assay with a 5% (p0.05) between-groups significant criterion.

Figure 1: An example of the toe spread index used to assess recovery.

Results

The surgical procedure and the laser, ultrasound application was well tolerated by all rats, and no animal died during the experiment. The study was conducted with a total of 18 footprint images in the different periods, preoperative, 7th, 14th, and 21st postoperative days. They were evaluated by the toe spread assay. These values are then analysed for their normality using Shapiro- wilk test and equal variance using brown-forsyth test. All the three groups passed the test. This indicates that all three groups have a similar baseline index and there is no potential bias among allocated subjects.

Toe spread assay value among all the three groups were found statistically significant comparing its pre-OP and 21st day value. The mean difference of group A is 8.95, group B is 5.81 and group C is 12.86. This shows that the LLLT group is very close to normal value showing significant difference than the other two groups. Tukey HSD test between all three groups proved that toe spread assay measure is statistically significant ($p<0.005$) between all three groups, this indicates that even the control group has shown improvement after 21 days of healing period. Hence toe spread assay has been improved in all three groups than its measure on 7th day but LLLT group has shown little more improvement then other two groups.

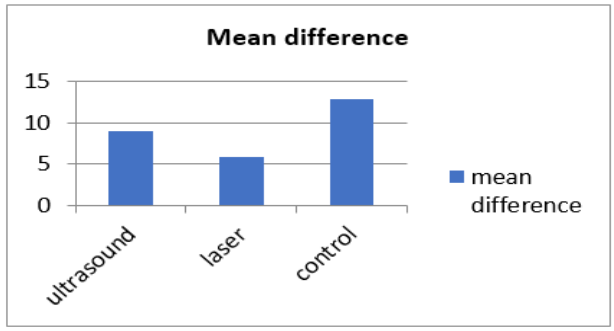

Fig 1: Representing the mean difference for Toe Spread Assay on Pre-op, day 2, day 14 and day 21.

Discussion

Some evidence states that peripheral nerves do in some manner respond to therapy with ultrasound, the results of early trials were not entirely conclusive, especially when it comes to the use of ultrasound in individuals.[11] High-intensity radiation would prevent neuron regeneration, whereas low-intensity therapeutic ultrasound delivered in minute doses would promote it, according to experimental research. This demonstrates that, as was shown in rats, ultrasonic exposure within a relatively narrow intensity range has a dose-related effect that is inversely proportionate. Suganthirababu P et al., states that ulnar nerve radiation to low level laser decreases the latency and increases the amplitude.[13]

The likelihood that therapeutic ultrasound could have a positive impact on nerve regeneration is what drove the current research. Since it is not always possible to compare the outcomes of several evaluation approaches employed by the same author or to draw immediate inferences from them, evaluating peripheral nerves that are going through regeneration can be difficult. Histologic, morphometric, and electrophysiological investigations make up the majority of research on peripheral nerve regeneration; while important, these techniques don't reveal much about the functional recovery itself. Depending on the severity of the nerve injury, injuries to the peripheral nerves result in significant dysfunctions and sometimes have lifetime consequences.[15]

Suganthirababu P et al., suggest that ultrasound therapy has a capacity to either assist or inhibit physiological activity by changing the nerve conduction velocity.[14]

In order to measure the effectiveness and speed of nerve conduction, nerve stimulation experiments include stimulating a nerve and monitoring the electrical impulses that occur. The treated group (getting ultrasound thermotherapy) would be measured and compared to control groups as the authors measured and analysed nerve conduction parameters. The quantitative information and conclusions from the nerve conduction studies would be presented in the study's results section. The differences in nerve conduction parameters seen between the treated group and the control groups are most likely to be discussed by the authors. The importance of the results in relation to nerve regeneration and functional recovery following compression neuropathy will be discussed in the discussion section.

Conclusion

Based on our samples, data, and techniques, it was possible to conclude that, when compared to LIPUS, LLLT had an advantageous effect on the functional rehabilitation of the right sciatic nerve. In the sciatic nerve damage, the healing process was improved after transcutaneously delivering therapeutic ultrasound and laser to the injury site. However, when compared, laser is more advantageous for nerve regeneration following a severe grade of peripheral nerve injury.

Ethical clearance: The Ethics Committee, BRULAC/SDCH/SIMATS/IAEC/01-2023/11, Saveetha University, India, approved the experimental study.

Funding: Self

Conflict of Interest: Nil

References

1. Frostick SP, Yin Q, Kemp GJ. Schwann cells, neurotrophic factors, and peripheral nerve regeneration. Microsurgery 18: 397–405, 1998.
2. Gordon T.et al., The role of neurotrophic factors in nerve regeneration. Neurosurgical Focus- Journal of neurosurgery; 26: 29, 2009.
3. Evans GR. Peripheral nerve injury: A review and approach to tissue engineered constructs. The Anatomical Record; 263:396–404, 2001.
4. Kouyoumdjian JA. Peripheral nerve injuries: A retrospective survey of 456 cases. Muscle and Nerve; 34:785–788, 2006.
5. Ishido M, Kami K, Mitsuhiko M. In vivo expression patterns of MyoD, p21 and Rb proteins in myonuclei and satellite cells of denervated rat skeletal muscle. American Journal of Physiology- Cell Physiology; 287:C484–C493, 2004.
6. Schaumburg HH, Berger AR, Thomas PK: Disorders of Peripheral Nerves. Philadelphia, FA Davis Co, 1992.
7. Omer GE Jr, Spinner M, Van Beek A: Management of Peripheral Nerve Problems. Philadelphia, W.B. Saunders Co., 1998.
8. Grant GA, Goodkin R, Kliot M: Evaluation and surgical management of peripheral nerve problems. Neurosurgery 44:825–840, 1999.
9. Fu SY, Gordon T: The cellular and molecular basis of peripheral nerve regeneration. Molecular Neurobiology; 14:67–116, 1997.
10. Gordon, T., Amirjani, N., Edwards, D.C. & Chan, K.M. Brief postsurgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients. Experimental Neurology; 223, 192–202, 2010.
11. Ebenbichler GR, Resch KL, Nicolakis P, Wiesinger GF, Uhl F, Ghanem AH, Fialka V: Ultrasound treatment for treating the carpal tunnel syndrome: Randomized "sham" controlled trial. The British Medical Journal; 316:731–735, 1998.
12. Suganthirababu P, Prathap L, Alagesan J, Kumaresan A, Srinivasan V, Ganesan D. Neurophysiological effect of low level laser therapy on Ulnar Nerve. Biomedicine. 2021 Oct 29;41(3):543-6.
13. Hong CZ, Liu HH, Yu J: Ultrasound thermotherapy effect on the recovery of nerve conduction in experimental compression neuropathy. Archives of Physical Medicine and Rehabilitation; 69:410–414, 1988.
14. Suganthirababu P, Alagesan J, Prathap L, Manikumar M, Kumaresan A, Banu N. Action of ultrasound therapy in altering motor nerve conduction velocity of ulnar nerve. Biomedical and Pharmacology Journal. 2019 Jun 25;12(2):843-8.
15. Fabrício Borges Oliveira et al, Action Of Therapeutic Laser And Ultrasound In Peripheral Nerve Regeneration, Acta Ortop Bras. 20(2): 98-103, 2012.