Journal of Medical Sciences

ISSN 1682-4474

Research Paper

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Azam Bakhtiarian,
Department of Pharmacology,
School of Medicine,
Tehran University of Medical Sciences,
Tehran, Iran

ANSInet
Asian Network for Scientific Information

J. Med. Sci., 7 (8): 1354-1357
15th November, 2007

Improved Regeneration of Injured Sciatic Nerve of Rats by He-Ne Laser

[1]Nasrin Takzare, [2]Mir-jamal Hosseini, [1]Mohammad Barbarestani, [2]Azam Bakhtiarian, [1]Hamid Reza Foroutan, [1]Bahram Montazeri, [1]Hamid Reza Kangarlou and [1]Alireza Takzare

Inability of the neural tissue to regenerate in response to trauma and the disability which the result has prompted a lot of effort in search for new means of neural rehabilitation. Low-energy Helium-Neon laser (He-Ne laser) irradiation has been proposed as a sensible choice. Low-energy laser therapy in specific wavelengths and affluence maintains the electrophysiological activity of injured peripheral nerve in rat and accelerating regeneration of the injured nerve. We studied the effect of low level laser irradiation (He-Ne laser) on regeneration of injured sciatic nerve of rats. In the current experiment, sciatic nerves of 20 adult rats were crushed surgically. The subjects were randomly assigned into case and control groups. The cases received daily laser irradiation (λ = 650 nm) for 4 min. The muscular function of the rats was tested by angle board each 3 days, since the 3rd day of post operation. On the 27th day, all the rats were sacrificed and the manipulated sciatic nerves were excised and studied histologically. The results showed significant improvement of neural structure and muscular function in laser treated rats over the controls, as revealed by angle board testing and microscopic examination. It seems that low-energy lasers can reduce the load of infection at the operation field, tissue inflammation and post operation pain. This study show that low-energy He-Ne laser greatly restores crushed sciatic nerves in rats and can be considered for clinical trails.

Key words: Helium-Neon laser (He-Ne laser), sciatic nerve, muscular function, angle board, microscopic examination, rat

[1]Department of Anatomy,
[2]Department of Pharmacology, School of Medicine,
Tehran University of Medical Sciences, Tehran, Iran

INTRODUCTION

Low level laser therapy is reported to influence soft tissue repair (Walker *et al*., 2000) and nerve regeneration (Rochkind *et al*., 1987a, b, 1989, 2001) and has there fore been used to treat a wide range of etiological diseases. Neural tissue is one of the most important tissues of the body which recovers extremely sluggishly after being injured (Walker *et al*., 2000; Rochkind *et al*., 1987a, b, 1989, 2001a, b; Nicolau *et al*., 2004). After the primary degeneration in response to trauma, its function is restored slowly and only the neural fibers are regenerated and have come in contact with the target organ (Rochkind *et al*., 2001a, b; Nicolau *et al*., 2004; Samoilov, 1991). The reason is the central nervous system has restricted regenerative capability. The medical researchers have been constantly endeavoring to find practical ways to restore the injured nerves (Walker *et al*., 2000; Rochkind *et al*., 2001; Nicolau *et al*., 2004; Nissan *et al*., 1986). Therefor, there have been some invasive procedures such as surgical methods and non-invasive ones like physiotherapy. Despite all efforts, heavy costs and the long time which it takes, the result is hardly more than slow neural regeneration and a doubtful outcome and the repaired nerve rarely resumes its optimal function. The application of low-energy lasers, especially He-Ne type, has opened up new windows of hope in neural injuries (Rochkind *et al*., 1987a, b, 1989, 2001; Samoilov, 1991; Andres *et al*., 1993; Rochkind, 1992; Midamba and Haaneas, 1993; Ijima *et al*., 1991). This method is absolutely non-invasive and its exclusive features have rendered it a relatively unique stance among all other methods (Rockindw *et al*., 1987; Schwartz *et al*., 2002; Jacobs and Fehling, 2003). Currently, low-energy lasers are widely used for neuromuscular lesions from dermatologic, rheumatologic and neurosurgical conditions. Low-energy lasers do not change the tissue temperature immediately but manipulate biological processes so their effect takes time to show (Mester, 1996; Babupour and Classbory, 1998). Today, many researchers have turned to this type of lasers and their clinical application is appraised (Passorolla and Gasomassina, 1984; Miloro *et al*., 2002). Experimentally, low-energy lasers have significantly helped regenerating the peripheral nerves and increasing the axonal density of the injured nerves after trauma and iatrogenic etiologies (Rochkind *et al*., 2001a, b; Bages *et al*., 2002; Shamir *et al*., 2001). In this study, we investigate the effect of He-Ne laser in regeneration of injured sciatic nerve of rats by simple method.

MATERIALS AND METHODS

Experimental procedure: This case-control study was performed on 20 Wister-albino rats (weighing 200-250 g). This strain used for many years in our laboratory in Tehran University, of medical science (2005-2006). They were kept in individual cages in a controlled room (temperature, 20-25°C humidity, 70 to 80%, exposed to 12 h of daylight). The rats were fed with standard rat food and tap water until experimentation. Twelve hours before the experiment the rats were stopped feeding but allowed free access to tap water. Limitation of food and water was not applied to the animals that were put into their cages after experimentation and were kept in separate standard cages under sterile conditions and the ambient air was kept at 25-30°C. All experiments were conducted in Tehran University according with the recommendations of the ethics committee on animal's experimentation of medical school.

Denervation: On the day of procedure, the operation area on the rat's skin was shaved and cleansed with Povidine-Iodine. Then the animal was anesthetized by ketamine. Under sterile conditions, an oblique incision was made in the paravertebral area near the hind legs just above the sciatic nerve and the skin and muscular fascia were carefully opened. Then the sciatic nerve was exposed and fixed by two pairs of forceps. Manual pressure was applied to the middle of the fixed part of the nerve, constantly for 90 sec using hemostats. Then traumatic nerve was replaced and the incision sutured (Rochkind *et al*., 2001a, b; Yueh *et al*., 2005). This procedure was performed on all the rats and the animals were randomly assigned into case and control groups.

Laser irradiation: Over a 27-day period on a daily basis, the rats in the case group were irradiated by low-energy He-Ne laser, at a wavelength (λ) of 650 nm, for 4 min.

Muscle function testing: Every 3 days since the 3rd day after the procedure, the rats of the case and control groups were tested for muscle force and coordination by means of a Riviline-Tatore angle board. After 27 days, all the rats were sacrificed and the traumatic portions of their sciatic nerves were excised for histological examination.

Statistical analysis: Values are expressed as Mean±Standard Error of the Mean (SEM) in tables. Statistical significance was evaluated by two-tailed student's t-test. The significance was set at $p<0.001$.

RESULTS AND DISCUSSION

The data collected from the angle board testing is shown in Table 1. This test is a simple method for assessing the function of sciatic nerve indirectly from the muscle power and coordination of the animal's hind legs.

Table 1: Mean muscle force of cases and controls on angle board (Comparative results between cases and controls on different days)

		Mean±SEM	
Days	n	Control group	Case group
3	10	1.3±0.68	4.5±0.60
6	10	7.2±0.69	12.5±0.78
9	10	11.3±0.68	16.2±0.88
12	10	16.9±0.72	24.7±0.93
18	10	27.7±0.79	35.2±1.61
21	10	35.5±0.75	42.5±1.34
24	10	35.5±0.90	48.2±1.30
27	10	41.3±1.01	55.5±1.16

There was significant increase in cases groups compared with control groups in muscle force on angle board test

Table 2: Diameter of myocytes in cases and controls

Groups	n	Mean diameter of myocytes±SEM
Case	10	2.20±0.59
Control	10	1.27±0.66

There was significant increase in cases groups compared with control groups in size of myocyte

As peripheral nerve lesions affects the pertinent tissues, leading to muscular atrophy and weakness, showed by microscopic changes in the target myocytes. This difference between in the cases and controls was compared in Table 2. The t-test analysis showed that the difference between the angle board results of cases and controls were statistically significant ($p<0.001$)

Low-energy laser acts as a focused source of energy and stimulates both the neurons and bystander cells. Low-energy laser irradiation in pain reduction has been observed in neurological studies. Recent studies have been suggested that the low-energy laser can improve recovery from nerve injuries in both rats and humans (Nicolau *et al*., 2004; Nissan *et al*., 1986; Samoilov, 1991; Andres *et al*., 1993; Rochkind, 1992; Midamba and Haaneas, 1993; Ijima *et al*., 1991). Mester and Mester (1996) reported the noticeable effects of low-energy laser irradiation on biological systems. It be demonstrated the regulatory role of laser beams on restriction of the primary inflammatory response (Bendov *et al*., 1999) and enhanced the healing of the injured tissue (Miloro *et al*., 2002; Rochkind *et al*., 2001a, b). The present experiment reproduced similar results revealing the significant difference between the neural regeneration in cases and controls. The microscopic specimens of the case group which received periodical laser irradiation clearly showed laser had prevented degeneration of the nerves and accelerated regenerative processes. Low-energy laser first introduced as effectiveness in biological systems (Mester and Mester, 1996). Studies have shown that certain wavelengths and energy densities of low-energy lasers can revive the neuronal function of the injured peripheral nerves, diminish degenerative changes in sensory and motor neurons and enhance the regeneration of traumatic nervous tissues (Bages *et al*., 2002; Shamir *et al*., 2001; Kimberly *et al*., 2005; Dong *et al*., 2003). Upon microscopic examination of the laser irradiated sciatic nerves, it was observed that the neural fibers had resumed their normal diameter and their Schwann cells and myelin coating were preserved. There were fewer macrophages as compared with the control group, which demonstrate reduced inflammatory reaction at the site of trauma. The muscular fibers with the injured sciatic nerves did not show the signs of atrophy when treated by laser, appeared normal diameter in size and color of the sarcoplasm. The function of muscles were improved as well as the control group which demonstrated by the angle board test results. The natural course of neural damage after the traumatic insult is observed in the control group. Primarily, the myelin sheath is fragmented into pieces to be taken up and digested by macrophages. As seen in the control group of rats, the degenerative and following regenerative changes are depicted by the numerous macrophages and Schwann cells. The natural consequence of neural deprivation of muscles was atrophic changes. The atrophic changes and lack of muscular function early after the operation in the control group of rats demonstratedin this Table 2.

This finding showed atrophic and microscopic changes in muscles and myocytes in the cases and controls similar to previous studies (Nicolau *et al*., 2004; Nissan *et al*., 1986; Samoilov, 1991; Andres *et al*., 1993; Rochkind, 1992; Midamba and Haaneas, 1993; Ijima *et al*., 1991; Mester and Mester, 1996). Present result suggests that low-energy lasers can reduce the load of infection at the operation field, tissue inflammation and post operation pain. As a condensed source of energy, it manipulates the cellular metabolic processes. Since it is a harmless vital stimulant, we recommend that He-Ne laser therapy be clinically applied after necessary clinical trials and considered as a very promising, Physiotherapeutic tool.

REFERENCES

Andres, J.J., R.C. Borke, S.K. Woolery and W.P.V. Merwe, 1993. Low power laser irradiation alters the rate of regeneration of the rat facial nerve. Lasers Surg. Med., 13: 72-82.

Babupour, R. and E. Classbory, 1998. Low-energy laser systems. Clin. Derma, 13: 87-90.

Bages, S., U. Comelekogla, G. Sahin, B. Bayukalilli, C. Erdogan and A. Kanik, 2002. Acute electrophysiology effects of pulsed Galliam-Arsenide low energy laser irradiation on configuration of compound nerve action potential and herve excitability. Lasers Surg. Med., 30: 376-380.

Bendov, N., G. Schefe, A. Lrinitchev, A. Wering, U. Oran and O. Halery, 1999. Low-energy laser irradiation effects satellite cell proliferation and differentiation *in vitro*. Biochim. Biophys. Acta, 1448: 372-380.

Dong, S.H., L. Eunju, K. Wom and K. Bumsun, 2003. Growth-associated protein-43 is elevated in the injured rat sciatic nerve. Neurosci. Lett., 344: 71-74.

Ijima, K., N. Shimoyama, M. Shimoyama and T. Mizuguchi, 1991. Evaluation of analgesic effect of low-power He: Ne laser on posthepetic neuralgia using VAS and modified McGill painquestionnaire. J. Clin. Laser Med. Surg., 9: 121-126.

Jacobs, W.B. and M.G. Fehling, 2003. The molecular basis of neural regeneration. Neurosurgry, 3: 43-47.

Kimberly, R., W. Ronald, K. Iiko, B. Lauren, S. Kimberly and J. Juanita, 2005. Light promotes regeneration and functional recovery and alters the immune response after spinal cord injury. Lasers Surg. Med., 36: 171-185.

Mester, E. and A.F. Mester, 1996. The biomedical effects of laser application. Laser Surg. Med., 5: 31-39.

Midamba, E.D. and R.H. Haanaes, 1993. Low reactive-level 830 nm diode laser therapy successfully accelerates regeneration of peripheral nerves in human. Laser Ther., 5: 125-129.

Miloro, M., L.E. Halkia, S. Mallery, S. Travers and R.G. Rashid, 2002. Low-level laser effect on neural regeneration in Gore-Tex tubes. Oral Surg. Pathol., 93: 27-34.

Nicolau, R.A., M.S. Martinez, J. Rigau and J. Tomas, 2004. Effect of low power 655 nm diode laser Irradiation on the neuuscular junctios of the mouse diaphragm. Lasers Surg. Med., 34: 277-284.

Nissan, M., S. Rochkind, N. Razon and A. Bartal, 1986. He-Ne laserirradiation delivered transcutaneeously: Its effect on the sciatic nerve of rats. Lasers Surg. Med., 6: 435-438.

Passorolla, S. and E. Gasomassima, 1984. Increase of proton electrochemical potential and ATP synthesis in rat mitochondria irradiated *in vitro* by He-Ne laser. FEBS Lett., 175: 95-99.

Rochkind, S., M. Nissan, L. Barrnea, N. Razon, M. Schwartz and A. Bartal, 1987a. Response of peripheral nerve to He-Ne laser: Experimental studies. Lasers Surg. Med., 7: 441-443.

Rochkind, S., L. Barrona and N. Rason, 1987b. Stimulatory effect of He-Ne low dose laser on injured sciatic nerves of rats. Neurosurgery, 20: 353-358.

Rochkind, S., M. Rousso, M. Nissan, M. Villarreal, L. Barr-Nea and D.G. Rees, 1989. Systemic effects of low-power laser irradiation on the peripheral and central nervous system, cutaneous wounds and burns. Lasers Surg. Med., 9: 174-182.

Rochkind, S., 1992. Central nervous system transplantation bene-fitted by low-power laser irradiation. Lasers Surg. Med., 7: 143-151.

Rochkind, S., M. Nissan, M. Alon and M. Shamir, 2001. Effects of laser irradiation on the spinal cord for regeneration of crushed peripheral nerves in rats. Lasers Surg. Med., 28: 216-219.

Rochkind, S., M. Nissan, M. Alon, M. Shamir and K. Salame, 2001a. Effects of laser irradiation of crushed peripheral nerve in rats. Lasers Surg. Med., 28: 216-219.

Rochkind, S., M.A. Nissah, M. Shamir and K. Salame, 2001b. Effects of laser irradiation on the spinal cord for the regeneration of crushed peripheral nerve in rats. Lasers Surg. Med., 28: 216-219.

Samoilov, N.G., 1991. Structure of skeletal in combined conditions of denervation, physical load and laser acupuneture. Arkh Anat Gistol. Embriol., 100: 81-85.

Schwartz, F., C. Brodie, E. Appel, G. Kazimirsky and A. Shainberg, 2002. Effect of Helium-Neon laser irradiation on nerve growth factor synthesis and selection in skeletal muscle cultures. J. Photochem. Photobiol., 60: 195-200.

Shamir, M.H., S. Rochkind, J. Sandbank and M. Alon, 2001. Double-blind rondomized study evalating regeneration of the rat transected sciatic nerve after suturing and postoperative low-power laser treatment. J. Reconstr. Mircosurg., 17: 133-137.

Walker, M.D., S. Rumpf, G.D. Baxter, D.G. Hirst and A.S. Loe, 2000. Effect of low intensity laser irradiation (660 nm) on a radiation-impaired wound-healing moel in murin skin. Lasers Surg. Med., 26: 41-47.

Yueh, S.H., F. Shang, W. Chin, G. Janng and Y. Chun, 2005. Effect of low-power pulsed laser on peripheral nerve regeneration in rats. Microsurgery, 25: 83-89.