Lasers Med Sci 2002, 17:62–67
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Acute Electrophysiological Effect of Pulsed Gallium–Arsenide Low-Energy Laser Irradiation on Isolated Frog Sciatic Nerve

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Abstract. We evaluated the acute electrophysiological effects of low-energy pulsed laser irradiation on isolated frog sciatic nerve measured by extracellular recording technique. A pulsed gallium–arsenide (GaAs) laser (wavelength: 904 nm, pulse duration 220 ns, peak power per pulse: 27 W, spot size: 0.28 cm^2, total applied energy density: 0.005–2.5 J/cm^2) was used for the experiment. Sixty isolated nerves were divided into six groups ($n=10$), each of which received a different laser dose. In each group, action potentials were recorded before laser irradiation which served as the control data. The extracellular action potentials were recorded for each combination of 1, 3, 5, 7, 10, 13 and 15 minutes of irradiation time and 4, 8, 16, 32, 64 and 128 repetition frequency by using a BIOPAC MP 100 Acquisition System Version 3.5.7 (Santa Barbara, USA). Action potential amplitude, area, duration and conduction velocity were measured. Statistical evaluation was performed using repeated measures variance analysis by SPSS 9.0. There were no statistically significant differences for action potential amplitude, area and conduction velocity among the laser groups and control data ($p>0.05$). The study showed that low-energy GaAs irradiation at 4–128 Hz repetition frequencies administered for irradiation times of 1–15 min generates no effect on action potential amplitude, area, duration and conduction velocity in isolated frog sciatic nerve.

Keywords: Electrophysiological effects; Frog sciatic nerve; Low-energy GaAs laser irradiation

INTRODUCTION

Low-energy laser therapy has been used for the treatment of various conditions such as wound healing, inhibition of plaque formation on teeth, reduction of oedema and the relief of pain. In spite of such wide clinical usage, the therapeutic value and efficacy are controversial [1]. Many studies have attempted to explain the mechanism of low-energy laser action, the findings have not yet yielded complete understanding of the process.

The effect of low-energy laser on the nervous system was first studied by Rochkind [2] who suggested that laser has biostimulational effect on traumatic nerve injury. Later, many researchers have used in vivo and in vitro nerve conducting models for this purpose [3–7]. Although some authors observed an increase in action potential and suggested that laser has a biostimulational effect on nervous system [2,4], others have not [6]. This contradiction may be related to differences in wave regimes (continue or pulsed), as well as in the power density and irradiation times used in the various studies.

The present study was designed to investigate acute electrophysiological effects of pulsed gallium–arsenide (GaAs) laser irradiation on the nervous system by using different doses in the frog sciatic nerve. The aim of this study was to help resolve some of the controversy about laser effects and expand our understanding of the process that occurs.

MATERIALS AND METHODS

The study design was approved by the ethic committee of University of Mersin, Faculty of Medicine.

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Table 1. The doses (J/cm^2) used for the 42 combinations of repetition frequencies and irradiation times. The doses were calculated as follows: average power pulse (W)=peak power (W) × pulse duration (s) × repetition frequency (s^{-1}); Energy (J)=average power pulse (W) × irradiation time (s); energy density (J/cm^2)=energy (J)/spot size (cm^2)

Irradiation time (min)	Repetition frequency (Hz)					
	4	8	16	32	64	128
1	0.005	0.01	0.02	0.04	0.08	0.16
3	0.015	0.03	0.06	0.12	0.24	0.49
5	0.026	0.05	0.10[a]	0.20	0.41	0.82
7	0.036	0.07	0.14	0.29	0.57	1.14
10	0.052	0.10	0.20	0.41	0.82	1.64
13	0.067	0.13	0.27	0.54	1.08	2.16
15	0.077	0.15	0.31	0.62	1.25	2.50

[a]This value was calculated as follows: average power pulse=27 W × 220 nsx16 s^{-1}=0.09 × 10^{-3} W; energy=0.09 × 10^{-3} W × 300 s=0.027 J; energy density=0.027 J/0.28 cm^2=0.1 J/cm^2.

Tissue Preparation

Thirty *Rana cameroni* frogs weighing 30–40 g were used in the experiments. After decapitation, the sciatic nerves were removed and placed in Ringer's solution. This solution was composed of 111.87 mM NaCl, 2.47 mM KCl, 1.08 mM $CaCl_2$, and 2.38 mM $NaHCO_3$. The isolated nerves (n=60) were randomly divided into six groups (n=10). All experiments were carried out at room temperature.

A pulsed GaAs laser (Petas, Turkey) was used. Laser parameters were: wavelength, 904 nm; spot size, 0.28 cm^2; pulse duration, 220 ns; peak power per pulse, 27 W. Irradiation parameters were: pulse repetition rate, 4, 8, 16, 32, 64 and 128 Hz; average power, 0.024–0.76 mW; total exposure duration, 1, 3, 5, 7, 10, 13 and 15 min; delivered energy, 0.0015–0.684 J; total applied energy density, 0.005–2.5 J/cm^2. Irradiation parameters were entered to and automatically controlled by laser equipment. All the experiments involved a total of 42 different exposure times and repetition frequency combinations (Table 1).

Electrophysiological Techniques

The experiments were carried out in vitro using extracellular recording techniques [8,9]. After 30 min of stabilisation in Ringer's solution, the nerve specimens were placed in a 5 cm × 15 cm plexiglas nerve chamber containing Ag/AgCl electrodes. The space between the electrodes was 0.5 cm. The nerves were stimulated with these electrodes. The stimulating voltage was set to produce a maximal compound action potential using single square pulses of supramaximal strength and 0.5 ms in duration.

The nerve action potentials were recorded using a BIOPAC MP 100 Acquisition System Version 3.5.7 (Santa Barbara, USA). Compound action potentials (CAP) from each nerve before laser irradiation served as the control data. After recordings of the control, nerves were irradiated by laser and action potentials were recorded after 1, 3, 5, 7, 10, 13 and 15 min of irradiation in all groups. BIOPAC Acknowledge Analysis Software (ACK 100 W) was used to measure CAP amplitude, area and total duration. Conduction velocity was measured from the latencies of action potentials recorded with supramaximal stimulation at two different points. The distance of these points was 1 cm.

Statistical Analysis

The data were analysed with repeated-measures analysis of variance by using. SPSS 9.0. The significance was set at $p<0.05$.

RESULTS

Low-energy GaAs laser irradiation was delivered to isolated frog sciatic nerves in 42 different irradiation doses (Table 1). There were no significant differences among the control and

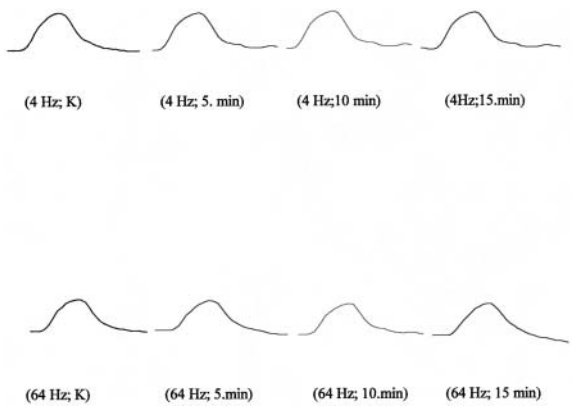

Fig. 1. Action potentials recorded after 5, 10 and 15 min of 4 Hz and 64 Hz GaAs laser irradiation.

Fig. 2. The recorded action potentials after 5, 10 and 15 min of (a) 4 Hz and (b) 64 Hz of GaAs laser irradiation, with control findings for each superimposed. There were no differences in action potential amplitude, area, or duration among the control and time–dose combination data.

Fig. 3. The figure shows the superimposed action potentials recorded after 5, 10 and 15 min for 4 and 64 Hz GaAs laser radiation. Note that irradiation time and dosage do not influence amplitude.

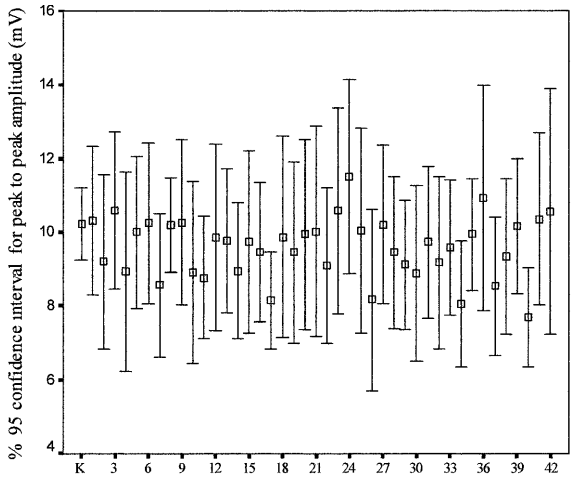

Fig. 4. Peak-to-peak amplitude values with 95% confidence limits around the median for all of the dosage/time combinations of GaAs laser irradiation in the study. K=Control groups.

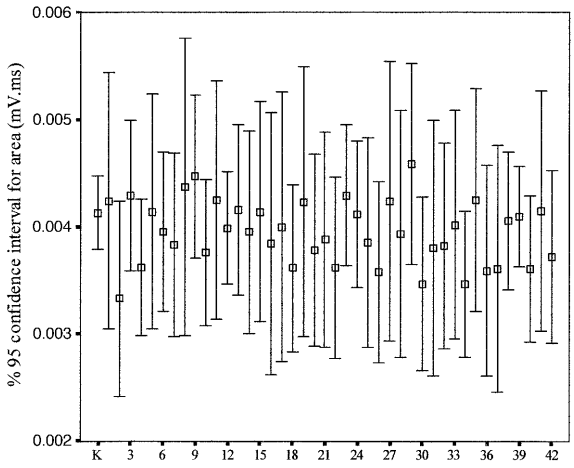

Fig. 5. The effect of GaAs laser on action potential area in isolated frog sciatic nerve. The laser irradiation did not affect the area in any of the 42 different experimental conditions. K=Control groups.

laser group data regarding CAP peak-to-peak amplitude, area, duration or conduction velocity. Figures 1–3 show the action potential recordings and Figs 4–7 show the calculated means (with confidence intervals) for the parameters. Each plot depicts the control data and the irradiation group findings for one of the four parameters. Since there were no significant differences among the control data and the data from all exposure time and dose combinations, the overall findings for each parameter were combined to derive the means (Table 2).

DISCUSSION

Nerve signals are transmitted by action potentials, which are rapid changes in cell membrane potential from the 'resting' or depolarised state. In the depolarisation stage, voltage-dependent Na^+ channels are activated, leading to a rapid flux of Na^+ ions into the nerve cell and action potential reaches its peak. A very short time after peak action potential, voltage-dependent K^+ channels open and K^+ ions rapidly exit to the extracellular space. As K^+ flows outward, the Na^+ channels gradually become deactivated, Na^+ flux drops off and membrane repolarisation has occurred [10]. Measurements of action potential amplitude, area, latency and conduction velocity may provide information about membrane Na^+ and K^+ transport. CAP amplitude, area and conduction velocity are positively

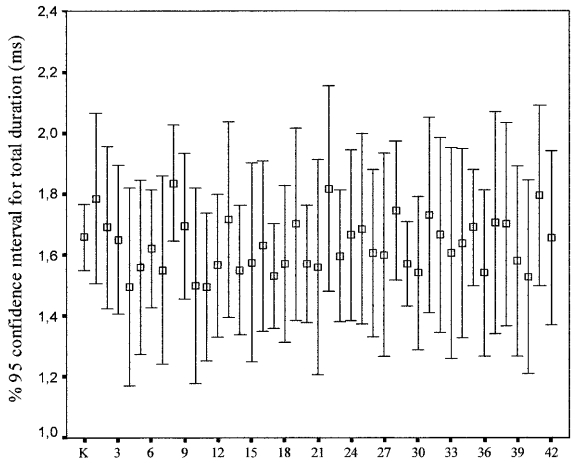

Fig. 6. The effect of GaAs laser on duration of action potential. Laser application did not influence duration of action potential. K=Control groups.

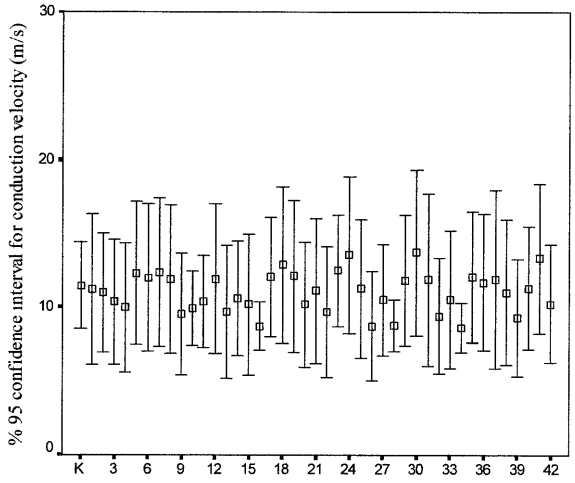

Fig. 7. The effect of GaAs laser on conduction velocity in the 42 different dosage/time combinations. K=Control groups.

correlated with sodium transport. In addition, the action potential amplitude and area recorded from nerve can be used to estimate the number of activated nerve fibrils [11].

In this study, we examined action potential characteristics in 42 different experimental conditions. Our aim was to investigate the acute effect of laser irradiation on peripheral nerve action potential. We used 42 different laser irradiation time and repetition frequency combinations (Table 1) on frog sciatic nerve and compared action potential's before and after laser irradiation in each group. There were no significant differences between control and experimental results with regard to CAP peak-to-peak amplitude, area, total duration or conduction velocity. As we could not find any difference between control and laser groups we suggest that laser irradiation does not alter membrane ion transport.

Although there are a lot of studies in the literature that try to explain the mechanism of low energy laser irradiation by modulation of peripheral nerve action potential characteristics, no mechanism has yet been advanced to explain this issue. In order to investigate the mechanism of low energy laser on peripheral nerve system, a well-characterised, reliable and robust peripheral nerve model is required [12]. In the present study in vitro frog sciatic nerve preparation was used for this purpose.

Helium–neon (HeNe, 632 nm), gallium–aluminium–arsenide (GaAlAs, 830 nm), and gallium–arsenide (GaAs, 904 nm) lasers are the most commonly used sources of low-energy laser irradiation. The biological effects of laser irradiation may be related to wavelength, laser dose and exposure time. Although no comparative investigation of these parameters has been described, different laser doses and exposure times have been used in various studies, which may explain the discrepancies in the literature regarding the effects of laser on the peripheral nervous system. Lowe et al.'s [7] study with GaAlAs laser showed that human median nerve CAP conduction velocity decreases significantly after 14 minutes of irradiation (1.5–6 J/cm^2) but it increases at higher doses (9–12 J/cm^2). Similarly, Tsuchiya et al. [13] noted suppressed amplitude of the slower conduction parts of action potentials (conduction velocity <12 m/s) with the use of GaAs laser in nerve fibre, and reported that this effect was dependent on irradiation time. This author also suggested that laser irradiation may selectively target fibres conducting at low velocities which include afferent axons from nociceptors. Rochkind et al. [4] used the HeNe laser on normal and injured sciatic nerve and found that there is a certain time/energy threshold. If the energy was under 3 J, there was no change in action potential; if the energy was higher than 8–9 J there was an inhibitory effect. Other authors [14,15] recorded significantly elevated action potentials in rat sciatic nerve after HeNe laser application at 3.5 J energy. Synder-Mackler and Bork [16] reported a significant postirradiation increase in conduction latency with HeNe laser (632.8 nm) at 19 mJ/cm^2. In contrast to these studies, Walsh et al. [6] (with GaAlAs laser), Ebert and Roberts [12] (with HeNe laser) and Lowe et al. [15] (with GaAlAs

Table 2. Descriptive statistics for action potential parameters studied

Variables	Mean	Standard deviation	Confidence interval 95%
Peak to peak amplitude (mV)	9.64	3.07	9.34–9.93
Area (mV·ms)	0.0039	0.001	0.0038–0.0040
Duration (ms)	1.63	0.36	1.6–1.67
Conduction velocity (m/s)	10.98	5.86	10.41–11.55

laser) demonstrated that dosage and irradiation time had no effect on amplitude and conduction velocity. In the present study GaAs laser is used at 0.005–2.5 J/cm^2 energy density and no effects on action potential parameters were found. Our laser doses are similar to those of the study by Synder-Mackler and Bork [16] and lower than those used in other studies. We used low doses because direct application of the laser light on nerve preparation eliminates absorption of laser by other tissues (skin, muscle).

Continuous or pulsed laser regimes must be considered as a significant related factor when discussing the effects of low-energy laser irradiation [6]. Most studies in the literature have dealt with the effects of continuous wave regime on the peripheral nervous system and the results of these studies were different from the results of pulsed lasers. Only Walsh et al. [6] and Lowe et al. [15] have investigated the impact of pulsed laser regimes on action potential and in these studies laser source, dose and exposure times were different from ours. Walsh et al. [6] used GaAlAs laser (9.55 J/cm^2) with pulsed wave in human superficial radial nerve and Lowe et al. [15] used a pulsed laser source (820 nm, 1.5 J/cm^2 and 9 J/ cm^2) in human median nerve and they could not find any significant laser-mediated effects. We used GaAs (904 nm, 0.005–2.5 J/cm^2) laser system with a pulsed wave regime and our results were similar to the findings of Walsh et al. [6] and Lowe et al. [15].

The effect of laser irradiance on healthy nerves may be different from that on injured nerves. Although Rochkind et al. [4] demonstrated significant healing in the peripheral nervous system with laser therapy, Parris et al. [17] and Khullar et al. [18] could not find any effect on mononeuropathic rats.

Action potential characteristics provide information about nerve signal transfer. The amplitude and area of an action potential is related to the number of activated nerve fibres which is linked to nerve function. Our results raise questions about the clinical usefulness of low-energy and pulsed-laser therapy in nervous system disorders. However, our study does not provide any information about the long-term effects of low-energy laser irradiation on peripheral nerves.

In conclusion, we suggest that low-energy laser irradiation has no acute effect on action potential parameters and more studies with in vivo models may be required to resolve this controversy.

REFERENCES

1. Pogrel MA, Chen JW, Zhang K. Effects of low-energy Gallium–Aluminum–Arsenide laser irradiation on cultured fibroblast and keratinocytes. Lasers Surg Med 1997;20:426–32.
2. Rochkind S. Stimulation effect of laser energy on the regeneration of traumatically injured peripheral nerves. The Krim National Medical Inst, Morphogenesis and Regeneration 1978;73:48–50.
3. Olson JE, Schimmerling W, Tobias CA. Laser action spectrum of reduced excitability in nerve cells. Brain Res 1981;204:436–40.
4. Rochkind S, Nissan M, Razon N, Schwartz M, Bartal A. Electrophysiological effect of HeNe laser on normal and injured sciatic nerve in the rat. Acta Neurochir (Wien) 1986;83(3–4):125–30.
5. Greathouse DG, Currier DP, Gilmore RL. Effects of clinical infrared laser on superficial radial nerve conduction. Phys Ther 1985;65:1184–7.
6. Walsh DM, Baxter GD, Allen JM. Lack of effect of pulsed low intensity infrared (820 nm) laser irradiation on nerve conduction in the human superficial radial nerve. Lasers Surg Med 2000;26:485–90.
7. Lowe AS, Baxter GD, Walsh DM, Allen JM. The effect of low-intensity laser irradiation upon skin temperature and antidromic conduction latencies in human median nerve: relevance of radiant exposure. Lasers Surg Med 1994;14:40–6.
8. Andrew Bl. Experimental Physiology. London: Churchill Livingstone, 1972.
9. Katz B. Nerve Muscle and Synapse. New York: McGraw–Hill Book, 1966.

10. Koester J, Siegelbaum SA. Propagated signalling: the action potentials. In: Kandel ER, Schwartz JH, Jessel TM (eds) Principles of Neural Sciences. New York: McGraw–Hill, 2000:151–70.
11. Daube JR. Clinical Neurophysiology. Philadelphia: F.A. Davis, 1996:60–8.
12. Ebert DW, Roberts C. In vitro frog sciatic nerve as a peripheral nerve model for studies of the mechanism of action of low energy lasers. Part one. Lasers Surg Med 1997;21:32–41.
13. Tsuchiya K, Kawatani M, Takeshige C, Sato T, Matsumato I. Diode laser irradiation selectively diminishes slow component of axonal volleys to dorsal roots from the saphenous nerve in the rat. Neurosci Lett 1993;161:65–8.
14. Nissan M, Rochkind S, Razon N, Bartel A. HeNe laser irradiation delivered transcutaneously: Its effect on the sciatic nerve of rats. Lasers Surg Med 1986;6:435–8.
15. Lowe AS, Baxter GD, Walsh DM, Allen JM. The relevance of pulse repetition rate and radiant exposure to the neurophysiologic effects of low intensity laser (820 nm/pulsed wave) irradiation upon skin temperature and antidromic conduction latencies in human median nerve. Lasers Med Sci 1995;10:253–9.
16. Synder-Mackler L, Bork CE. Effect of helium–neon laser irradiation on peripheral sensory nerve latency. Phys Ther 1988; 68:223–5.
17. Parris WC, Janichi PK, Johnson BW, Matheus L. Infrared laser diode irradiation has no behavioural or biochemical effect on pain in sciatic nerve ligation-induced mononeuropathy in rat. Anesth Prog 1994;41(4):95–9.
18. Khullar SM, Brodin P, Barkvoll P, Haanaes HR. Preliminary study of low-level laser for treatment of long-standing sensory alterations in the inferior alveolar nerve. J Oral Maxillofac Surg 1996;54:2–7.

Paper received 26 February 2001;
accepted after revision 24 August 2001.