Ga-As (904nm) Lazer Irradyasyonunun Ratlarda Siyatik Sinir Hasarı Üzerine Etkileri

The Effects of Ga-As (904nm) Laser Irradiation on Injured Sciatic Nerves of Rats

Lazer Irradyasyonunun Sinir Hasari Üzerine Etkileri / The Effects of Laser Irradiation on Injured Nerves

Arzu Erbilici[1], Ece Ünlu[1], Yusuf Sinan Şirin[2], Ömer Besalti[3], Aytul Cakci[1]

[1]Department of Physical Therapy and Rehabilitation, Ministry of Health Ankara DışkapıYıldırım Beyazıt Education and Research Hospital, Ankara,

[2]Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Surgery, Burdur,

[3]Ankara University, Faculty of Veterinary Medicine, Department of Surgery, Ankara, Turkey.

Özet

Amaç

Bu çalışmanın amacı Ga-As (904 nm) lazerin hasar oluşturulan sinir bölgesindeki ve ilişkili spinal kord segmentindeki etkilerini araştırmaktır.

Gereç ve Yöntemler

Bu çalışmada ağırlıkları 180-240 g olan otuz Albino Wistar erkek rat kullanıldı. Siyatik sinir orta seviyeden bir anevrizma klip (Aesculap FE 751; Tuttingen, Germany) 4 dakika süreyle uygulanarak hasara uğratıldı. Ratlar üç gruba ayrıldı. Birinci grupta, lazer irradyasyonu ilişkili spinal kord segmentinin (L3-L6) traş edilen derisine temas ettirilerek dört noktadan uygulandı. Grup ikide, hasarlı bölgenin irradyasyonu bölgedeki traşlı deriye temas ettirilerek uygulandı. Üçüncü grupta aynı prosedür emisyonsuz olarak uygulandı. İlk lazer tedavisi cerrahiden iki gün sora uygulandı ve izleyen 21 gün boyunca günde bir kez tekrarlandı. Ölçülen Elektrofizyolojik parametreler bileşik kas aksiyon potansiyeli (CMAP), distal motor latansı (DML) ve motor sinir ileti hızıdır (NCV). Ölçümler hasar oluşturulmadan hemen önce, tedavinin 21. gününde ve takip süresinin 42. gününde alınmıştır. Siyatik Fonksiyon İndeksi (SFI) ve Parmak Açma Testi (TSA) postoperatif 2, 7, 14, 21 ve 42. günlerde uygulandı.

Bulgular

DML ve CMAP amplitüdü yönünden gruplar arasında belirgin bir fark bulunmadı. Birinci grupta NCV 21. ve 42. günlerde diğer gruplardan belirgin şekilde daha hızlı bulundu. SFI ve TSA yönünden 1, 7, 14, 21 ve 42. Günlerde gruplar arasındaki farklılık önemsiz bulundu (p>0.05).

Sonuç

Sonuç olarak Ga-As lazerin kullanımı hasarlı bölge üzerinden irradyasyon uygulanan gruptaki sinir iletimi üzerine olumlu etkisi dışında sinir rejenerasyonu veya fonksiyonel gelişme üzerine herhangi bir belirgin fayda sağlamamıştır.

Anahtar Kelimeler

Elektrofizyolojik Parametreler, Lazer, Sinir Rejenerasyonu, Siyatik Fonksiyon Indeksi, Parmak Açma Testi.

Abstract

Aim

The purpose of this study was to investigate the effects of Ga-As (904 nm) laser on the crushed nerve region compared with laser of the corresponding spinal cord segments. Material and **Materials and Methods**

Thirty Albino Wistar male rats weighing 180-240 g were used in this study. The sciatic nerve was crushed at the middle level with an aneurysm clip (Aesculap FE 751; Tuttingen, Germany) for 4 minutes. The rats were allocated into three groups. In group 1, laser irradiation was applied at the four points along the corresponding spinal cord segments (L3-L6) in contact with the shaved skin. In group 2, the crushed area in contact with the shaved skin was irradiated. In group 3, the same procedure was performed without emission. The first laser treatment was performed on the second day of surgery and was repeated once daily for 21 consecutive days. The measured electrophysiological parameters included compound muscle action potential (CMAP), distal motor latency (DML), and motor nerve conduction velocity (NCV). Measurements were taken just before crushing and were repeated on the 21st day of the treatment and the 42nd day of the follow-up period. Sciatic Functional Index (SFI) and Toe Spread Analysis (TSA) were carried out on postoperative days 2, 7, 14, 21 and 42.

Results

There was no significant difference between groups regarding DML and amplitude of CMAP. The NCV was significantly faster in group 1 on the 21st and 42nd days than in the other groups. The differences between groups regarding SFI and TSA on the 1st, 7th, 14th, 21st, and 42nd days were insignificant (p>0.05).

Conclusion

In conclusion, the use of Ga-As laser did not provide significant benefit on nerve regeneration or functional improvement except for its positive effect on nerve conduction in the crushed area irradiated group.

Keywords

Spread Electrophysiological Parameters, Laser, Nerve Regeneration, Sciatic Functional Index, Toe Analysis.

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Corresponding Author: Ece Ünlü, Department of Physical Therapy and Rehabilitation, Ministry of Health Ankara Dışkapı Yıldırım Beyazit Education and Research Hospital, 06110, Ankara, Turkey. **GSM:**+905065595122 **E-mail:** dreceunlu@yahoo.com

Introduction

Recovery after injuries to the peripheral nerves remains an important problem, since it is not complete in most cases [1, 2]. The extent of the atrophy of the target muscle following peripheral nerve injury worsens in conjunction with the longevity of the recovery and regeneration period [3, 4]. Other usual results after such an injury are degeneration of the axons and retrograde degeneration of the corresponding neurons of the spinal cord [5, 6]. The enhancement of faster regeneration of the injured axons, preventing anterograde degeneration and preserving the proximal stump from retrograde degeneration and muscle atrophy, are the major goals in peripheral nerve injury recovery [5].

There have been many studies by Rochkind [3-6] related to the use of low–power laser irradiation (LPLI) in the treatment of injured peripheral nerve and spinal cord. The results of his studies have shown an increase in the amplitude of compound muscle action potential (CMAP) in both intact and injured peripheral nerves. Histological studies showed an increase in the blood supply and the number and diameter of the axons in the crushed nerve as well as inhibition of scar tissue [6]. Contrary to these important findings, some researchers have been unable to determine any positive effect of LPLI on the recovery of the injured nerves [7, 8]. On the other hand, LI of the crushed peripheral nerve mitigates the degenerative changes in the corresponding neurons of the spinal cord and induces proliferation of neuroglia both in astrocytes and oligodendrocytes. This change represents the higher metabolism in corresponding neurons of the related spinal cord segment and may facilitate regeneration [3,6].

The aim of this study was to investigate the effects of Ga-As (904 nm) laser on the functional and electrophysiological recovery of crushed rat sciatic nerve comparing two different applications, either on the crushed nerve region or on the corresponding spinal cord segments.

Material and Methods

The present study was carried out on 30 Albino Wistar male rats weighing 180-240 g each. Animals were housed in smooth-bottomed plastic cages at 22oC with a 12:12 h light-dark cycle. Standard laboratory diet and water ad libitum were available. Animals were randomly divided into three groups (n=10 rats each). The rats were anesthetized intraperitoneally with xylazine hydrochloride 10 mg/kg (Rompun, Bayer; Turkey) and ketamine hydrochloride 50 mg/kg (Ketalar, Parke Davis; Turkey) for both surgical and electrophysiological procedures. This study was approved by the local ethics committee.

The left thigh along its lateral side and the thoracolumbar region of the spine were shaved. The left sciatic nerve was exposed and separated from the surrounding muscles from sciatic notch to the furcation area. The sciatic nerve was crushed at the middle level with an aneurysm clip (Aesculap FE 751; Tuttingen, Germany) for 4 minutes. The skin and subcutaneous tissues were sutured with atraumatic 3/0 catgut sutures. After surgery, the rats were assigned an identification number on their ears and the crushed point was indicated with Chinese ink.

Ga-As laser (Petaş; Turkey) was applied with 904 nm wavelength, spot size 0.28 cm2 in diameter, 220 ns pulse duration and 27 W peak powers per pulse. In group 1, the lumbar area was irradiated at the four points along the corresponding spinal cord segments (L3-L6) in contact with the shaved skin. In group 2, the LI was applied to the crushed area in contact with the shaved skin. The incidence angle was 90o to the irradiation surface. The parameters for LI were: pulse repetition rate 1000Hz, average power 0.76 mW, and delivered energy density 19 joul/cm2. In group 3, the same procedure was performed without emission. Each treatment session lasted 10 minutes. The first laser treatment was performed on the second day of surgery and was repeated once daily for 21 consecutive days. The treatment was stopped after 21 sessions and the rats were followed until postoperative day 42 without medication.

The effects of laser were determined by electrophysiological means using an electromyography (EMG) machine (Medelec Synergy 5 Channel; Oxford, UK). A pair of monopolar needle electrodes was used as recording electrode (Medelec, disposable subdermal needle, 18 mm length, 0.30 mm diameter, 017K019), which was placed on the plantar muscles. Another pair of monopolar electrodes was used for stimulation. The sciatic nerve was stimulated supramaximally with a single square pulse (intensity 10V, duration 0.5 ms) from the distal and proximal sites of the injured area. The stimulation sites were 2 cm distal or proximal to the crushed area. The ground electrode was placed subcutaneously between the stimulation and recording electrodes. The measured electrophysiological parameters included CMAP (peak to peak amplitude, mV), distal motor latency (DML) (ms) and motor nerve conduction velocity (NCV) (m/sn, calculated by dividing the distance between the stimulation sites by the difference in latency of the responses).

The electrophysiological investigation was carried out just before crushing, which served as the baseline parameters. Measurements were repeated on the 21st day of the treatment and the 42nd day in the follow-up period. All three recordings were done using the same technique.

Sciatic Functional Index (SFI) was used to quantify the functional recovery in rats after injury to the sciatic nerve. All animals underwent walking track analysis as described originally by de Medinaceli et al. [9] and modified for mice by Inserra et al. [10]. In summary, the rats' hind paws were dipped into X-ray film developer and they were allowed to walk without assistance along a corridor (8.2 cm [w] x 42 cm [l] x 12 cm [h]). The X-ray film was placed in the path of the rats on the walking track. The formula for SFI, which was standardized by Bain et al. [11], is calculated as follows:

SFI=-38.3(EPL-NPL)/NPL + 109.5(ETS-NTS)/NTS + 13.3 (EIT-NIT)/NIT -8.8

where EPL= Experimental print length, NPL=Normal print length, ETS= Experimental toe spread, NTS=Normal toe spread, EIT=Experimental intermediate toe spread, and NIT=Normal intermediate toe spread.

Toe spread analysis (TSA) was another parameter used to observe the functional improvement after sciatic nerve injury. It is a reflex elicited as the rat was picked up from the body and the legs allowed to hang free and was scored as 0-3 from no spreading of toes to normal full toe spread [12).

SFI and TSA were determined on postoperative days 2, 7, 14, 21 and 42.

Statistical Analysis

Wilcoxon test was used to determine the differences in SFI and TSA from the 1st to 42nd postoperative day. Kruskal Wallis Variance Analysis was used for comparison between the groups. The change in the electrophysiological parameters over time was determined by paired t test, and one way ANOVA was used for investigations between groups. For all data, p< 0.05 was accepted as significant.

Results

Three rats in group 1 and 5 rats in group 2 died after the 21st day. Infection was seen in 1 rat in group 2 (hind foot) on the 21st day and in 1 rat in group 3 (skin over the incision area).

There was no significant difference between groups regarding DML and amplitude of CMAP. However, the differences in CMAP amplitude between baseline and the 21st and 42nd day values were significant. NCV was significantly faster in group 1 on the 21st and 42nd days than in the other groups, and the differences between baseline and the 21st and 42nd days were significant. When the improvement was analyzed within the groups from the beginning till the 42nd day, there were no significant differences in DML and amplitude of CMAP. However, CMAP was significantly lower than the baseline values (Table 1).

The differences between groups with respect to SFI at the 1st, 7th, 14th, 21st, and 42nd days were insignificant (p>0.05). Within groups, significant improvement was determined in groups 1 and 2 between days 1-7, and in each group between days 7-14, and 14-21 (Table 2).

Table 1. Comparison of the electrophysiological findings of each group on days 21 and 42 with respect to DML, the amplitude of CMAP and NCV.

Group	Variables	Baseline (Mean±SD)	21st day (Mean±SD)	42nd day (Mean±SD)
Group I	Amplitude of CMAP (mV)	9.66 ± 1.41		4.72 ± 1.52
	DML (ms)	1.19 ± 0.14	1.51 ± 0.14	1.45 ± 0.13
	NCV (m/s)	60.46 ± 6.75	52.23 ± 4.97	53.57 ± 6.66
Group II	Amplitude of CMAP (mV)	8.54 ± 2.64	3.77 ± 1.60	4.76 ± 2.10
	DML (ms)	1.29 ± 0.24	1.60 ± 0.40	1.32 ± 0.19
	NCV (m/s)	60.43 ± 8.27	43.00 ± 3.74	46.79 ± 8.60
Group III	Amplitude of CMAP (mV)	9.29 ± 2.36	2.50 ± 1.62	3.36 ± 1.21
	DML (ms)	1.10 ± 0.08	1.68 ± 0.60	1.45 ± 0.34
	NCV (m/s)	56.57 ± 4.59	43.50 ± 5.50	46.28 ± 4.90

DML: Distal motor latency, CMAP: Compound muscle action potential, NCV: Nerve conduction velocity

Table 2. Comparison of the Sciatic Functional Index (SFI) between groups.

Groups (mean)±SD (median)	1st day	7th day	14th day	21st day	42nd day
Group 1	-55.57±17.89 (-49.29)	-77.90±17.07 (-68.21)	-22.04±18.22 (-19.43)	-7.06±11.74 (-5.81)	-5.84±12.25 (-5.08)
Group 2	-60.14±15.87 (-57.29)	-43.39±25.94 (-42.31)	-25.78±20.04 (-29.28)	-6.69±19.84 (-0.45)	-8.96±5.47 (-8.82)
Group 3	-65.01±21.19 (-73.36)	-60.19±26.32 (-64.58)	-27.96±17.31 (-24.67)	-10.90±10.84 (-12.02)	-8.96±5.93 (-9.80)
P	0.377	0.07	0.66	0.54	0.76

When TSA was compared between groups on days 1, 7, 14, 21, and 42, there was no significant difference. Significant improvement was found between days 1-7 in groups 1 and 2, in each group between days 7-14 , and in groups 1 and 3 between days 14-21 (Table 3).

Table 3. Comparison of Toe Spread Analysis (TSA) scores within groups.

Groups (p)	Days 1-7	Days -14	Days 14-21	Days 21-42
Group 1	p=0.046	p=0.004	p=0.047	p=0.083
Group 2	p=0.015	p=0.006	p=0.317	p=0.317
Group 3	p=0.180	p=0.010	p=0.059	p=0.059

Discussion

The results of this study showed that low-power Ga-As laser, applied over the skin either at the spinal segment or at the injured sciatic nerve, did not produce significant improvement according to the electrophysiological parameters and the functional scores in comparison with the control group. However, NCV improved more in group 2 after the 21st day.

Many researchers have tried to determine the mechanism by which LPLI affects tissues. Kovacs [13] and Mester [14] suggested that LI increases blood supply and neovascularization of the epithelial tissues. Kiernan [1] emphasized the diffusion of plasma proteins at the site of injury. The newly formed vessels might be more permeable to proteins, which could be an explanation for the effect of LPLI. It has been found that laser induces Schwann cell

proliferation, affects nerve cell proliferation and induces sprouting of cellular processes [15]. In our study, Ga-As LPLI using, significant improvement in nerve regeneration was not observed according to the parameters studied in a model of crush injury of the sciatic nerve of rats.

The biological effects of laser depend on the wavelength and laser dose. Bagıs et al. [8] evaluated the effect of Ga-As laser (904 nm wavelength) on crush-injured sciatic nerve with different repetition rates of 16, 128, 1000 Hz and with energy densities of 0.31, 2.48, 19 J/cm2, respectively, using both electrophysiological parameters and histopathological examination. Their results showed that low energy Ga-As irradiation did not have any effect on nerve regeneration. In our study, we used the same wavelength and energy intensity but we applied irradiation at two different sites, either on the related spinal segment or on the crush injury site, and no positive effect on nerve regeneration was determined at either site with respect to DML and CMAP amplitude. However, in the spinal cord irradiated group, there was a significant improvement in the NCV. This interesting finding might be the positive effect of LI on remyelination of the injured nerve. Because of the short distance, which can confound the results of NCV, the results should be accepted cautiously. Rochkind [3] et al. investigated the effect of He-Ne LI on the corresponding segments of the spinal cord after crush injury of the sciatic nerve. They found that CMAP amplitude increased approximately up to the pre-crush levels and remained so for an extended period. They suggested that applications on the spinal cord induced the regeneration of the injured peripheral nerve. In our comparisons between the groups, we could not find a significant increase in the amplitude of CMAP but we observed improvement in the NCV in the spinal cord irradiated group. Within the groups, the CMAP amplitude increased between days 21-42 but never reached pre-crush levels.

Energies of He-Ne laser below 3.5 J/cm2 or above 7 J/cm2 were found to be non-effective transcutaneously [4]. Rochkind [5] mentions that there is no effect below a threshold of energy influx, whereas beyond a maximum threshold the effect is reversed. On the other hand, there are different studies by different authors reporting contradictory data. Khullar [16] used Ga-As laser (830 nm) on an injured sciatic nerve and found no difference in the amplitude of CMAP and the histopathological data. Pogrel et al. [17] found that Ga Al As laser had no stimulatory effect on fibroblast and keratinocyte cultures.

SFI is a well-known index used to quantify functional recovery in rats after sciatic nerve injury. Our results showed that though there was improvement in all data within groups, there was no significant difference between groups indicating that Ga-As laser is effective. In a very recent study, Dos Reis et al. [18] analyzed the influence of AlGaAs laser (660 nm) on the myelin sheath and functional recovery of the sciatic nerve in rats. Although they found significant changes in morphometric investigations in the nerve, they could not find improvement by means of functional recovery using SFI. Mohammed et al. [19] in their study assessed the effect of LPLI on regeneration of the peroneal nerve of rabbits. They found that laser therapy produced a significant amount of structural and cellular changes in the nerves, which they demonstrated in histopathological analyses. Nevertheless, they did not have functional data supporting their findings. Histopathological evaluation is one of the most valuable investigations to support nerve regeneration. One of the limitations of our study is the lack of histopathological data.

There are many studies supporting the effectiveness of LPLI on injured nerve regeneration. Rochkind [20], in their recent pilot study, investigated the effect of LPLI (780 nm) in patients suffering from peripheral nerve and brachial plexus injuries. In this prospective study, they found significant and electrophysiological improvement in these patients. These encouraging results will support the clinical use of this treatment in patients with nerve injury. We believe the contradictory results of the various studies published previously are due to the difference in type, wavelength, intensity and the irradiation site of the LPLI. Further researches should be planned to enlighten the conflicting data of different types of LPLI on nerve regeneration.

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