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Muscular Trauma Treated with a Ga-Al-As Diode Laser: In Vivo Experimental Study

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Abstract. The aim of the study was to verify in an experimental model the effects of laser therapy performed with Ga-Al-As diode lasers (780 nm, 2500 mW) on traumatised muscles. Forty adult New Zealand male rabbits were divided into four groups (A, B, C and D) of ten animals each. Each group of animals was further divided into two subgroups of five animals each. The animals were submitted to muscular trauma for 7 min by clamping the posterior muscles of the left thigh under general anaesthesia. Four days later, the rabbits in the B_1, B_2, C_1, C_2, D_1 and D_2 subgroups started daily laser therapy. The parameters utilised were: 150 J/cm^2 energy density, 3 W, 50 Hz in group B; 250 J/cm^2, 3 W, 100 Hz in group C; and 800 J/cm^2, 3 W, 0 Hz (continuous output) in group D. The animals in subgroups A_1 and A_2 were used as untreated controls and allowed to heal spontaneously. In order to prepare samples for histological, histochemical and histomorphometrical studies, dissection of the posterior muscle of the thigh was performed under general anaesthesia and before sacrifice, after five days of laser therapy in the subgroups B_1, C_1 and D_1 and after ten days of laser therapy in subgroups B_2, C_2 and D_2. The samples of untreated subgroups A_1 and A_2 were subjected to the same procedure and at the same times as the corresponding laser-treated groups. The following parameters were analysed on muscular samples: qualitative histological aspect (lactate dehydrogenase (LDH), cytochrome oxidase, acid phosphatase and alkaline phosphatase concentration with histoenzymatic methods) and quantitative histomorphometric evaluation of muscular damage and tissue repair. Blood samples were drawn from each subgroup before the trauma and again before sacrifice to measure the creatine phosphokinase (CK) and LDH levels. The results obtained in the tables are shown. Analysis of the results showed a better qualitative and quantitative healing process in traumatised muscles treated with Ga-Al-As diode laser therapy than in spontaneously healed ones. The results obtained with laser therapy were confirmed as haematic, histoenzymatic and histomorphometric values. According to these results, there is a positive relationship between the biostimulation properties of the laser and the healing of traumatised muscular tissue.

Keywords: Diode laser; Experimental model; Laser biostimulation; Low energy laser therapy; Muscle trauma

INTRODUCTION

Since Mester et al. [1] first produced evidence of the biostimulatory effect of low power laser light, it has been applied in the biological and medical fields. Different types of lasers have been used for many years to produce biostimulative–modulative effects. These include the ruby, HeNe, Argon-ion, IR and CO_2 lasers operating in continuous wave, interrupted and pulsed modes [2].

The biological effects of low intensity laser irradiation depend on both the characteristics of the light source (e.g. wavelength, energy, pulse duration) and the structure of the tissue. Recently, low-energy laser irradiation has been found to modulate biological processes in humans and animals. Many authors [3–5] have investigated the biostimulatory effects of laser applications in several fields such as basic research, cell culture, wound healing, hormonal or neural stimulation, antiphlogistic reactions and pain relief.

One of the principal uses of low-level laser therapy is in the treatment of injury after trauma. Because the possible effects of low-energy laser on muscular tissue or reparative processes in muscle tissue have not been investigated previously, further studies in order to verify such biological changes need to be made [6].

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The aim of the present study was to investigate the possible effects of a new model Ga-Al-As diode laser (780 nm, 2500 mW) on skeletal muscle after trauma, assessed by means of blood tests, a qualitative histological analysis of lactate dehydrogenase (LDH), cytochrome oxidase, acid phosphatase and alkaline phosphatase determination with histoenzymatic methods, and quantitative histomorphometric evaluation of muscular damage and tissue repair.

Previous Ga-Al-As diode lasers used for tissue biostimulation operated at a low power output of the order of a few mW. The laser used in this study can reach a power of 2500 mW and can therefore apply higher doses of energy (J/cm^2) to the irradiation sites. The laser used in our study was a portable monoiodic 2500 mW power laser, with either a continuous or modulated output and fed by rechargeable batteries and/or a mains supply of 110–240 V at 50/60 Hz. The active material of the device was Ga-Al-As, with wavelength of 780 nm and an output power of 2500 mW. The device is in the isolated class 1 type B and in laser class III B. Settings can be adjusted in the following ranges: from 0 to 99′59″; energy from 0 to 999 mJ/cm^2, frequency, from 0 to 999 Hz; and duty cycle exposed up to 50%. In this study the laser was used in both continuous output mode (group D) as well as modulated output (groups B and C).

The laser therapy parameters used in this study were specially designed to demonstrate the biostimulation effect of the device by delivering high energy densities without creating thermal damage in the treated tissue.

MATERIALS AND METHODS

Forty adult New Zealand male rabbits (2.5 ± 0.5 kg body weight (bw)) were housed singly in stainless-steel cages and provided with commercial rabbit pellets, hay and water ad libitum during the stabling period. The animals were maintained in windowless facilities at 20–22°C, a relative humidity of 40–60% and a photoperiod of 12/12 h light and dark. Anaesthetic induction was obtained with 35 mg/kg bw of ketamine (Ketavet, Farmaceutici Gellini, Aprilia Lt, Italy) and 5 mg/kg bw of xylazine (Rompun, Bayer Italia SpA, Milano, Italy) administered by intramuscular injection. Anaesthesia was maintained by means of a mixture of 2% halothane (Flouthane, Zeneca Ltd, Macclesfield, UK) and oxygen (0.3 l/min) delivered by an automatic ventilator using a specially designed face mask. Arterial blood pressure was continuously recorded, and the heart rate and respiratory rate were monitored every 5 min. During all procedures the temperature of the surgery theatre was maintained at 19 ± 1°C. The animals were divided into four groups of ten animals each, A, B, C and D. The animals in group A were used as untreated controls and allowed to heal spontaneously whereas the animals of groups B, C and D were treated with three different settings of the Ga-Al-As diode laser.

Muscular trauma was created, under general anaesthesia, with a Foerster clamp used at the same setting each time in order to guarantee the same compression pressure on the muscular mass of the rabbit. After outlining an area measuring 2.5×2.5 cm on the third medial of the posterior region of the left thigh of the rabbit with an inedible ink brush the Foerster clamp was positioned for 7 min over a full thickness region of thigh. This procedure was repeated in all the animals used in the study.

Each group of animals was further divided into two subgroups of five animals each. Four days after being submitted to muscular trauma, the rabbits in subgroups B_1, B_2, C_1, C_2, D_1 and D_2 started daily laser therapy by using the laser beam on the marked, traumatised area. Therapy consisted of bringing the laser point to a perpendicular position about 1 cm from the traumatised area until the chosen power density for the therapy was reached and then using it on to the target tissue.

The parameters selected for laser therapy groups were:

Group B: 150 J/cm^2 of energy density, 3 W and 50 Hz (modulated output);
Group C: 250 J/cm^2 of energy density, 3 W and 100 Hz (modulated output);
Group D: 800 J/cm^2 of energy density, 3 W and 0 Hz (continuous output).

The animals in subgroups B_1, C_1, D_1 were sacrificed after five days of laser therapy together with the animals of the untreated control subgroup A_1. The animals of the subgroups B_2, C_2 and D_2 were sacrificed after 10 days of laser therapy together with the animals of the untreated control subgroup A_2. No other type of therapy (pharmacological or physical) was given to the animals during the study.

Two blood samples, of 2 ml each, were taken from each subgroup, before the trauma and before sacrifice, in order to measure levels of CPK (creatine phosphokinase) and LDH. The base value in all the groups is a mean value of the animals utilised.

The posterior thigh posterior muscles were dissected for histological evaluation in all the treated subgroups (B_1, B_2, C_1, C_2, D_1 and D_2) and in the untreated subgroups (A_1 and A_2) at the end of therapy, under general anaesthesia and before the sacrifice of the animals.

The biopsies were rapidly frozen in isopentane ($-59°C$) and stored at $-80°C$ until processing. Samples of all groups were sectioned with a cryostat microtome at $-25°C$ (thickness 9 μm) and processed for histological and histoenzymatic studies.

The H&E stain was utilised in histological studies in order to evaluate the shape and dimension of muscular fibres, the position and appearance of cellular nuclei and the presence of muscular degeneration and/or regeneration.

Among the histoenzymatic reactions permitting an evaluation of the cellular metabolism, LDH cytochrome *c* oxidase, acid phosphatase and alkaline phosphatase determinations were chosen for our study. These reactions provide information about the natural activity of the enzymes and their location. LDH was also monitored in the blood samples. LDH is a mitochondrial enzyme depending on NAD which has a crucial role in the glycolysis reaction. This enzyme causes a catalytic reversion of the oxidation of lactate to pyruvate and from lactic acid to pyruvic acid, allowing cells to overcome a temporary reduction in oxygen supply. We determined LDH presence in cryostat sections using a histoenzymatic method. An increase in LDH level indicates cellular damage caused by anaerobic conditions, as does the increase in acid phosphate, which is a marker of muscular damage (necrosis, phagocytosis). Alkaline phosphatase, usually absent from muscle cells, is present primarily in the cellular membrane during active transport processes and during muscle regeneration. Cytochrome oxidase is a mitochondrial enzyme that indicates active oxidative metabolism within the cells.

The histomorphometric study was performed by a blinded investigator using a semiautomatic Kontron Electronic Imaging System KS 300 (Kontron Electronic, Munchen, Germany) on five randomly selected fields for each specimen.

Diameter variations in the muscle fibres was also measured. In order to minimise the possible distortion due to an oblique cut, the smallest diameter fibres at the centre of the specimen were measured [7]. A total of 200 cells were evaluated from each sample.

All experiments were performed following the International Guiding Principles for Biomedical Research involving animals and Italian law concerning animal experimentation.

Statistical evaluation of data was performed using the software package SPSS/PC+ Statistic TM 4.0 (SPSS Inc., Chicago, IL, USA); Student's *t*-test was used to compare data and the significance level was set at $p<0.01$.

RESULTS

The data in the tables are the average of all of the values obtained in each subgroup of animals. The blood levels of CPK and LDH (Table 1) show that the most statistically significant results were obtained by group D, treated with continuous laser energy output. These data are also confirmed by the histoenzymatic value (Table 2) measured directly from the muscular tissue in group D.

Table 1. Average value in blood of CPK and LDH in all groups

Group	CPK			LDH		
	Normal	5 days	10 days	Normal	5 days	10 days
A Untreated	846	2323	853	245	1054	1711
B 150 J/cm^2/50 Hz/2500 mW	846	1146	1085	245	247	162
C 250 J/cm^2/100 Hz/2500 mW	846	770	989	245	297	175
D 800 J/cm^2/0 Hz/2500 mW	846	644	828	245	175	118

CPK, creatine phosphokinase; LDH, lactate dehyrogenase.

Table 2. Average value in muscular cells of LDH, COX, acid phosphatase and alkaline phosphatase (means ± SD, no. of samples = 5)

Group	LDH		COX		Acid ph.		Alkaline ph.	
	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
A Untreated	6.20 ± 0.5	5.3 ± 1.36	15.6 ± 4.31	14.2 ± 2.6	++	++	−	−
B 150 J/cm^2/50 Hz/2500 mW	4.87 ± 2.13	7.18 ± 1.69	10.05 ± 1.06	13.42 ± 3.22	+	+	−	+/−
C 250 J/cm^2/100 Hz/2500 mW	4.67 ± 1.79	6.07 ± 1.14	10.43 ± 2.84	9.59 ± 2.43	+/−	+/−	−	+/−
D 800 J/cm^2/0 Hz/2500 mW	3.92 ± 1.53	4.01 ± 0.97	6.52 ± 1.27	11.88 ± 4.81	+/−	−	+/−	+/−

++, high positivity; +, positivity; +/−, low positivity; −, absent.

Table 3. Histomorphometric evaluation of muscle tissue modification after trauma during healing processes

Group	Regular nucleus		Regular fibres		Necrosis		Fibrosis		Diameter	
	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
A Untreated	No	No	No	No	Yes	Yes	Yes	Slight	44	49
B 150 J/cm^2/50 Hz/2500 mW	No	No	No	No	Yes	No	Slight	No	62	49
C 250 J/cm^2/100 Hz/2500 mW	Yes	Yes	Yes	Yes	Yes	No	Slight	No	61	45
D 800 J/cm^2/0 Hz/2500 mW	Yes	Yes	Yes	Yes	No	No	No	No	47	55

Normal diameter = 40/60 μ.

The values reported in Table 1 were calculated, for blood specimens, before the trauma and before sacrifice 5 and 10 days after laser therapy. The values reported in Tables 2 and 3 were calculated from biopsies taken 5 and 10 days after trauma and laser therapy.

Changes in blood enzyme levels demonstrate a modification of muscular metabolism in the tissue receiving laser treatment when compared to the controls. Results show that in the treatment groups lower enzyme levels (LDH, acid phosphatase) demonstrate that muscular damage was more evident in the untreated group. Group D_1 demonstrated a significant difference in LDH levels compared to untreated group A_1 ($p=0.0058$) five days after the start of laser therapy. The other analyses repeated at 10 days did not demonstrate any significant differences. The cytochrome *c* oxidase test results demonstrate a significant difference between the untreated groups A_1 and all the treated groups B_1, C_1, D_1 (A_1–B_1, $p=0.012$; A_1–C_1, $p=0.0339$; A_1–D_1, $p=0.0006$). The alkaline phosphatase, on the other hand, was higher in the treated groups where the repair of the traumatised tissue was most pronounced. In particular, the best results were observed in group D where the difference was statistically significant. These data seem to be the best compared to that of other groups, in particular to groups A_1 and A_2 (spontaneous healing).

The histomorphometric results (Table 3) show that in each subgroup findings that are closer to an optimal regeneration of the muscle are reached at the end of the treatment period (10 days).

DISCUSSION AND CONCLUSIONS

A relationship between the biostimulation properties of the laser used and the healing of muscular tissue has been demonstrated.

Analysis of the results shows that the best results from a histochemical and histomorphological point of view were obtained in group D where the muscular tissue was treated with continuous output laser light, compared to groups B and C treated with pulsed output laser light.

The biochemical and histomorphometric evaluation of the animals in group D, demonstrate better results when compared to group A. They present a statistically significant difference of enzymatic values (LDH, $p=0.0058$; COX, $p=0.0006$) and better and quicker healing of the traumatised tissue, as seen by the increase in alkaline phosphatase and from the appearance and size of the muscle cells.

Group D results are better when compared to the other treated groups, even without using statistics.

The main biological effects of the laser that we used in our research include increased blood flow, improvements in capillary hydrostatic pressure, emptying of intertissue sacs and the stimulation of electrolyte exchange. The effects of this diode laser are particularly efficient compared to lasers previously used for biostimulation. We could attribute this change to the increased power of this laser.

The parameters used in our laser therapy were selected to demonstrate the biostimulation effects of the laser by distributing high densities of energy without creating thermal damage in the treated tissue. We did not find any thermal damage. 'Biostimulation' is a response of living cells and cellular interactions and it is strictly dose-dependent and cumulative [2]. For this reason, we believe that the better results in group D were due to the higher density of energy transferred to the irradiated tissues in a continuous fashion.

The application of this experimental experience to a clinical field could be useful in establishing the most appropriate parameters to the employed not only in treating different types of muscular trauma but also for other types of pathologies, such as inflammatory or degenerative pathologies. The different parameters that this laser offers could be further studied especially in the field of pulsed output.

The versatility of this type of laser allows one to adapt the parameters to clinical needs. If these experimental data are extended to the clinical field, we could expect a functional recovery of the patient in a shorter period of time. With little exercise, this would allow the patient to go back to work more quickly, providing obvious benefits for the community.

Our conclusions [8–10] are that the possibility of using a Ga-Al-As diode laser for the treatment of muscular trauma is important for speeding up the regeneration process of the muscle tissue damaged by trauma and with evident advantages relating to functional recovery.

We agree with other authors [11] that diode laser systems will be important therapeutic devices in the future, particularly because of their compact size and simplicity of use as well

as for the wide choice of devices obtainable which use diode laser technology [12].

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