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Does laser prevent physiologic muscle atrophy? An histological study.

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ABSTRACT

Physical therapists usually confront with the dilemma of physiologic muscle atrophy, especially after limb immobilization. Exercises as well as electric stimulation are the preferred treatment routine to prevent disuse muscle atrophy. In the histological study here presented, twenty albino rats, equally divided into two groups: (1) control group, and (2) laser group were used. In all the albino rats the whole right leg was immobilized in a plaster cast, started above the foot and ended below the hip joint. For the experimental group, a window was opened on the front of the thigh over the quadriceps muscle for treatment intervention. The experimental group received daily LASER treatment for one month. At the end of the study, animals were sacrificed and a histological study was done to measure the dependent variable of the study, which is the size of the muscle fiber. The aim of the study was to determine the effect of laser therapy on prevention of muscle atrophy in simple physiological immobilization versus control group. Results of this study revealed that immobilization for one month produced a relative muscle fiber size variation (atrophy) of 30.7% in the immobilized limb, and 20.4% in the immobilized limb exposed to the laser treatment. In conclusion, laser therapy may have a significant effect in prevention of muscle atrophy in physiological immobilization.

INTRODUCTION

The role of Physical Therapy during the period of immobilization is to prevent or decrease the deleterious effects of immobility and disuse on bone, joint and soft tissue structures. Muscle atrophy is one of the most common problems seen at the end of immobilization period. The longer the immobilization period is, the more the adverse effects on soft tissues are. The main goal of treatment during the immobilization period is to keep the skeletal muscles and their blood supply, like pumping effect, in or around its normal level [1]. Physical therapists usually apply active exercises and/or electrical stimulation for preventing of muscle atrophy for the immobilized muscles. Laser is a relatively new therapeutic tool. It has been used to reduce pain and to accelerate healing process of wounds. In a Russian research study, Nemtsev reported that HeNe laser may be recommended for atrophy prophylaxis and treatment in cases of hypokinesia and weightlessness induced atrophy [2]. Whilst the Food and Drug Administration (FDA) in the USA has still to approve laser therapy, the modality has found increasing application by physical therapists, dentists, acupuncturists, and some physicians, for a range of conditions including the treatment of open wounds, soft tissue injuries, arthritic conditions and pain associated with various aetiologies [3]. The potential of relatively low intensity laser irradiation applied directly to tissue in modulating certain biological processes, in particular to photobiostimulate the wound healing process, has been mentioned [4].
The aim of the study here presented was to determine the effect of laser therapy on prevention of muscle atrophy in physiological immobilization.

MATERIALS AND METHODS

DESIGN OF THE STUDY. It was 2x1 pre-test/post-test research design. Laser therapy and sham treatment "control group" were the independent variables, and the muscle fiber size was the dependent variable.
EXPERIMENTAL MODEL. The experiment was carried out on 20 male albino rats. Their body weight ranged between 120-150 grams at the beginning of the study. The rats were divided into two groups:
Group I "Control group". Ten rats were immobilized by a plaster cast covering the whole right lower leg, started above the foot and ended below the hip joint, keeping the ankle joint & the hip joint free from immobilization. The period of immobilization continued for one month without any treatment.
Group II "LASER therapy". Ten rats were immobilized (same as in group I), but a window was opened on the front of the thigh over the quadriceps muscle to apply daily laser therapy for ten minutes during the period of immobilization (one month).
INSTRUMENT. Laser therapy unit LTU-904 is a portable, simple-to-use unit. Activated by a finger-tip control, the hand held Laserex LTU- 904 consists of a pulsed infra-red laser whose radiation penetrates into the affected tissues to a depth of 20-

30 mm. The advantages of a pulsed Ga-As infra-red laser (λ=904 nm), as compared to a continuous He-Ne laser (λ=632 nm) are in the greater depth of penetration and selective biological absorption. Specifications for LTU-904 are:

- Laser type: Gallium arsenide (Ga-As) Laser diode
- Laser wavelength: 904 nm
- Peak power: 5 W
- Mean Output power: 5.0 mW
- Pulse repetition rate: 5000 Hz
- Pulse duration: 200 ns
- Warning signals: Inbuilt emission detector and visual/audible warning signal.
- Mean fluence: 20 mW/cm2

PROTOCOL.

All the rats were kept in the same conditions, except laser treatment. The rats in the experimental group received daily treatment of laser therapy for ten minutes through the window in the plaster cast, which was opposit to the motor point of the quadriceps muscle. The rat in the control group received a sham treatment. At the end of the experiment, all the animals were sacrificed with an overdose of ether and the quadriceps muscles were dissected out for histological examination. Jaffe [5] method was used for tissue preparation. Histological examination were subjected to the morphometric study "the qualitative description of a structure" [6, 7]. Measurement of the muscle fiber diameter was made by means of a micrometer disc that was placed in the ocular disc of the microscope. The disc is usually calibrated as a line divided into 100 units. This calibration is done for standardization of the measurements taken by the ocular micrometer[8]. All the results were tabulated and the mean number of the maximum muscle fiber diameters was calculated.

RESULTS

The difference in size of muscle fibers between immobilized and non-immobilized limb in control and laser-therapy groups was calculated to detect the percentage of muscular atrophy in the right immobilized limb in relation to the left non-immobilized one. The change in muscle fiber size in each group was compared to determine the effect of laser-therapy on prevention of muscle atrophy in physiological immobilization.

GROUP I (CONTROL).

The data reported in Table I show that seven animals survived and were investigated. The maximum muscle fiber diameter (MMFD) in the right immobilized limb ranged between 11.8 and 18.1μ with a mean value of 14.6 with SE=0.2. The MMFD in the left non-immobilized limb ranged between 16.7 and 26.7μ with a mean value of 21.3μ with SE=0.3. The difference in MMFD between the right and left side ranged between 4.8 and 8.7 with a mean value of 6.6 with SE=0.2. The percentage of atrophy in this group ranged between 15.4 and 42.3% with a mean value of 30.7% with SE=0.5. The decrease in muscle fiber size due to muscular atrophy in the right side was statistically significant as t=19.5 and p<0.00001 (Table III). The microscopic examination of a Transverse Section (TS) by a photomicrograph showed a great decrease in muscle fiber size in the right immobilized limb compared with the left non-immobilized side in the control group.

GROUP II (TREATED).

Seven animals survived and were investigated. The difference in MMFD between the right and left side ranged between 2.0 and 11.2μ, with a mean value of 5.3 with SE=0.2 (Table II). The decrease in muscle fiber size, due to muscular atrophy in the right side, was statistically significant as t=9.5 and P<0.00001 (Table III).

The decrease in muscle fiber size in the right immobilized limb, when compared with the left non-immobilized side, was analyzed by photomicrograph of a TS with the same magnification for each side.

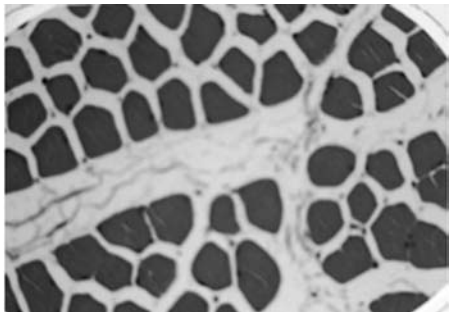

Fig.1. A photograph of a TS of the normal muscle fiber size (left side) from a male albino rat showing normal muscle fibers with peripheral nuclei. The connective tissue endomysium & perimysium are normal (H&Ex200)

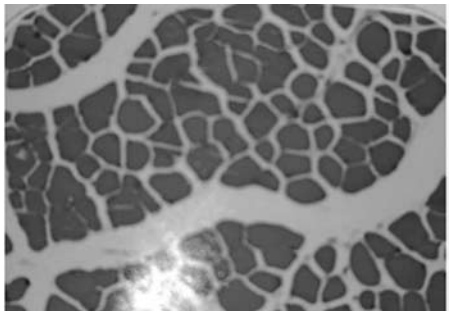

Fig.2. A photograph of a TS of the immobilized muscle fiber size (right side) from a male albino rat showing atrophic muscle fibers with peripheral nuclei (H&Ex200)

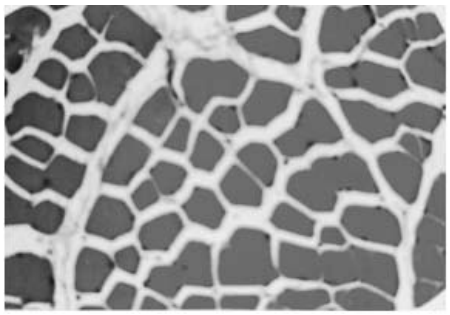

Fig.3. A photomicrograph of a TS of the normal muscle (left side) from a male albino rat showing normal muscle fiber size with peripheral nuclei. The connective tissue endomysium & perimysium are normal (H&Ex200)

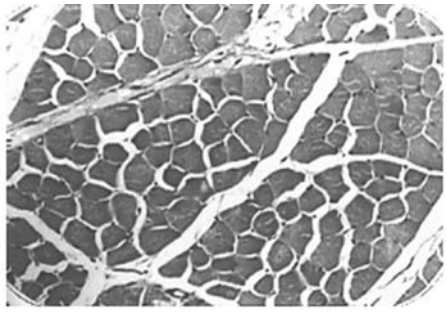

Fig.4. A photomicrograph of a TS of the immobilized muscle fibers (right side) from a male albino rat showing the difference in muscle fiber size after laser- treatment (H&Ex200)

DISCUSSION

Limb immobilization is commonly used to protect fractured bones and injured tissue. The most common complication of immobilization is the muscle wasting that occurs due to a decreased muscle use. The results of the current study supports the deleterious effects of disuse and immobilization on the muscle fiber size, which lead to atrophy of the immobilized muscles. In fact, in both the groups studied there was a significant decrease in the MMFD of the immobilized limb in comparison with the non-immobilized one.

As regards the effect of laser-therapy on muscle fiber size, our findings might support what was reported first by Nemtsev [2], who recommended laser-therapy for atrophy prophylaxis and treatment in cases of hypokinesia and weightlessness induced atrophy. In fact, even if the reduction in muscle atrophy by lasertherapy cannot be considered significant from the statistical point of view ($p \geq 0.05$), however we found that the decrease in the diameter of muscle fibers in the immobilized leg of treated animals was about 20% versus the 30% monitored in control animals. The positive effect the laser treatment seemed to have on trophism of muscle fibers is in agreement with data shown by other authors.

The biophysical effects of lasertherapy on muscle have been widely reported in literature [9, 10]. Recently, the efficacy of 780-nm lasertherapy on peripheral nerve regeneration has been demonstrated [11]. Moreover, low level laser therapy seems also to affect growth and regeneration of capillaries [12].

Many studies have been carried out on the effects of laser on cell growth. The data showed that the growth stimulation was accomplished by an increase in the respiration activity and by synthesis process in the cell, while accumulation of the toxic intermediate of oxygen metabolism and degenerative processes decreased. [13]. Recent research confirmed that laser irradiation can enhance proliferation and metabolic processes in cells [14, 15].

Table I. Maximum Fiber diameter of the quadriceps in control group.

Animal #	Right	Left	Differ.	%
1	15	22.4	7.4	33.1
2	13.7	18.7	5.0	26.7
3	18.1	26.7	8.7	32.4
4	16.5	19.5	3.0	15.4
5	14	24.2	10.2	42.3
6	13.2	20.7	7.8	36.2
7	11.8	16.7	4.8	29.0
Mean	14.6	21.3	6.7	30.7
SD	2.1	3.4	2.5	8.4
SE	0.2	0.3	0.2	0.5

The difference in MMFD between the right and left side ranged between 15.4%-42.3% with a mean value of 30.7 with SE=0.51. The decrease in muscle fiber size due to muscular atrophy in the right side was statistically significant as t=19.5 and P<0.00001.

Table II. Maximum Muscle Fiber Diameter (MMFD) of the quadriceps in the laser-treated group.

Animal #	Right	Left	Difference	%
1	22.3	25.9	3.6	13.8
2	16.8	23.6	6.7	28.6
3	20.7	22.7	2.0	8.8
4	21.0	25.3	4.3	17.1
5	19.3	24.5	5.1	21.1
6	22.5	33.7	11.2	33.1
7	16.6	20.8	4.2	20.4
Mean	19.9	25.2	5.3	20.4
SD	2.4	4.1	3.0	8.4
SE	0.3	0.4	0.2	0.7

The values show the difference in MMFD between the right immobilized limb and the left non-immobilized limb in the laser-treated group.

Table III. Mean values of MMFD in right and left side in the two groups.

	Control Group		LASER Group	
Side	Right	Left	Right	Left
Mean	14.6	21.3	19.9	25.2
SD	2.1	3.4	2.4	4.1
SE	0.2	0.3	0.3	0.4
t	19.5		9.5	
P	<0.0001	<0.0001	<0.0001	<0.0001
Sig.	*	*	*	*

It is showing the significant different. The results indicated that there was a significant difference in size between the right and left sides in all groups.

In conclusion, this experimental study provides the clinicians with a scientific piece of information about the application of laser therapy for preventing muscle atrophy. Prolonged immobilization for one-month lead to 30% muscle atrophy of its original size, but the daily exposure to laser treatment reduce muscle atrophy to 20%. Further studies are in progress, with an increased number of cases, in order to improve the statistical significance of the results.

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