Effect of Low Level Laser Therapy (830 nm) With Different Therapy Regimes on the Process of Tissue Repair in Partial Lesion Calcaneous Tendon

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Background and Objective: Calcaneous tendon is one of the most damaged tendons, and its healing may last from weeks to months to be completed. In the search after speeding tendon repair, low intensity laser therapy has shown favorable effect. To assess the effect of low intensity laser therapy on the process of tissue repair in calcaneous tendon after undergoing a partial lesion.
Study Design/Materials and Methods: Experimentally controlled randomized single blind study. Sixty male rats were used randomly and were assigned to five groups containing 12 animals each one; 42 out of 60 underwent lesion caused by dropping a 186 g weight over their Achilles tendon from a 20 cm height. In Group 1 (standard control), animals did not suffer the lesion nor underwent laser therapy; in Group 2 (control), animals suffered the lesion but did not undergo laser therapy; in Groups 3, 4, and 5, animals suffered lesion and underwent laser therapy for 3, 5, and 7 days, respectively. Animals which suffered lesion were sacrificed on the 8th day after the lesion and assessed by polarization microscopy to analyze the degree of collagen fibers organization.
Results: Both experimental and standard control Groups presented significant values when compared with the control Groups, and there was no significant difference when Groups 1 and 4 were compared; the same occurred between Groups 3 and 5.
Conclusion: Low intensity laser therapy was effective in the improvement of collagen fibers organization of the calcaneous tendon after undergoing a partial lesion. Lasers Surg. Med. 41:271–276, 2009. © 2009 Wiley-Liss, Inc.

Key words: calcaneous tendon; diode laser; lesion tendon; low level laser therapy; physical therapy; repair tissue

INTRODUCTION

The calcaneous tendon is one of the most frequently injured tendons in human beings, followed by digital flexors, due to overuse, trauma caused by firearm wounds, and sharp objects [1].

Owing to the slow pace of healing, the rupture of the calcaneous tendon is considered a serious injury, and it has drawn the attention of several researchers [2].

Spontaneous rupture of the calcaneous tendon occurs between 2 and 6 cm of its insertion into the calcaneous bone. Histological examination has suggested that such tendons had already undergone primary degeneration [3] and showed important alterations in the type of collagen fibers [4].

In order to observe blood supply to the calcaneous tendon, CARR & NORRIS (1989) [5] verified that the number of blood vessels varies along the length of the tendon and their highest concentration occurs in the calcaneous insertion and up to 4 cm above it, considering that neoangiogenesis is a vital part of the healing process, as it restores normal circulation and carries more cells and nutrients to the injured location, thus limiting ischemic necrosis and allowing tissue repair [6].

Due to its low blood supply, the calcaneous tendon is a structure that can take weeks or even months to heal completely [2,7].

During the period of the lesion, it is customary for the patient to remain immobilized in order to prevent a new rupture, which could generate countless functional complications, including ultra-structural and biomechanical alterations in the tendon [8,9].

Such complications, caused by prolonged immobilization, can be minimized by shortening the duration of the tendon repair [3].

Trying to accelerate tendon repair, several physical agents such as ultrasound [10], electrical stimulation [11], and low level laser therapy [12] have shown beneficial effects.

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Accepted 10 February 2009
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20760

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Among those, low level laser therapy has been fairly resorted to by physiotherapists over the last 20 years, with significant effects, such as an increase in the proliferation of fibroblasts and collagen synthesis [13], cutaneous neovascularization [14,15], and tendon repair [16].

The use of laser therapy to heal tendon damage was investigated by several groups of researchers through studies both in vivo and in vitro, with both positive and negative findings [12]. However, prior studies are controversial due to the variation of parameters for the use of irradiation, leading to a lack of consensus on which should be the ideal parameters to be used for laser therapy.

Due to these contradictions found in inconclusive findings in the literature, more studies are needed to compare and to standardize ideal parameters for the use of low level laser therapy, as well as to determine the frequency of treatments.

MATERIALS AND METHODS

This study was carried out with 60 male Wistar rats (*Rattus norvegicus: var. albinus, Rodentia, Mammalia*), weighing from 260 to 320 g. The animals were kept in individual cages with a 12-hour light—dark cycle, and they were given suitable ordinary feed as well as water ad libitum. This was approved by the Research Ethics Committee (Comitê de Ética em Pesquisa) of UNIFESP—EPM.

The animals were randomly divided by computerized draw (Urn Randomization) in five groups with 12 animals each. In Group 1 (Standard Control), the animals were not subjected to the traumatic lesion and received no treatment. This group was used as reference for the analysis. Group 2 (sham) was subjected to a lesion of the calcaneous tendon, by direct trauma, and received a placebo treatment with the equipment turned off, whereas groups 3, 4, and 5 were also subjected to a lesion of the calcaneous tendon, and then treated with low level laser therapy (LLLT) during 3, 5, and 7 consecutive days, respectively.

Procedure to Produce the Partial Lesion of the Calcaneous Tendon

The animals were anaesthetized with an intraperitoneal injection of tyletamine hydrochloride and zolazepam hydrochloride with a dosage of 50 mg/kg. The skin around the left foot's calcaneous tendon was trichotomized and positioned in the equipment developed by the machine workshop at Universidade Federal de São Carlos (UFSCar—Brasil) (Fig. 1a). Light traction was exerted on the right calcaneous region with the ankle in dorsiflexion, and a weight of 186 g was released perpendicularly from a height of 20 cm above the animal's tendon. The potential kinetic energy on the tendon was equal to 364.9 mJ (Fig. 1b).

Immediately after this procedure, the weight was removed and the location of the lesion was marked by drawing a circle around it with a ball-point pen, in order to carry out precise laser applications always in the same location.

a

b

Fig. 1. Equipment for partial lesion of the calcaneous tendon (**a**), moment in which the lesion occurs through contusion on the tendon after the weight is released (**b**).

For treatment, the animals were placed in a contensor (a polyvinyl chloride tube—PVC) in order to insulate the limb to be treated.

All the animals were killed on the 8th day after being subjected to the lesion on the calcaneous tendon.

Laser Treatment

The animal's right tendon received low level laser therapy with an infrared diode, with wavelength of 830 nm (GaAsAl), power of 40 mW, power density of 1.4 W/cm^2 and beam cross section area of 0.028 cm^2 with continuous waveform. DMC® brand.

The animals in experimental groups 3, 4, and 5 received low level laser therapy with a flow of 4 J/cm^2, total energy of 0.12 J every day during treatment, always in the same period, during 3, 5, or 7 days starting on the day the lesion was produced.

On the eighth day after the lesion, the tendons were surgically removed by dissection from the calcanear insertion until the junction with the tendon muscle.

In order to perform the analysis, the histological slices were chosen randomly, so as to avoid any identification with the corresponding animal when birefringence measurements (blind analysis) are taken.

The analysis of collagen fibers was carried out using optical anisotropic properties (birefringence) by means of polarization microscopy.

The measurements of optical delay (OR in nm) were made with polarized light microscopy in the Leica microscope, with a Pol 10×/0.22 objective, 0.9 condenser, 1/4 lambda Sénarmont compensator, lambda = 546 nm monochromatic light, obtained by means of a Leica interference filter. In order to carry out the measurement, the long axis of the tendon was kept at approximately 45° in relation to the microscope polarizers. In this position, the collagen fibers introduced the highest OR.

OR measurements were taken at different points in the central areas of the tendons (partial lesion area), for each group investigated. The resulting measurements in degrees were transformed to nm by multiplying the degrees by 3.03.

Data Analysis

The analysis of variance method with a fixed factor (ANOVA) where $P \leq 0.05$. To better detail possible differences between the groups, the Bonferroni multiple comparisons method was used.

Whenever the calculated statistic presented significance, it was marked with (*) to offset it, whereas nonsignificant results were represented with NS.

RESULTS

The results of this study show that the groups treated with LLLT experienced significant improvement in terms of realignment of collagen fibers, according to birefringence analysis.

Group II (control) presented optical delay values of 33.19 nm, demonstrating a disorganization of collagen fibers in comparison with the groups treated with LLLT and the standard control group.

Group I, which gives the standard birefringence value for normal tendon tissue, did not undergo any type of lesion or intervention and had values of 63.09 nm (Table 1).

It can be observed that the average of Group I values (63.09 nm) does not present a statistically significant difference in relation to Group IV ($P<0.999$), showing that treatment with LLLT during 5 days produced optical delay values equal to those observed in normal tissue (Fig. 2a,b).

It can be observed that no significant difference was found in the comparison between Group III (3 days) and Group V (7 days), with $P<0.999$ (Fig. 3).

TABLE 1. Descriptive Measurement of Optical Delay (nm) Corresponding to Alignment of Collagen Fibers

Group	Average (nm)	SD
Group I	63.09	5.44
Group II	33.19	3.21
Group III	42.38	5.98
Group IV	62.18	9.27
Group V	46.22	2.59

Group I (standard); Group II (control); Group III (3 days); Group IV (5 days); Group V (7 days application).

DISCUSSION

The actual mechanisms by which the laser stimulates tendon repair are still not completely explained. Nonetheless, some authors believe that cellular responses depend on choice and combination of their parameters, such as wavelength, energy density or flow, power density, beam cross section area, application technique, irradiation time, and treatment intervals [12–14].

The wavelength used in this study is within the ideal wavelength range found by Karu [17], which is near

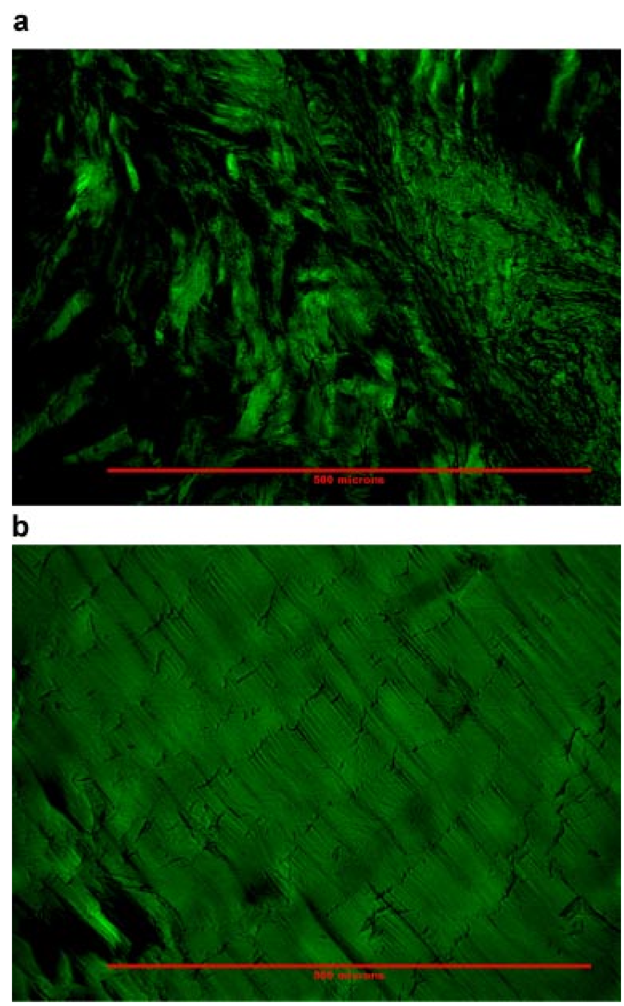

Fig. 2. **a**: Birefringence analysis of calcaneous tendon with partial lesion (Group II—Control) showing disorganization of collagen fibers. **b**: Analysis of calcaneous tendon (Group 4) treated with LLLT during 5 days, showing organization of collagen fibers.

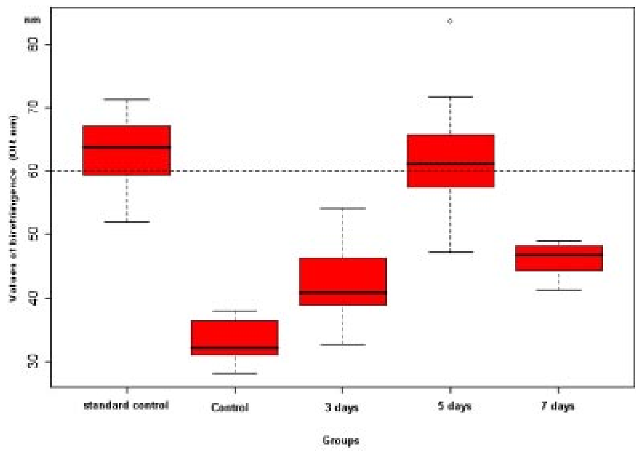

Fig. 3. Distribution of the values of collagen fiber alignment.

760 nm, 810 and 840 nm, as in these bands of the electromagnetic spectrum, superficial chromophores have weak absorption, and therefore, there is greater penetration in the skin. Because of this, in clinical practice infrared laser is the most used in cases of orthopedic and sport injuries.

In this study, a flow of 4 J/cm^2 was used, as there are reports demonstrating that flows between 1 and 4 J/cm^2 are insufficient to promote significant therapeutic effects [18].

Friedman et al. [19] report that low flows intensify electrochemical formation of the transmembrane by taking protons to the mitochondrium, followed by release of mitochondrium calcium inside the cytoplasm through the antiport process, which in turn, triggers subsequent mitosis and cellular proliferation. On the other hand, high dosages lead to the release of a large amount of calcium, causing hyperactivity of the adenosine triphosphate-calcium (ATPase) and of the calcium pumps, so depleting the ATP reserve in the cell and inhibiting cellular metabolism as a consequence. For these reasons, a dosage of 4 J/cm^2, considered to be intermediary, was used in this study.

Salate et al. [16] demonstrated in their study to verify tendon repair with different powers (10 and 40 mW), that the group irradiated with a power of 40 mW showed precocious neovascularization and a greater number of vessels in comparison with the other groups, thus agreeing with the positive results obtained with the same power used in this study.

In this investigation, total energy was equal to 0.12 J and only one point was used, which matched exactly the location of the partial lesion marked with a ball-point pen. Carrinho et al. [20] used different energy values (0.09 and 0.28 J) to repair the calcaneous tendon and had better results with the lower energy (0.09 J), whereas Salate et al. [16] had better results in repairing the calcaneous tendon using the higher energy (0.4 J).

All this diversity of parameters in the different studies analyzed clearly shows that many questions still need to be answered.

Another factor that may influence tendon repair is the choice of the suitable time to carry out laser treatment. This study chose to stimulate the initial phase of the healing process, remodeling it at an early stage because the strength of the healed tissue increases significantly in this phase, as attested by some authors who described the anti-inflammatory effect of laser in the initial healing phase [21].

The evaluation method proposed was the birefringence analysis, which according to Vidal [22] is the best method to detect and describe the orientation of collagen fibers in the tendon. The evaluation of collagen fibers orientation employed in this research was used in various forms by several authors in the study of therapeutic agents and factors such as heating, exercise and other physiological processes that have an influence in the tendon repair process [18,23].

The results of this study consistently demonstrate that collagen fibers, during the initial stages of the repair process, responded beneficially to the use of low level laser after the lesion produced on the calcaneous tendon, thus corroborating the findings of Agaiby et al. [6] and Carrinho et al. [20].

In addition, the results also showed that the Optical Delay (OR) values (nm), corresponding to total birefringence, were greater in Group I (Standard Control) when compared to OR values in the other injured groups. These data may be explained by the high degree of aggregation and organization of collagen fibers in the tendons in this group, considering that they did not suffer any type of lesion, that is, their tendons are undamaged, thus corroborating the findings of Vidal and Carvalho [24].

However, no significant difference emerged from the comparison of Group I with Group IV (treated during 5 days). Results that may better explain the high degree of organization of collagen fibers in Group IV are those presented by Salate et al. [16], where after the 5th day of irradiation the tenotomized tendons were found to have more vessels. Therefore, an increase in the number of vessels may indicate an improvement in tissue repair, and consequently an improvement in the degree of the collagen fibers organization.

On the other hand, OR values presented by Group V were differently significant ($P \leq 0.05$) in relation to Group II and Group IV, being respectively higher and lower, whereas no significant difference was found in relation to Group III. These results show that a gradual increase of OR values, that is of the degree of collagen molecules organization, occurred between 3 and 5 days of laser applications, yet with seven applications this organization decreased in comparison with the 5-day group.

These results corroborate those presented by the same study mentioned above by Salate et al. [16], which also report that the group of animals irradiated during 7 days with 40 mW had fewer blood vessels than those in the 5-day group. In addition, that study observed that the fibroplasia and fibrillogenesis period began around the seventh day, when the number of blood vessels decreased and got back to normal, in agreement with the study by Enwemeka [25].

In addition, Enwemeka et al. [26] report that the mechanical load imposed early on the tissue accelerates parallel alignment and polymerization of fibrils within the collagen fibers. Thus, the fibrillar alignment process can begin 4–5 days after tendon rupture. This fact may also explain the better alignment observed in the groups treated during 5 days with respect to those treated during 3 days, as the animals were kept loose in the cages and discharged weight onto the injured limb.

Another possible explanation for the decrease in collagen fiber alignments between 5 and 7 days of irradiation may come from the bioinhibitory effect of laser, studied by several authors [18,27]. Labbe et al. [27]; Karu [28], suggest that induction by light stimulation (laser) and the inhibiting effects, respectively, result from the absorption of light by the flavins and cytochromes in the mitochondrial respiratory chain, leading to alterations in electron transfer in redox pairs located in this area.

Based on the findings of the above mentioned authors, as well as on other authors, it was observed that the majority of studies on the effects of laser on the cell attest to specific intracellular modifications [19,29]. Most of those modifications have pointed to calcium metabolism, which is presumably affected in terms of either concentration or of intracytoplasmatic transport. Such alterations presumably stimulate cellular division to the detriment of cellular protein synthesis, which might explain why cell growth increased and pro-collagen remained unchanged [29].

The important finding of this study is that it showed that the treatment per 5 days was better than 7 days to only collagen fibers orientation, and we did not evaluate the inflammatory phases and the cells. But it is important to remember that laser can provide an inflammatory modulation that may improve the healing process. However, the laser application in the early phases is important, but there are some studies, like the important study by Ng et al. [30], showing that early laser application was less favorable than a sequential mode of application. The present study pointed out that the sequential application per 5 days is important.

This study attempted to analyze and determine the most adequate laser parameters to be used in the initial stages of a partial lesion in the calcaneous tendon. However, more studies need to be done in order to elucidate the effects of low level laser on tendon repair, not only to verify the alignment of collagen, but also to verify tensile force and to count inflammatory cells and blood vessels in the different inflammatory phases, thus gaining an understanding of cellular metabolism and its regenerating potential, and enabling to transfer it to clinical practice.

CONCLUSION

Low intensity laser therapy is effective to improve repair of the calcaneous tendon of rats subjected to a partial lesion.

ACKNOWLEDGMENTS

We thank the Plastic Surgery Department of UNIFESP and CAPES for supporting this study.

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