Effect of MLS® Laser Therapy for the treatment of experimentally induced acute tendinopathy in sheep – a preliminary study.

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ABSTRACT

Tendon injuries are common in human athletes and sport horses. Unfortunately, traditional treatments are limited in their ability to completely heal injured tendons. Recent advances in low-level laser therapy (LLLT) have shown promising results. This study evaluated the effect of Multiwave Locked System (MLS®) laser therapy in collagenase-induced tendon lesions in sheep. Six animals were randomly assigned to two groups, with group 1 receiving ten MLS® Laser Therapy treatments at 5 J/cm² on the left hind limb and group 2 receiving the same number of treatment at 2.5 J/cm² on left hind limb. The right hind limb was considered a control for both groups. Clinical follow-up, ultrasonography and histological examinations were performed on the injured tendons. Clinical and histological evaluations demonstrated that using a therapeutic dose less than 5 J/cm² resulted in an anti-inflammatory effect. Moreover, the histological examinations showed a statistically significant reduction in cell number in both treated groups and a significant decrease in vascularization in the treated tendons in group 2. MLS® Laser Therapy appears to be an effective tool to improve collagen fiber organization in the deep digital flexor tendon.

INTRODUCTION

Overuse tendinitis and other tendon injuries are common among athletes [1,2] and represent a frequent cause of lameness in sport horses [3,4]. In the human medical field, Low Level Laser Therapy (LLLT) has been used to treat acute and chronic musculoskeletal pain and foster wound healing [5]. However, few studies have evaluated its effectiveness in treating patients with acute tendonitis and other tendinopathies [6].

As demonstrated in the literature, LLLT acts on two phases of the healing process[7]. First, it reduces PGE2 concentrations and inhibits cyclo-oxygenase [8,9,10,11]. Secondly, it modulates fibroblast metabolism and collagen deposition due to its anti-inflammatory effect. Histological changes observed in tendons receiving LLLT include increased collagen production [12], improved collagen bundle organization [13,14] and an increased number of small blood vessels [5,15].

Animal models are commonly utilized in tendon disorder research [16] and the collagenase-induced tendinitis model has been used to study acute inflammatory responses [8]. This model has been used in rats, sheep and horses, and mimics a traumatic tendon injury [17,18]. The sheep is recognised as a model for human and equine orthopaedic injuries, including tendinopathy, due to the similar connective tissue structure of the flexor tendons in these species [19,20,21]. Numerous authors have described the positive effects of LLLT in experimental trials in rats [9,16,22,23], mice [13,24] and rabbits [12]. However, to our knowledge, there are no studies investigating the effect of LLLT on experimentally induced tendinitis in sheep. This preliminary study was designed to investigate the effect of MLS® (Multiwave Locked System) laser therapy on an experimental model of collagenase-induced tendinitis in sheep in order to evaluate a specific treatment for human and animal athletes.

MATERIALS AND METHODS

This study was approved by the University Ethics Committee for Animal Experimentation (CEASA) and by the Italian Ministry of Health on 17 May 2010 (DM no. 97/2010-B). Six healthy adult female Bergamasca sheep weighing 50-60 Kg were included in the study. Prior to enrollment,

clinical and ultrasound examinations were performed to confirm tendon integrity.

A defect was produced in the deep digital flexor tendons (DDFT) of both hind limbs by collagenase injection as previously described [21,25]. Intravenous administration of 10 µg/kg of medetomidine (Sedator®, Ati srl Ozzano dell'Emilia, Italy), and 2 mg/kg of propofol (Rapinovet® Intervet Italia, Peschiera Borromeo, Italy) were used to anaesthetize the animals. After aseptic disinfection and placement in lateral recumbency, 500 IU of sterilized bacterial collagenase type 1A (C-9891; Sigma, Milan, Italy) in 0.13 ml of saline solution was injected bilaterally (left and right hind limbs) into the DDFT under ultrasound guidance. A 23-gauge needle was used to perform the injection. The needle was introduced 15 cm distal to the calcaneal bone and was inserted into the full thickness of the DDFT using a lateral approach with the hock joint flexed at 90°. A suture was applied close to the injection site to mark the precise location for treatment and tendon harvesting. Antibiotic therapy using amoxicillin-clavulanic acid (Synulox® Pfizer Italia, Rome, Italy) at a dose of 12.5 mg/kg SC was started and continued for 5 consecutive days. Buprenorphine (Temgesic® RB Pharmaceuticals, Slough, UK) at a dose of 0.01 mg/kg IM BID for 5 days was used to provide analgesia.

Seven days after collagenase injection, the 6 sheep were divided into two groups (group 1 and 2) and treated using MLS® Laser Therapy. The MLS® Laser Therapy was performed using an Mphi veterinary laser device (ASA, Arcugnano-VI, Italy), equipped with combined, synchronized and overlapping continuous and pulsed emissions from a single handpiece. Continuous emissions or continuous interrupted emissions were produced by an InGa(Al)As diode laser with the following parameters: wavelength of 808 nm, peak power of 1000 mW for continuous wave, mean power of 500 mW for continuous interrupted wave, spot area of 3.14 cm^2, spot diameter of 2 cm. Pulsed emissions were produced by an InGaAs/GaAs diode with the following parameters: wavelength of 905 nm, peak power of 25 W, mean power of 54 mW at 1500 Hz, pulsed wave, spot area of 3.14 cm^2, spot diameter of 2 cm. Following the protocol of Bjordal and Lopes-Martins (2013), the applications were performed daily for 5 days followed by 2 days of no treatment and then daily for 5 additional days [26]. All treatments were conducted by the same individual. Scan modality was based on the size and shape of the treatment area (Fig. 1). The equipment was calibrated before the start of every session using the Powermeter Ophir Nova II Display S/N 573995. In group 1, the left hind DDFT received MLS® laser treatment at a dose of 5 J/cm^2. In group 2, the left hind DDFT received MLS® laser treatment at a dose of 2.5 J/cm^2. The right hind DDFT was considered an internal control (without treatment) for both groups.

Fig. 1 MLS® Laser Therapy

Sheep in both groups were monitored daily by evaluating the circumference, swelling and heat of the limb at the point of injury. Pain on limb palpation and degree of lameness were also assessed using a previously developed scoring system ranging from a grade of 0 to 4 [27]. Tendon thickness and echogenicity of the wound area were evaluated ultrasonographically [17,28]. Ultrasound examinations, using a GE Medical System LOGIQ P5 machine and linear 6-10 MHz probe, were performed 7, 21 and 37 days after lesion creation.

At day 37 after tendon lesion creation (30 days after the first laser treatment), the animals were sedated and anesthetized as previously described. The animals were subsequently euthanized using an intravenous injection into the auricular vein of 10 ml of a combination of drugs approved for euthanasia (Tanax®, Intervet, Milan, Italy). After euthanasia, the tendons were surgically removed from the calcaneus to the end of the metatarsal region and the DDFT of both hind limbs harvested for histological analysis. Tendons were removed 5 cm proximally to 5 cm distally of the lesion previously marked by a cutaneous surgical stitch. Harvested DDFTs were cut into 1 cm pieces and the proximal–distal orientations were marked. Tissue samples for histology were fixed in 4% parformaldehyde (PFA) and embedded in paraffin. Sections were cut into 5 µm slices, mounted on microscope slides and stained using Harris hematoxylin and eosin (HE). Sections were analyzed for cell density, vascularization and tissue organization using specific markers to evaluate fibroblast and tissue/matrix organisation characteristics. A quantitative analysis was performed to compare differences in cell number between groups. Differences in vascularization were also evaluate by looking at the ratios of blood vessel areas. Three segments were processed from each tendon, with 5 slides taken from each segment and three microscopic fields examined per slide, resulting in a total of 540 fields evaluated.

Analyses were performed using STATISTICA 9 (StaSoft) software, and data were assessed for normality using a Shapiro–Wilk test. Differences among the experimental groups within each sampling were evaluated using a Kruskal–Wallis Test. In all analyses, a $p < 0.05$ value was considered significant.

RESULTS

After the collagenase 1A injection, an inflammatory reaction with a mild localized thickening of the DDFT was detected in all subjects. Lameness, ranging from grade 3 to 4, and pain (detected by palpation) remained evident for the first 3-5 days. A localized

increase in temperature around the point of injury was detected manually for the first 3 days. From day 7 of the MLS® Laser Therapy, an inflammatory reaction was observed in the treated limbs of group 1, with about 1 centimeter increase in wound circumference and an increase in the temperature of the metatarsus (Fig.2); whereas no worsening of lameness or pain was observed. This inflammatory response was not observed in the treated limbs of group 2.

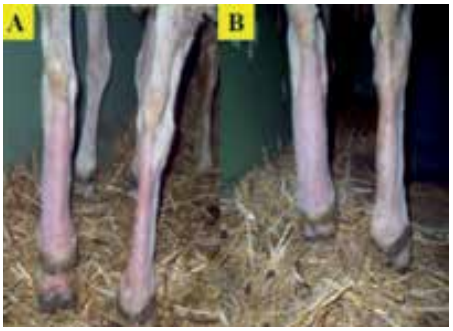

Fig. 2 Inflammatory reaction observed in the treated limbs of group 1 during MLS® Laser Therapy in left limb: A after collagenase induction, B 7 days after MLS® Laser Therapy

A progressive reduction in limb circumference was observed by the end of treatment for both groups. Limb circumference returned to a value close to the starting size only in group 2. In addition, local temperature, lameness and pain decreased in all subjects. The treated left DDFT showed a more rapid reduction in local inflammation compared to the right DDFT in all sheep. Ultrasound examinations detected the presence of a lesion in the DDFT 7 days after lesion creation and during the entire follow-up period. Better collagen-fiber alignment and more uniform filling of the lesions were observed in the treated limbs compared to the control limbs. During the follow-up period, group 1's treated limbs showed a marked thickening of the DDFT compared to the control limbs. Histological analysis revealed that there was disorganization of the extracellular matrix, increased vascularization and increased cell density in the DDFTs of the control limbs. In contrast, the sections obtained from tendons treated with MLS® Laser Therapy showed a more uniform and organized extracellular matrix, a lower number of cells and a better realignment of collagen fibers. The quantitative analysis revealed a significant decrease in fibroblasts in the treated legs compared to the control legs in group 1 (5 J/cm²). However, the ratio of the vessel areas did not differ between the control and treated tendons. In group 2 (2.5 J/cm²), the treated legs also had a decrease in fibroblast number compared to the control legs. A significant decrease in vascularity was observed in the treated tendons compared to the control tendons.

DISCUSSION

This is the first experimental study to evaluate the effect of LLLT on the tendon healing process in a sheep model. We evaluated the effects of two different doses of MLS® Laser Therapy in the acute phase of induced tendon lesions in order to determine a suitable therapeutic range for physiotherapy in human and veterinary medicine. The latest reviews on the effectiveness of LLLT in human medicine[5,6], highlighted the need to identify a specific treatment protocol for tendinopathy. Several authors [13,14,15,16,23,29,30,31] have reported the efficacy of LLLT in increasing the calcaneal tendon's mechanical properties as well as increasing the alignment of collagen fibers and angiogenesis, with doses between 3 and 5 J/cm². In the present animal model study, we initially decided to use 5 J/cm², which is the average value reported in the literature for treatment of acute tendinitis in human medicine. A 50% reduction in radiant fluence was elected for the second group (2.5 J/cm²) because the clinical symtomps and ultrasonographical data demostrated an increase of inflammatory response in group 1 during and after MLS® treatment. The tendon circumference in the first treatment group did not return to normal after a month of follow-up. In contrast, in the second group, the tendons' external morphology returned to the physiological state by the end of the trial. In particular, the clinical manifestations of two sheep worsened slightly in the initial treatment phase, with an increase in circumference at the injection site, a local rise in temperature and a slow remission of symptoms. The aggravation of the inflammatory condition, observed during the applications of MLS® Laser Therapy in group 1, appears to indicate that a dose of 5 J/cm² was excessive for treatment of acute tendinitis with this type of laser emission. Instead, the dose of 2.5 J/cm² (group 2) appears to be suitable to produce an anti-inflammatory and biostimulating effect on tendon healing. Results from histological examinations indicated that both treatments induced a statistically significant decrease in cell number, although the values only returned to normal in the second group. Moreover, the MLS® dose of 2.5 J/cm² (group 2) caused a significant decrease in blood vessel area and better improvement of collagen fiber organization in deep digital flexor tendon compared to group 1 and the control group.

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AUTHOR DISCLOSURE STATEMENT

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