High Intensity Laser Therapy during chronic degenerative tenosynovitis experimentally induced in chicken broiler

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ABSTRACT

The aims of this study was the safety and the efficacy of *High Intensity Laser Therapy* (HILT) on chronic degenerative tenosynovitis.
We have effectuated the histological evaluation and seroassay (C reactive protein) on 18 chickens affected by chronic degenerative tenosynovitis experimentally induced.
We have been employed a Nd:YAG laser pulsed wave; all irradiated subjects received the same total energy (270 Joule) with a fluence of 7,7 J/cm^2 and intensity of 10,7 W/cm^2.
The histological findings revealed a distinct reduction of the mineralization of the "choral" matrix, the anti-inflammatory effect of the laser, the hyperplasia of the synoviocytes and ectasia of the lymphatic vessels.

Keywords: Nd:YAG laser, tenosynovitis, hyperplasia synovicytes.

1 INTRODUCTION

The treatment of the teno-desmic pathologies in veterinary medicine, is a topic that needs to be extended and studied in order to reduce the long recovery periods of the horse and the high percentages of relapses. Virginia B. Reef[1] shows that the prognostic success in tenitis, tenosynovitis, desmities and desmo-synovitis in the horse is between 20 - 90% of the cases and the time needed for the clinical recovery is between 8 - 12 months. This broad range of data changes according to the gravity of the lesion; in the racehorse, the Author[1] specifies that, the higher the stress, the lower the probability of success of tendon therapy. It is easy to understand the importance of research to better understand the complex physio-pathological mechanism of tendon repair in the hope of reducing both the prognostic range and the percentage of relapse (10 – 80%).
In literature we have found papers that describe the employment of the laser in order to stimulate the tendon repair[2] and the production of collagene tissue[3]. We have found a plethora of reports, some negative[4,5,6,7,8,9,10,11,12,13] and some positive[14,15,16,17,18,19,20,21,22,23,24] in the clinical treatment by *Low Level Laser Therapy* (LLLT). In particularly one paper[25] describes no clinically effect of LLLT on wound healing in horses. It was therefore investigated the *High Intensity Laser Therapy* (HILT) on experimentally induced chronic degenerative tenosynovitis in an animal model, since this method has been

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confirmed in sports traumatology[26,27,28,29] .
Most of the studies of laser therapy conducted over the past thirty years have been carried out using equipment of low or medium power (*Low Level Laser Therapy*: LLLT), with wavelengths in the red and near infrared (600 - 900 nm). In this spectrum, the laser beam is partially absorbed by natural chromophores, such as melanin, which retain part of the radiated energy. On the contrary, our study is based on the use of the Nd:YAG, a maximum laser in power therapy (HILT), which is characterized by wavelengths (1064 nm) that permit it to penetrate the tissue and diffuse through it with greater facility[30], as it does not have an endogenous chromophore. Moreover, with the Nd:YAG pulsed wave laser, power peaks up to 1000 Watts can be delivered for times of 200μ seconds: very high peak intensities (W/cm^2) in very short times. Such high intensities in very short times prevent thermal accumulation on the part of the tissue, as instead occurs with the use of the Nd:YAG with a continuous impulse[28,29] . All this is expressed in a greater diffusive capacity of the laser beam through the tissues, with a very low risk of histological damage.
In addition, in HILT, quantities of energy (Joule) and fluence (J/cm^2) are delivered that are not so very different from those delivered in LLLT, but with an intensity (power density: W/cm^2) that is even up to 1000 times greater.
Turner at al.[31] says that: the acid solubility of the collagen of cockerel tendon demostrates a similar degree of intermolecular acid-labile cross-linkage to that found in adult human tendon, implying similar maturity of the collagen. By contrast, rabbit tendon collagen demostrates a dissimilar degree of cross-linkage suggesting immaturity of the collagen. Since this cross-linkage is likely to be related to mechanical properties, the cockerel may well be a better animal than the rabbit for the study of tendon healing[31] . Therefore we have chosen the chicken as the animal model for our tendon study in order to literature[31,32] .
The objective of this study was to evaluate the safety of the power laser with regard to surface and deep structures.

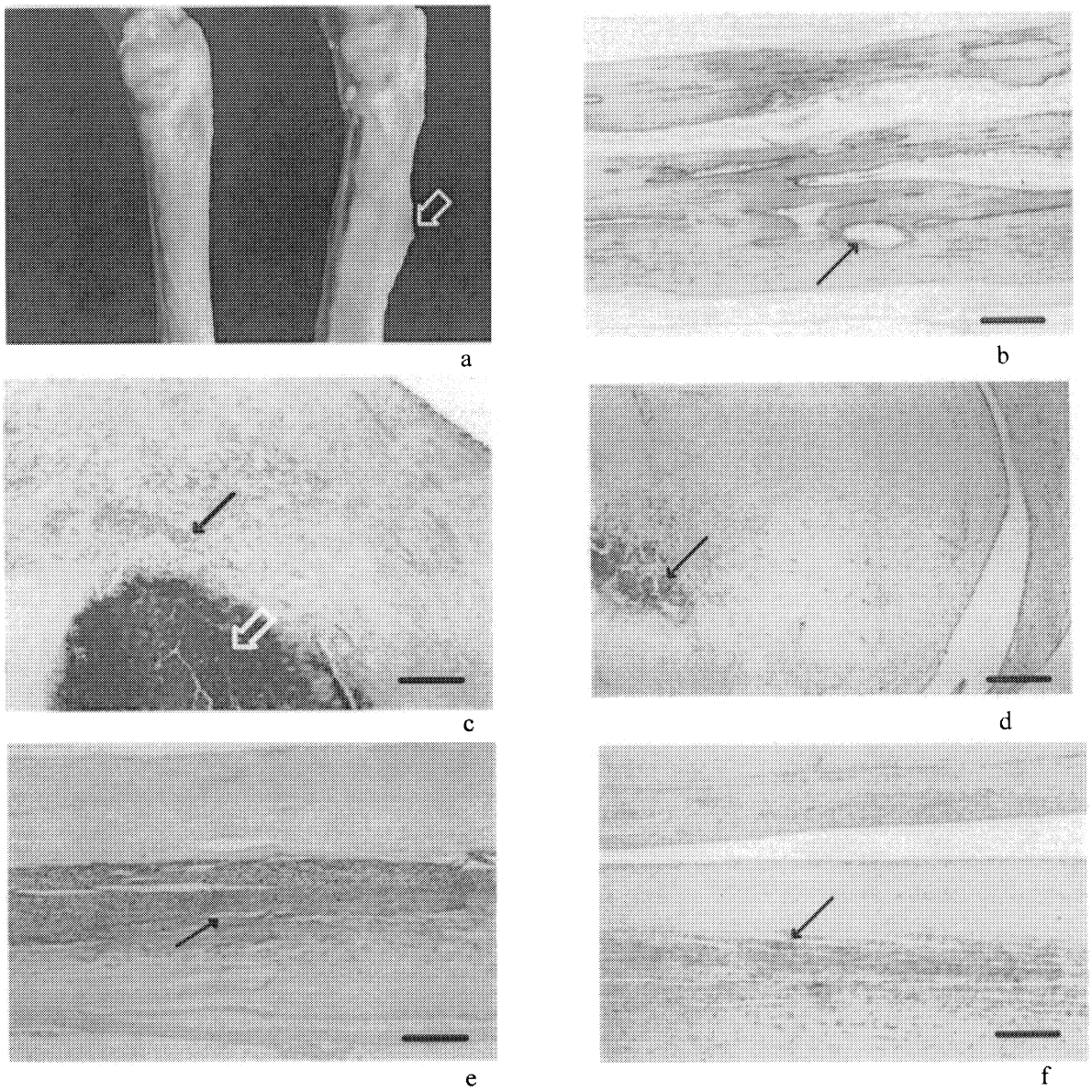
2. MATERIALS AND METHODS

2.1 Laser device

An Nd:YAG (Smart Beam by DEKA Mela - El.En. Group, Calenzano, Florence-Italy) mean power of 6 Watt, with a wavelength of 1064 nm, spot size of 0,12 cm^2 and pulse duration of 200 μSec was used.
All subjects of the Treated group has been irradiated with the same parameters: 270 Joules, fluence of 7,7 J/cm^2 and intensity of 10,7 W/cm^2.
All subjects had 6 treatment cycles a week for 2 weeks, altogether 12 cycles, according to Tam[14] and Lubich[27] protocols.

2.2 Experimental procedure

The study was carried out on 18 xenobiotic heavyline female broiler chickens, raised in free range conditions. When they were purchased, the subjects were 6 weeks old and had a homogeneous body weight (mean weight: 200 g).
At the age of 10 weeks, the subjects were infiltrated in the tendon sheath, right limb, (Fig. 1/a) with 0.5 ml of Freund's complete adjuvant[37] .
In the same tendon a second infiltration was administered at the age of 14 weeks, using a 1:1 mixture of Freund's complete adjuvant +10% sterile buffered formalin.
The induced phlogistic process was first monitored on the 16th week. After euthanasia, 4 subjects were submitted to necroscopy: macroscopic and histological surveys were carried out on the tendons involved and the healthy contra-lateral ones.
On the 17th week, the 14 subjects were subdivided into two groups.
Treatment began on the 18th week and ended at the end of the 19th week of life.



Description of Figure 1:

Composite picture with photograph and photomicrograph of Control and Treated groups all sampled at the 19th week.

a) Photograph of Control group. Right limb: site of infiltration (open arrow) with severe modification of tendon profile. Left limb: physiological tendon profile.
b) Photomicrograph of Control group. Sagittal section: wide bone-methaplasia with reconstitution of Haversian's system (arrow); Haematoxilin – Eosine (H. & E.), scale bar 100 micron.
c) Photomicrograph of Control group. Coronal section: wide mineralization coral zone (open arrow); presence of phlogistic infiltrate (arrow). H. & E. scale bar 100 micron.
d) Photomicrograph of Treated group. Coronal section: minimal mineralization coral zone (arrow); hyperplasia of synovial membrane with synoviocytes on more layers. Absence of phlogistic infiltrate. H. & E. scale bar 100 micron.
e) Photomicrograph of Control group. Sagittal section: mineralization coral zone (arrow); H. & E. scale bar 100 micron.
f) Photomicrograph of Treated group. Sagittal section: minimal mineralization coral zone (arrow); hyperplasia of synovial membrane with synoviocytes on more layers. Absence of phlogistic infiltrate. H. & E. scale bar 100 micron.

2.3 Macroscopic and histological examination

Morphological tests were carried out after necroscopy.
The flexor tendons of phalanges of each animal were examined externally for morphological and functional investigation. Then they were opened for a morphological evaluation of the tendon lesion and also the characteristics of the synovial fluid.
Bioptic samples were taken, then fixed in 10% buffered formalin, embedded in paraffin, and cut into 3μ-thick sections for histopathological studies.
In particular two samples were harvested: one of the coronal plane and the other on the sagittal plane in order to have a precise idea of the size of the lesion and its spatial location.
The histological sections were stained with *Haematoxylin - Eosine* (H. & E).
Histomorphological tests were carried out using a light microscope (*Leika*), to analyze the homologous fields for performing a cell count at 400x.

3. RESULTS

3.1 Autoptic examination 16 th week

The four Control showed, externally and internally, severe tenitis, chronic tenosynovitis characterized by severe thickness of the tendon sheath, increase of synovial fluid and adherence between the two tendon flexors of the phalanges.

3.2 External morphological examination at 19 th week

In the 19 th week of life, we noted macroscopically that there were considerable differences between the Control group and the Treated group.
The external morphological examination of the tendons showed, in the Control, a marked swelling with modification of profile. In the Treated group the tendon region recovered its physiological profile.
In the Control group the tendon sliding test showed a block, even total, of the functionality because of the severe adherence between the visceral surfaces of the flexor tendons. This was not the case in the Treated subjects.
In the Treated group the constant presence of dense synovial fluid of a light yellow color, although not always in a large quantity, was observed.

3.3 Histological Examination

In the *Haematoxylin - Eosine* (H. & E.) stained samples the following morphological variations within the groups were possible to observe.

3.3.1 Control group

In coronal and sagittal sections we observed the presence of abundant phlogistic chronic infiltrate, while in the coral tendons we found severe mineralization and calcification zone (Fig. 1/c, 1/e); in one case we observed a wide bone-metaplasia with reconstitution of Haversian's system (Fig. 1/b). In the synovial membrane we noted the synoviocytes arranged in monolayers (Fig. 1/e).

3.3.2 Treated group

We observed intense hyperplasia of synovial membrane, the presence of synoviocytes in active synthesis characterized by their round shape and arranged in more layers. On the tendons the phlogistic infiltrate in each preparation and the mineralized coral zones were minimal (Fig. 1/d, 1/f). Zones calcified or with bone-metaplasia were not observed.
Numerous ectasic lymphatic vessels were identified.

4. DISCUSSION

The histological images revealed a clear distinction between the Treated group and the Control. In the Treated group we did not find any phlogistic infiltration as in the Control group. In the Control we observed the evolution of the degenerative chronic phlogosis with the phlogistic infiltration and foci of mineralization of the core; in one case the degenerative process even reached bone metaplasia (Fig. 1/b). The anti-inflammatory effect of the laser opposes the degeneration process by the chronic phlogosis, and this fact probably leads to the reduction of mineralization of the matrix (fig. 1/d, 1/f) and oppose bone metaplasia. In fact, in all subjects of Treated group bone metaplasia was never observed.
There was a higher quantity of synovial fluid in the subjects treated with the laser compared to the Control. In particular especially in the histologic images of the Treated group we observed hyperplasia of the synovial membrane and absence of the inflammatory infiltration.
It is interesting to notice that the laser stimulates the proliferation of synoviocytes and promotes indirectly the synthesis of synovial fluid in spite of the severe degenerative process in act. It is particularly interesting because the synovial fluid is fundamental to ensure the correct trophism and the functionality of the tenodesmic system.
Minimal variation in the physical-chemical characteristics of the synovial fluid may induce suffering and stress to the tendons and delay the any repair process.
Another interesting element that came from this research concerned the ectasia of the lymphatic vessels noticed in the Treated group. It is known that this laser, at precise frequencies, has an antiedemic effect in clinical practice.
Lastly we can say that chickens represent a valid animal model for the study of the chronic degenerative tenopathies. Compared to the rat or the rabbit this species appears to be easily manageable. The fact that the tendons of the heavyline broiler chickens are so big, allows easy intravaginal inoculations. The flexor superficial and deep tendons of the phalanges of the chicken are in the metatarsic palmar region following the same pattern of the horse.
The basal metabolism of the chickens, and particularly of the broiler chicken in growth if properly fed (force feeding), allows a valid model of degenerative chronic tenitis to be obtained in a short time,.

5. CONCLUSIONS

It would appear that the power laser (HILT) is able to exert an anti-inflammatory effect and in the course of degenerative chronic tenitis it can reduce or fully arrest the evolution of any tissue mineralization.
It also seems that the laser is capable of stimulating synovial hyperplasia with consequent increasing of the synthesis of synovial fluid. The last but not least consideration refers to the absolute safety of HILT regarding the delicate tenovaginalis structures.

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