Nd:YAG laser in experimentally induced chronic degenerative osteoarthritis in chicken broiler, pilot study.

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ABSTRACT

The *Low Level Laser Therapy* (LLLT) has been widely tested in arthritis disorders, but there is still some disagreement in the results, therefore in this study we have investigated *High Intensity Laser Therapy* (HILT).
The degenerative arthritis was induced in 18 chickens by intra-articular inoculation of Freund's complete adjuvant.
Clinical studies were carried out (weight increase and grades of lameness), as well as morphological (macroscopic and histological) tests and seroassay (C Reactive Protein).
The Nd:YAG pulsed wave was employed.
The serologic data revealed the anti-inflammatory effect of the laser, with a highly significant difference between those treated and the control group. No lesion on the skin, i.e. burn, or in depth has been observed in the Treated group.
Heavyline of broiler chickens in growing age has been revealed a good animal model of O.A.. The Nd:YAG Pulsed Wave it is safe on these structures. The anti-inflammatory effect of the HILT it seems to contrast the destructive degenerative process.

Keywords: High Intensity Laser Therapy, degenerative arthritis, neochondrogenesis, animal model

1. INTRODUCTION

The literature offers a plethora of reports, some negative[3,4,5,6,7,8,9,10,11,12] and some positive[13,14,15,16,17,18,19,20,21,22,23] in the treatment of arthritis and rheumatic disorders by *Low Level Laser Therapy* (LLLT). In this study, we have investigated the possibility of using *High Intensity Laser Therapy* (HILT) on an animal model of osteoarthritis, since this method has been introduced into sports traumatology[24,25,26,27].
Most studies of laser therapy conducted over the past thirty years have been carried out using equipment of low or medium power (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 powerful laser (HILT), which is characterized by wavelengths (1064 nm) that permit it to penetrate the tissue and diffuse through it with greater facility, as it does not have an endogenous chromophore. Moreover, with the pulsed Nd:YAG laser, power peaks up to 1000 Watts can be delivered for 200μ seconds: very high peak intensities (W/cm^2) in very short times. Such high intensities in very short times prevent thermal accumulation by the tissue, as occurs with the use of the continuous wave Nd:YAG[26,27] . All this enables effective diffusion of the laser beam through the tissues, with a very low risk of histological damage.
In addition, the amount of energy (Joule) delivered by HILT are similar to those delivered by LLLT, but with an intensity (power density: W/cm^2) that is even up to 1000 times greater.

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The primary objective of this study was to evaluate the cicken animal model of OA[1,2] and the safety of the power laser with regards to surface and deep structures.
The secondary objective was to verify its clinical efficacy in line with what has already been reported in the literature relative to the biological effects observed *in vitro* and *in vivo*. In particular, we evaluated the laser's analgetic[29] and anti-inflammatory[30] effects.

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 treated subjects, had the similar area (35 cm^2), irradiated with the same quantity of energy (270 Joules) and intensity (10.5 W/ cm^2). All subjects had 6 treatment cycles a week for 2 weeks, altogether 12 cycles, according to Tam[11] and Lubich[23] protocols.

2.2 Experimental procedure

The study was carried out on 18 xenobiotic heavyline of 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 tibiotarsal-metatarsal joint (right limb) with 0.5 ml of Freund's complete adjuvant[31].
In the same joint a second administration was made 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 of both the joint involved and the healthy contra-lateral joint were carried out.
On the 17th week, the 14 subjects were subdivided into two groups: 7 Control and 7 Treated.
Treatment began on the 18th week and it is ended at the end of the 19th week of life.

2.3 Test examination

At the end of the 19th week of life the following tests were carried out on all subjects:
- a clinical evaluation of lameness;
- a blood sample for the assay of C reactive protein (CRP);
- measurement of weight after suppression.

The lameness was evaluated by well-known clinical parameters, with 4 grades:
- grade zero: the subject has no walking problems;
- grade one: evident lameness;
- grade two: severe lameness;
- grade three: the does not walk and keeps the affected limb raised.

Table 2 shows the averages of the parameters (trend of CRP, lameness grade, bodyweight increase) for each group.

2.4 Statistical Analysis

Data are presented as mean ± SEM (Table 1). Statistical analysis was performed by student's paired *t*-test (Table 2). Differences were considered statistically significant at $P<0.05$.

2.5 Macroscopical and histological examination

Morphological tests were carried out after necroscopy.
The tibio-tarsal joints of each animal were examined externally for morphological and functional investigation (articular excursion). Then they were opened for a morphological evaluation of the articular 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.
Prior to dissection of the tibial diaphysis in its third distal portion and of the tarsal diaphysis in its third proximal portion, after being fixed, the joints were placed in a decalcifying mixture of chloridric acid and sodium hydroxide for four days. After this, they were processed like the other tissues. The histological sections were stained with haematoxylin-eosin (E. & E.). Histomorphological tests were carried out using a light microscope (*Leika*), to analyse the homologous fields for performing a cell count at 400x.

3. RESULTS

3.1 Autoptic examination 16th week

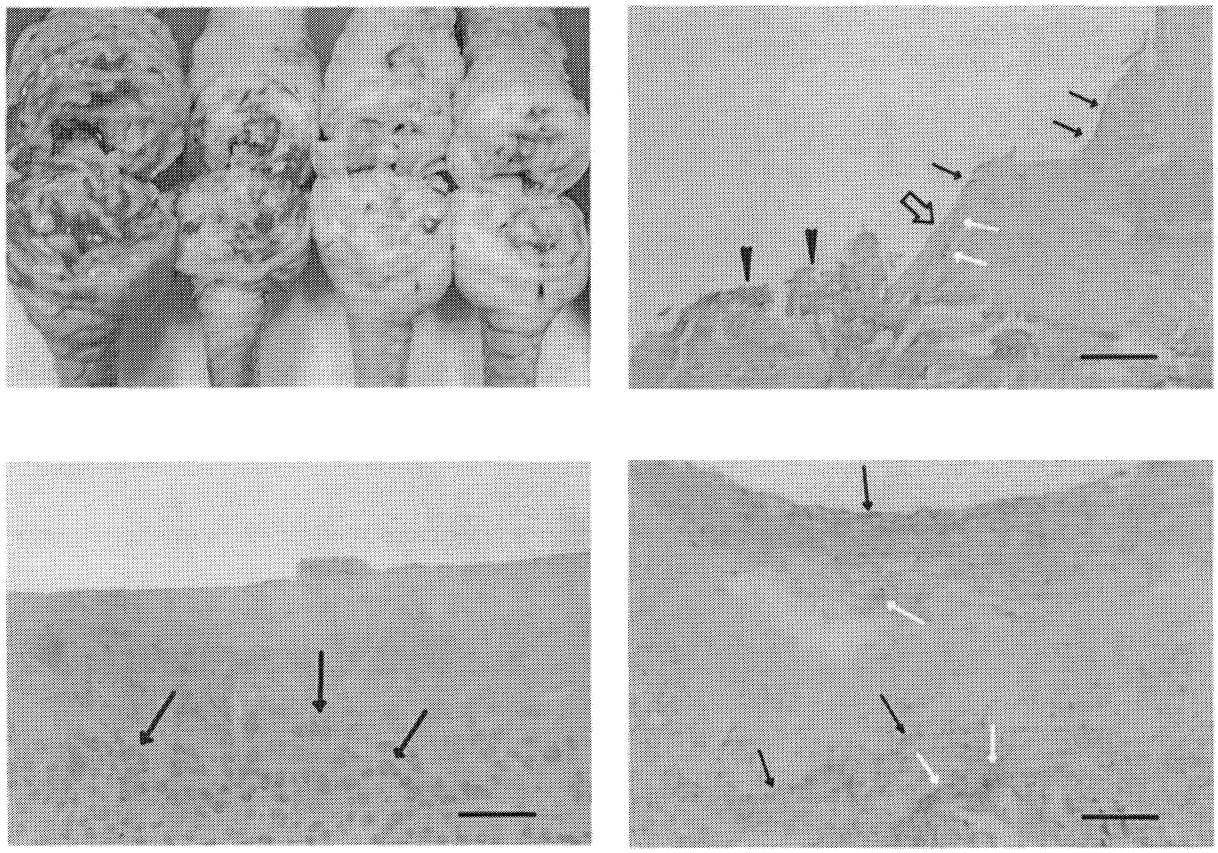
The four Control showed, externally and internally, severe arthritis, comparable to at least a 4th grade of *Ahlback* (32), characterized by fissuration-detachment of the articular cartilage, eburnation of the underlying bone, newly-formed cartilaginous processes protruding into the articular cavity, chronic synovitis (Fig. 1/A). This process determined an angular rotation of the limb.

3.2 External morphological examination and articular functionality at 19th week

In the 19th 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 joints showed, in the Control, a marked axial and angular deviation, with a spherical modification in the joint - analogously in the coronal and sagittal diameters.
The test of articular functionality showed a severe ankylosing arthropathy for the Control and, in some cases, a complete ankylosis that never it has not been observed in the Treated group. In the Control group no synovial fluid was found - except in very reduced quantities. On the contrary in the Treated group the constant presence of dense synovial fluid of a light yellow colour - although not always in a large quantity - was observed.
Morphological macroscopic alterations at at 19th week

3.2.1 Control group

Complete modification of the articular structure, due to the presence of central osteophytic proliferations, ankylosing, haemarthrosis (Fig. 1/B), the absence of synovial fluid, ulcerative-eburnean areas on the articular condyles (Fig. 1/B), and marked thickening of the articular capsule, which had acquired a fibrous consistency (Fig. 1/B).



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 and Treated groups.
From left: two Control's group subjects. Ulcerative-eburnean areas on the articular condyles, absence of synovial fluid, presence of haemarthrosis, marked thickening of the articular capsule.

The other two subjects are Treated group. The articular surface appeared to be preserved, traces of blood in the joint, modeste increase of synovial fluid.

b) Photomicrograph of Control group. Presence of abundant phlogistic infiltrate (head arrow), with polymorphonucleates (arrow) and giant "Muller" type cells (white arrow). Presence of the intra-articular synovial "pannus" (open arrow). Haematoxilin – Eosine (H. & E.), scale bar 100 micron.

c) Photomicrograph of Treated group. No phlogistic infiltrate and the lack of giant cells, presence of a low regenerative-hyperplasia activity of the chondroblasts (arrow). H. & E. scale bar 100 micron.

d) Photomicrograph of Control group. Ulcerative areas with the presence of phlogistic infiltrate (arrow) and giant "Muller" type cells (white arrow). H. & E. scale bar 100 micron.

3.2.2 Treated group

A general aspect of circumscription of the chronic osteoarthritic process was observed, that could be attributed to the regenerative cartilaginous phenomenon of the ulcerative-eburnean areas present on the articular condyles. The articular surface appeared to be preserved, and no osteophytic proliferations were observed. There was traces of blood in the joint. A modest increase in the synovial fluid, and a thickened and fibrous articular capsule, were found. (Fig. 1/B).

3.3. Histological examination

3.3.1 Control group

The articular appearance was distinguished by the presence of chronic-active phlogosis, characterized by the peri- and intra-articular infiltrate of mononucleates (lymphocytes and macrophages) and polymorphonucleates (essentially heterophiles), localized in large quantities over the contact areas of the intra-articular synovial "pannus" and in the surrounding ulcerative areas of joint cartilage (Fig. 2/A). In particular, in the necrotic-ulcerated cartilaginous areas giant "*Muller*" type cells in the infiltrate (Fig. 2/B), as well as the presence of a moderate osteoclastic activity at the level of the subchondral bone were seen. Cartilaginous fragments of cartilage were observed in the articular lumen (riziform bodies). The 400x cell count and the search for mitoses did not indicate chondroblastic regeneration in the peri-ulcerated areas.

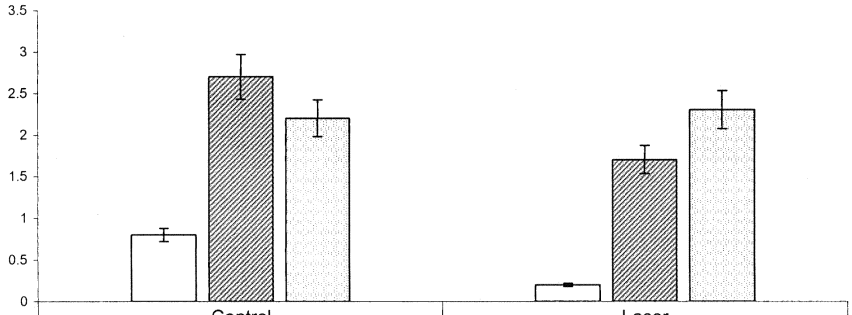
3.3.2 Treated group

The articular appearance was characterized by the absence of intraluminal free necrotic fragments, clear-cut margins of the ulcerated areas. No phlogistic infiltrate and the lack of giant cells was observed (Fig. 2/D). The phlogistic infiltrate in each preparation was minimal or lacking; nor was any osteoclastic activity observed at the epiphyseal and metaphyseal levels. We have observed a low regenerative-hyperplasia activity of the chondroblasts (Fig. 2/D). This activity was evaluated by means of a 400x cell count and a count of the number of mitoses per microscopic field. The finding of intense hyperplasia in the synovia remained constant, with synoviocytes with an intumescent appearance arranged in several rows, seemingly in active synthesis.

3.4 Other examination

As far as the weight surveys are concerned, the graph below (Table 1) shows the distribution of the averages of the chickens sub-divided by group.

Table 1: Average of parameters: CRP, Lameness and Weight Increase of Body



	Control	Laser
☐ Trend of CRP	0.8	0.2
▨ Lameness Degree	2.7	1.7
☐ Weight of Body	2.2	2.3

Table 2: means and statistical analysis						
	CRP		Lameness Degree		Weight of Body	
Groups	Control	Laser	Control	Laser	Control	Laser
	0,82	0,11	3	1	2	2
	0,9	0,13	3	2	2,5	2,65
	0,7	0,06	3	2	2,6	2,2
	0,82	0,13	2	2	2,2	2,5
	0,99	0,78	3	2	1,8	2,5
	0,68	0,16	3	2	2,1	2,1
	0,97	0,21	2	1	2,2	2,3
Mean	0,8	0,2	2,7	1,7	2,2	2,3
p<0.05	0,000118899		0,00118899		0,198011473	

4. DISCUSSION

In the light of the results, we consider that heavyweight chickens, while growing, represent a valid animal model for the study of inflammatory-type degenerative arthropathies. In our opinion, for this type of investigation, the heavyweight chicken is preferable to other animal species because it has a bipedal gait that exposes the joints to greater stresses with respect to quadrupeds. It has large joints that are easy to study; it is extremely docile and manageable, and has a high pain threshold. Furthermore, since this chicken's basal metabolism is high, particularly while during growth, we were able to obtain a chronic active process with degenerative evolution in a relatively short time (6 months).

The most significant fact that emerged from this study is the ability of the power laser (HILT) to antagonize the degenerative process and to give a weak neochondrogenic response.
From the histological examination a total absence of inflammatory infiltrate in the laser-treated subjects was found. This correlates with the trend of the C reactive protein (CRP). From the graph in table 2, it appears evident that the Control group has mean CRP values that were 4 times greater than Treated group.

The statistical analysis of the variation in the CRP indicates that there was a highly significant difference ($p<0.0001$) between the Treated group and the Control group. A separate consideration must be made regarding the lameness trend. For the Nd:YAG, it has been reported[29] the ability of this laser of to give a good analgetic response. In this case it is our opinion that the grade of lameness is correlated to the joint functionality, rather than to the direct analgetic effect or antinflammatory. In fact, we observed macroscopically a distinct decrease in the osteophytic proliferations, and a reduction in the grade of ankylosis, with the partial conservation of the articular planes or surfaces and a reinstatement of synovial fluid in the treated subjects with respect to Control and then a better clinical condition.

It seems probable therefore that, in the Treated group the treatment blocked the catabolic condition and favored the anabolic one. In untreated animal this effect may be lowered by reduction of serological levels of of GH and of IGF-1 as reported by Calderon et al.[33] in different animal models, during the degenerative process in arthritis exerimentally induced with the Freund adjuvant.

5. CONCLUSION

High Intensity Laser Therapy in the doses that we used has been shown to be safe in the treatment of articular pathologies, and not to induce lesions to surface and deep structures.
Our study indicates that the laser is capable of antagonising experimentally-induced arthritis having antiinflammatory effect.

We have also observed a low neochondrogenic effect and low synovial hyperplasia. Moreover from this study it is emerged that the chicken it is a good animal of osteoarthritis.

As this is a pilot study, we consider that further detailed study is essential. In particular, we confirm that it would be useful to investigate the nature of the newly-formed cartilage (hyaline cartilage or fibrocartilage), and to identify the correct irradiation intensities of the Nd:YAG power laser: activation threshold, efficacious dose and, possibly, toxic dose. Moreover, we believe it would be appropriate to verify the experiment on spontaneous pathologies of osteoarthritis and on other animal species.

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