High Intensity Laser Therapy in arthrosis: experimental investigations on animal models

Introduction

Arthrosis is a disease with a high social impact as it affects 30-35% of the population. An arthritic patient costs the public health services approximately 4,000 euro on average a year, touching peaks of almost double this amount in the most severe cases.

Conventional therapy foresees the administration of anti-inflammatory, antalgics, and decontraction agents. The current trend is to use chondroprotective drugs with often encouraging results.

The international bibliography provides results that are often contrasting with regard to the clinical effectiveness of the Low Level Laser Therapy (LLLT) in the treatment of arthritic and rheumatic complaints, and some even express negative opinions, while others are positive. Over the last few years the High Intensity Laser Therapy (HILT) has been making its mark with excellent results in sports traumatology and pain therapy, for this reason we decided to assess the possibility of also transferring this method to the cure of arthritic ailments and therefore prepared an animal model with an arthritic pathology in line with the indications of the various Authors.

The majority of studies conducted over the last thirty years in Laser therapy have been carried out with medium and low intensity Laser devices (Low Level Laser Therapy: LLLT), with wavelengths in the infrared and near infrared (600 - 900 nm). Within this spectrum the Laser beam is partially absorbed by the natural chromophores, like melanin, which withhold part of the energy irradiated.

Our study on the other hand is based on the use of a Nd:YAG High In-
tensity Therapy (HILT) Laser, characterised by a wavelength (1064 nm) that allows it to penetrate and spread more easily through the tissue due to not having an endogenous chromophore. Moreover, with the pulsed wave Nd:YAG it is possible to deliver power peaks of up to 1000 Watt for times of 200µ seconds: extremely elevated peak intensity (W/cm²) in very brief times. Such a high intensity in such a short time prevents the heat accumulation by the tissues as happens with the use of Nd:YAG with constant emission (Parra 29, 30). This all extrinsicates in a greater spreading capacity of the Laser beam through the tissues with a very low histolesive risk.

In other words, quantities of energy (Joules) and fluence (J/cm²) are delivered in the HILT that are not dissimilar to the ones delivered with the LLLT but there is an intensity (power density: W/cm²) of even up to 1000 times higher.

The objective of this study was to assess the safety of intensity Laser at various power intensities (10, 30, 50 and 80 W/cm²) used on the superficial and deep structures.

The secondary objective was that of verifying the biological effects in vivo of the three different types of Laser: CO₂, Diode, Nd:YAG; and more specifically, we assessed the antalgic 32, antinflammatory 33 and cytoproliferative 34 effects of the Laser.

**Materials and methods**

**Lasers used**

Three types of Laser were used: CO₂ (10.600 nm), Nd:YAG pulsed wave (1.064 nm), Diode (830 nm) produced by El.En. S.p.A. (Calenzano - Florence). Table 1 shows the powers used by the three lasers assessed.

**Investigation population**

According to Bentley 3 the ideal animal model for the study of arthrosis should have the following characteristics:

- the presence of precocious lesions and action mechanisms similar to

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Average intensity used (watts)</th>
<th>Spot area (cm²)</th>
<th>Power density (W/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser Nd:YAG</td>
<td>1.064</td>
<td>1.9</td>
<td>0.19</td>
</tr>
<tr>
<td>Laser Nd:YAG</td>
<td>1.064</td>
<td>5.7</td>
<td>0.19</td>
</tr>
<tr>
<td>Laser Nd:YAG</td>
<td>1.064</td>
<td>9.5</td>
<td>0.19</td>
</tr>
<tr>
<td>Laser Nd:YAG</td>
<td>1.064</td>
<td>10</td>
<td>0.125</td>
</tr>
<tr>
<td>Laser CO₂</td>
<td>10,600</td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>Laser DIODE</td>
<td>830</td>
<td>1</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 1. Types of lasers and dosimetric Parameters used.
those described for the human pathology;
- evidence of an initial loss of the cartilaginous matrix and subsequent appearance of fissures, fibrillations and erosions;
- the cartilaginous lesions must therefore be followed by sclerosis of the subchondrial bone;
- the alterations described must be readily reproducible and identifiable in the living animal;
- the induction method of the lesions must be valid for different animal species and articular sites and free from systemic effects.

In this study we chose chickens of the heavy breed, bred with the open range system to allow ample possibility for deambulation. This species was preferred over others as it has a bipedal gait similar to man, ample articulations capable of supported heavy loads and an elevated basal metabolism that allowed us to obtain chronic degenerative lesions in relatively brief times, a good-natured disposition making it easy to treat with Laser. Moreover, it expresses a range of cytokins and chemokins that can be compared to those of humans.

Table 2 illustrates the breakdown into groups of the population investigated.

Investigation protocol
The investigation was performed in compliance with the Helsinki Declaration and the International Standards governing research on animals. The chronic degenerative arthrosic phenomenon was induced via double inoculation in the lower right limb of each subject with Freund’s Complete Adjuvant (FCA) + formaldehyde at 10%.

The inoculations were administered at one-month intervals. Eight months after the second infiltration the Laser therapy was commenced. Following is a list of the activities in chronological order with the specific examinations performed;
A) acquisition of subjects;
B) one month’s growth;
C) 1st inoculation with FCA;

### Table 2. Breakdown of the subjects in the investigation groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>12</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
</tr>
<tr>
<td>Nd:YAG 10 W/cm²</td>
<td>12</td>
</tr>
<tr>
<td>Nd:YAG 30 W/cm²</td>
<td>12</td>
</tr>
<tr>
<td>Nd:YAG 50 W/cm²</td>
<td>12</td>
</tr>
<tr>
<td>Nd:YAG 80 W/cm²</td>
<td>12</td>
</tr>
<tr>
<td>DIODE</td>
<td>12</td>
</tr>
<tr>
<td>CO₂</td>
<td>12</td>
</tr>
</tbody>
</table>
D) one month's growth;  
E) 2\textsuperscript{nd} inoculation with FCA;  
F) growth after 8 months;  
G) beginning of Laser therapy:  
- T/0 => on all subjects: Rx, clinical evaluation of the lameness, weight, blood tests; bioptic analysis on 8 controls (after euthanasia with general anaesthetic).  
- T/1 => beginning of Laser therapy.  
- T/2 (3 weeks after T/1) => end of Laser treatment: in all 15 Laser sessions were performed spread over three weeks.  
- T/3 (2 weeks after T/2) => on all the remaining subjects: Rx, clinical evaluation of the lameness weight, blood tests; bioptic analysis (after euthanasia with general anaesthetic).  

Assessments carried out:  
- X-rays in both lat-lateral and antero-posterior of both limbs of each subject.  
- Serological analyses (ELISA) for: PCR, IL 1 beta, ILGF 1, TGF beta.  
- Macroscopic examination via photographic acquisition.  
- Microscopic examination: histological and immunohistochemical examination (IHC): histological staining with hematoxilin-eosin, Herovici polychrome solution and Alcian PAS blue.  
- In IHC we performed assessments for: Type II Collagen, ILGF 1, MMP1, TIMP2.  

The stainings with Herovici were performed in order to highlight the presence of protocollagen (pale blue) as a demonstration of the age of the cartilage: the protocollagen precedes the formation of collagen. The synthesis activity of the mucopolysaccharidic matrix was instead assessed via the Alcian PAS blue. The data collected were entered onto an electronic spreadsheet and analysed statistically with the t-Test.
Results

Anti-inflammatory effect
The graph in fig. 1 illustrates the mean of each subject treated with Laser with a comparison between the Controls and the Healthy subjects.

Neochondrogenic effect
The neochondrogenic effect was documented histologically and immunohistochemically (IHC). Figures 2 and 3 show the histological images referring respectively to a Control (fig. 2) and a subject treated with Nd:YAG at 50 W/cm² (fig. 3).

Fig. 2 shows the almost completely dextruded cartilage with partial covering of the subchondral bone tissue where in fact the haversian systems can be observed. In fig. 3 instead, there is neoformed cartilage structured according to the physiological architecture on the subchondral bone tissue; basal globiform isogen groups can be recognised which on moving towards the surface tend to arrange themselves parallel to the articular surfaces.

Discussion

From an analysis of the graph in fig. 1 it is apparent that all the types of Laser used have carried out an anti-inflammatory effect (see the curve of the IL 1beta).

As far as the historegenerative effect on the articular cartilage is concerned however, we observed a different effect between the different types of Laser. The CO₂ Laser offered less biostimulation. The diode Laser offered greater stimulation compared to the CO₂ but failed to induce the synthesis of very active isogen groups which were however very dishomogeneous in shape and distribution. Moreover, the immunohistochemical examination of the Type II collagen indicated that it was fibrocartilage.

It is a completely different situation with the Nd:YAG Laser which proved
Having tried various power intensities we were able to observe a linear trend between the therapeutic response and the dose supplied. In fact at 10 W/cm² we observed the presence of the activation threshold with the proliferation of the basal isogen groups, at 30 W/cm² homogeneity was observed in both shape and spatial distribution of the isogen groups, at 50 W/cm² we identified the most effective dose for stimulating the physiologically structured hyaline cartilage, while at 80 W/cm² we observed tissular regression above all on the surface, and the lack of chondrocyte action of the Type II collagen (table 3).

The curve of the ILGF-1, MMP1 and TIMP2 were particularly interesting in immunohistochemistry. As far as the IGF-1 is concerned, an expression was observed in the CO₂ which was comparable to that of the CTR group. The degree of expression of the growth factor with the Diode Laser was not dissimilar to that of the CO₂. With regard to the degree of expression in the subjects treated with the Nd:YAG Laser, this faithfully reflects the expression of the Type II collagen with a better expression than subjects treated with 50 W/cm².

The trend of the MMP1 and TIMP2 in immunohistochemistry was also very interesting. In this case a sharp difference was observed between the MMP1 and TIMP2 in the CTR, which was less marked with the CO₂, diode, and Nd:YAG at 30 W/cm², whereas it was highly significant with the Nd:YAG at 50 W/cm². Obviously this different expression of the MMP1 and the TIMP2 has opposed trend between the CTR and the Nd:YAG group at 50 W/cm². In fact, in the CTR we obtained a high value of MMP1 and a low value of TIMP2, while in the Nd:YAG at 50 W/cm² these were exactly the opposite.

**Conclusion**

From this study it has emerged, in primis, that the High Intensity Laser Therapy, when administered at suitable doses, is safe in the treatment of articular pathologies and does not induce lesions to the surface and
deep structures.
This study indicates that the Laser is capable of antagonising the experimentally induced arthrotic phenomenon to stimulate the neochondrogenic activity with the formation of hyaline cartilage and to induce sinovial hyperplasia.
These effects are closely linked to the dose supplied. More specifically, we varied the intensity (power density: W/cm$^2$) and maintained constant energy (Joules) and fluence (energy density: J/cm$^2$).
It was therefore observed that the low intensity only has an anti-inflammatory effect while the high intensities have a neochondrogenic and sinovial hyperplastic effect as well as the anti-inflammatory effect.
As this was a pilot study we believe that further investigation and confirmation are indispensable. We are also of the opinion that it would be important to perform verifications on spontaneous arthrotic pathologies in animals.

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