THE RELATIVE EFFECTS OF He-Ne LASER AND MELOXICAM ON EXPERIMENTALLY INDUCED INFLAMMATION

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Certain crystals cause synovial tissue inflammation and variability of inflammatory indicators like plasmatic prostaglandin E₂ (PGE₂), fibrinogen (PF) and synovial tissue PGE₂. We evaluated Helium-Neon Laser efficacy on PF, plasmatic PGE₂ and synovial PGE₂ and its ability to induce involution of anatomopathological (AP) lesions in induced granulomatose process, alone and combined with a meloxicam injection. Rats were assigned to five different treatment groups. 5 mW He-Ne Laser radiation (632.8 nm) and 8 J.cm² energy density were used in the treated groups. PF and plasmatic PGE₂ and synovial PGE₂ levels decreased in granulomatose arthritis groups treated with low level laser therapy (LLLT) or with a meloxicam injection or a combination of both therapies; the differences being statistically significant ($p < 0.01$), as compared with the non-treated control group. In the combination therapy group, involution of granulomatose inflammation was observed. We conclude that He-Ne laser treatment or meloxicam administration after experimentally induced inflammation, normalizes plasmatic PGE₂ and fibrinogen levels, but produces similar histological lesions as non-treated controls.

Key words: biostimulation, inflammation, laser therapy, meloxicam

Introduction

Certain crystals, like hydroxyapatite, were used as inflammatory irritants to examine the anti-inflammatory effect of Helium-Neon laser. Hydroxyapatite, a kind of calcium phosphate hydroxide, causes synovial tissue inflammation and produces local granuloma associated with an increase in synovial tissue prostaglandin E₂ (PGE₂)⁽¹⁾. Crystals, like hydroxyapatite, have positively charged hydrogen atoms on the surface, which can bind with complementary arrays of negatively charged protein. Plasmatic proteins, like fibrinogen, can increase or decrease the inflammation process⁽²⁾. It is also possible that irregular or sharp crystal surfaces may induce mechanical damage to cell membranes⁽³⁾. Anionic crystals, on the basis of their negative electron charge, have the ability to activate the kinin system resulting in

pain, vasodilatation and increased vascular permeability⁽⁴⁾. Interaction with synovial fibroblasts and macrophages may induce the release of different cytokines, such as IL₁ and IL₆, resulting in the formation and release of prostaglandins, acute phase proteins, prostacycline and superoxide radicals which can act on the neighboring cells and vessels, thus increasing the inflammatory process⁽⁵⁾. The amount of inflammatory mediators, such as plasma fibrinogen (PF) and prostaglandin E₂, can rise in acute synovitis, thus providing a valuable tool for evaluating the outcome of therapy⁽⁶⁾.

In previous works, we demonstrated that the increased levels of plasma fibrinogen, typical of tissue injury, returned to normal levels with He-Ne laser irradiation of the wounded area. Furthermore, He-Ne laser therapy inhibited the synthesis of

PGE₂ in rats with induced inflammatory processes^(7,8,9).

Meloxicam can be a selective Cox-2 inhibitor, with a potent anti-inflammatory effect⁽¹⁰⁾. Several studies have tested the efficacy of LLLT on acute inflammation, however there have been very few reports about the anti-inflammatory effect of laser in vivo⁽¹¹⁾. Photo-irradiation at low energy levels can generate significant bio-effects manifested by biochemical, physiological, and proliferation phenomena in various enzymes, cells, tissues, organs, and organisms^(11,12). The LLLT energy produces insignificant and minimal temperature changes⁽¹³⁾. Studies on the anti-inflammatory effect of LLLT are still at the point where more experimental cases are needed. Therefore, we evaluated the effects of meloxicam and laser therapy on plasma fibrinogen, plasma prostaglandin E₂, and synovial tissue prostaglandin E₂ in rats. Furthermore, we studied the ability of a meloxicam injection and laser therapy to induce involution of histopathology by provoked lesions.

Materials and Methods

Animals and preparation of experimental material
Rats were divided into 5 groups, each containing 12 animals.

1. Controls (normal)
2. induced arthritis
3. induced arthritis treated with meloxicam
4. induced arthritis treated with He-Ne laser
5. induced arthritis treated with He-Ne laser + meloxicam

The 60 female Suquia rats, weighing 280-320 gm, fed with balanced food containing 20% protein were given ether inhalation anesthesia on the 4th day after hydroxyapatite injection and were bled by decapitation. Blood was immediately transferred to centrifuge tubes containing an anticoagulant dry mixture of ammonium oxalate and potassium oxalate (2:1) and immediately centrifuged to 3000 rpm.

Posterior right foot joint of each rat was sectioned and arranged in a solution containing 75 mg Diclofenac diluted in 500 ml lactate Ringer solution, and the posterior left foot was fixed in buffered 10% formalin.

Induced inflammation

Inflammation was induced by injecting a 2 mg / 0.05 ml intraarticular hydroxyapatite physiological solution into each one of the two hind leg joints.

Treatment

Meloxicam, a NSAIDs, was given to rats by intramuscular injection of 0.065 mg / Kg /dose, immediately following hydroxyapatite injection, 24 h, and 48 h after inducing the inflammatory process. Helium Neon laser, wavelength 632.8 nm, was used at tunable pulse rate, up to a continuous beam. During laser treatment, the tip of the laser probe was applied directly on the posterior foot articulations. The procedure was subsequently repeated every day for two minutes immediately following hydroxyapatite injection, 24 h, and 48 h later with an incident energy density of 8 J/cm².

Experimental processing of the biological material

Plasma fibrinogen was determined by spectrophotometry using the method of Ratnoff and Menzie⁽¹⁴⁾. A Metrolab 1700 Spectrophotometer (UV. Vis. spectro) was used. Results are expressed in mg/100 ml. Plasma and synovial tissue Prostaglandin E₂ levels were measured using high pressure liquid chromatography (HPLC). Results are expressed in ug/ul.

In 8/12 rats per group, the left hind legs were fixed in buffered 10% formalin, then stained according to the hematoxylin-eosin technique and studied under an optical microscope. The hydroxyapatite crystals were visualized with polarized lens.

Data Analysis

Results of the experimental studies are reported as mean $\pm$ SE. Differences were analyzed using the ANOVA lineal model, and multiple comparison was tested using REGWQ Multiple Range. The histopathological data were analyzed with the Mann Whitney's U test. A value of $p < 0.05$ was regarded as significant.

Results

The results obtained are presented in Table 1. PF, plasmatic PGE₂, synovial tissue PGE₂ values in groups 1, 3, 4 and 5 significantly diminished when compared with the induced arthritis group ($p < 0.01$).

In each treated group, PF, plasmatic PGE₂ level and synovial PGE₂ decreased in the same way as in the controls after the meloxicam injection or laser therapy, but histopathological lesions of these groups were similar to those in non treated animals (group 2) (Fig.1 and 2)). The histopathological lesions observed in articular tissues and in adipose peripheral tissue were mono and polymorphonuclear infiltrates, granulomatose nodule formed by macrophage increase and giant cells reactions of strange body. However, when both therapies were associated (Group 5) (Fig. 3 and 4) PF, plasmatic PGE₂ and synovial tissue PGE₂ levels virtually returned to normal when compared to controls. Furthermore, an important granulomatose involution was detected.

Table 1: Effect of Helium-Neon laser and meloxicam in granulomatose process

Groups	PF* mg/100ml	PGE ₂ * ug/ul (plasma)	PGE ₂ * ug/ul (synovial)
1. Normal (control)	221.64 $\pm$ 10.68	6.77 $\pm$ 0.26	57.67 $\pm$ 3.55
2. induced arthritis	369.00 $\pm$ 17.30	12.90 $\pm$ 1.04	178.19 $\pm$ 9.35
3. arthritis + meloxicam	246.32 $\pm$ 7.61	6.75 $\pm$ 0.24	52.46 $\pm$ 6.03
4. arthritis + He-Ne laser	234.18 $\pm$ 8.47	6.07 $\pm$ 0.42	45.36 $\pm$ 1.11
5. arthritis + meloxicam + He-Ne laser	213.25 $\pm$ 9.95	6.81 $\pm$ 0.54	66.19 $\pm$ 4.57

* $X \pm ES$

$p < 0.01$ Groups 2 vs. 1,3,4 and 5

N.S Groups 1 vs. 3 vs. 4 vs. 5

Fig.1- Wide granulomatose nodule with giant cell reactions of foreign body type (panoramic view 4X Hematoxyline and Eosine stain).

Fig. 2 - High power view with prominent giant cell of foreign body type multinucleated with intracytoplasmic crystal (40X Hematoxiline-Eosine).

Fig. 3 - Soft subcutaneous tissue with chronic inflammation and few multinucleated giant cells of strain body (10X Hematoxiline-Eosine).

Fig. 4 - Soft subcutaneous tissue with moderate chronic inflammatory infiltrated, granulomas formation unobserved (10X Hematoxyline - Eosine)

Discussion

The specific role of crystals in producing synovitis is complex, since they can be identified in joint effusions after any inflammation or even in the absence of an inflammatory articular reaction. Occasionally, severe acute synovitis can also be found with few or atypical crystals, as in the experimental model⁽¹⁵⁾.

Laser therapy has been studied for modulating several processes in biological systems, including tissue healing (especially cutaneous wounds), pain control, nerve function, arthritis and other lesions⁽¹⁶⁾. Currently, many investigators believe that the effects of laser photostimulation on cells are caused by a photochemical and/or photophysical process, which begins with the absorption of light by a photoreceptor or chromophore. Karu has proposed a photosignal transduction chain from absorption of light by a photoreceptor to the cellular response⁽¹⁷⁾. He-Ne laser may induce chemical and/or physical changes in the photoreceptor molecule, and may cause a cascade of reactions directly. However, secondary reactions including photochemical signs and amplification of the first response after irradiation have been observed. These changes do not need direct light activation and occur in darkness minutes and hours after the photostimulation⁽¹⁸⁾.

Our results indicate that in the acute stage of hydroxyapatite granulomatose inflammation, low power laser inhibits the rise of inflammatory mediators and histopathological lesion in joints. He-Ne

laser may inhibit the emergence of chemostatic factors in the early stages of inflammation, or may interfere with chemical mediators, thus inhibiting Cox-2/Cox-1 relation, as does meloxicam, a new preferential cyclooxygenase 2 (Cox-2) inhibitor currently used in treating inflammatory processes.

Inflamed tissue is characterized by extravasation of fluid, and probably by a decreased in Ph values when compared with non-inflamed tissue. Such conditions are ideally suited to "trap" NSAIDs from the circulation⁽¹⁹⁾. In this experimental model, meloxicam showed its efficacy acting on some of its chemical mediators, like plasmatic prostaglandin E₂ and plasmatic fibrinogen. The occurrence of inflammatory effects in the very initial inflammatory stage seems to have critical significance in the development of subsequent inflammation^(20,21).

We conclude that He-Ne laser treatment or meloxicam administration after experimentally induced inflammation, normalizes plasmatic PGE₂ and fibrinogen levels, but produces similar histological lesions as non-treated controls. Both therapies normalized not only chemical mediators and synovial tissue, but also induced histological granulomatose involution.

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