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Low-intensity laser therapy efficacy evaluation in mice subjected to acute arthritis condition

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ABSTRACT

Acute arthritis is an inflammation that affects many joints. The principal treatment options comprise drugs (corticosteroids), invasive and painful surgery. The objective of this study was to evaluate the efficacy of low intensity laser therapy (LILT), a non-invasive treatment, in a murine model of acute inflammation model. 48 mice received a synovial injection of Zymosan A into one knee. Mice were treated with LILT by three different wavelengths, either as a single (S) or dual (D) application immediately after the injury or after 24 h following initiation of an inflammatory response. The histological analysis aimed at identifying inflammatory infiltrate and the structure of the articular surfaces as an indicator of a long-term damage due to inflammation. Statistical analysis (Kruskal-Wallis test), did not allow to reject the null hypothesis. However, LILT promoted histological alterations in some treatment groups. Histological evidence (Median and confidence interval) of anti-inflammatory effects was especially noticeable in knees of mice irradiated with lasers emitting moderate intensity and continuous 660 nm (S = 18.5 (11.4; 27.6); D = 16.0 (6.93; 27.0)) and high intensity and pulsed 905 nm (S = 17.5 (10.2; 24.79), with decrease of the resorbed region. However, the 905 nm pulsed laser was responsible for exacerbation of inflammation for multiple LILT sessions with a short delay (D = 45.0 (22.84; 63.83)), tending to aggravate the resorption of the articular surface ($p < 0.05$). LILT showed signs of an anti-inflammatory effect when applied once, but promoted increased resorptive area when used for two sessions, indicating the importance of a controlled LILT protocol to reach therapeutic effects.

1. Introduction

Inflammation of the joints space, be it acute or chronic as in rheumatoid arthritis can lead to the destruction of the cartilage and ultimately the bone. As such, rheumatoid arthritis is an autoimmune inflammation of unknown etiology that manifests clinically by deformation and destruction of the articular surfaces in an affected joint. Next to surgery including joint replacement, treatment of chronic inflammation such as rheumatoid arthritis consists of drugs, inhibiting inflammatory infiltration, mainly targeting lymphocytes [1]. Treatments for acute injuries are more difficult to treat due to the time delay until chronic damages are clinically manifest.

Minimal or non-invasive and inexpensive therapies using systemic chemotherapeutics as well as locally acting physical therapies are under investigation [2,3]. Preclinically testing of new therapies often used murine acute arthritis models, such as a Zymosan A injections into a knee joint. This product is made from the polysaccharide wall of *Saccharomyces cerevisiae* and composed mainly of glucana, a ligand of the Toll-Like2 receptor and activator of the alternative complement pathway, thus activating the innate immune system. The administration has the consequence of an acute inflammation of the joint, due to the migration of leukocytes, production of cytokines and inflammation mediators at the site of injection [4].

While in rheumatoid arthritis the inflammatory processes driven by the production of various cytokines is coordinated through a common element such as NF-κB (nuclear factor kappa B). The signaling pathway in acute inflammation is less clear, and further analysis is required.

Among the locally acting physical therapies, LILT is intensely researched whereby biologically active proteins absorb a photon's quantum energy. A wide range of possible targets was discussed, such as enzymes, lipids changing their reaction kinetics or conformation and thus aided in ATP production or affecting known signaling pathways influencing proliferation, growth, and development. The delivered photon [eV cm^{-2}] or energy dose [J cm^{-2}] is known to be modulating

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the biological effect with an often apparent biphasic dose response, suggesting the existence of an optimum photon or energy density for the maximum attainable biological benefit.

LILT has been shown to influence biology at the molecular, cellular, and tissue level. For example, LILT causes vasodilation, relevant factor for articular inflammation because it increases the local support of oxygen and contributes to the migration of immune cells [5]. Wavelengths between 600 and 700 nm are adequate to treat small joints and superficial tissues, whereas longer wavelengths 780–950 nm, experiencing less attenuation by tissue and hence achieve a larger tissue penetration, are used for deeper, highly perfused tissues and larger joints. Therefore, maximizing beneficial tissue response needs to consider the laser wavelength [nm], irradiation dose both in power density or irradiance [mW cm^{-2}] and energy density or radiant exposure [J cm^{-2}] whereby continuous exposure or pulsed exposure need to be considered separately. The number or frequency of treatment sessions is another efficacy determining confounder [6].

Despite all the advances, LILT mechanisms for most indications remain poorly understood. LILT can lead to cellular apoptosis, migration, and proliferation. The appropriate dose for a particular tissue is often questionable and finding the optimum dose requires extensive in vivo studies. Few double-blinded clinical trials are available and difficult to conduct, particular for visible LILT wavelength below (700 nm). Energy density at target depth (fluence rate [mW cm^{-2}] and fluence [J cm^{-2}] are rarely reported [6].

Moriyama et al. previously demonstrated in the Zymosan A an acute inflammation model an increased transcription of iNOS associated with a reduction of infiltrating macrophages in the synovial fluid [7] following 660 and 905 nm LILT. Ying-Ying Huang et al. conducted a study in which they developed acute arthritis in mice with a Zymosan A injection, followed by 810 nm mediated LILT for five days. The results lead them to propose three possible explanations for the biphasic response. First, an excess of reactive oxygen; second, an excess of nitrous oxide; and third, activation of a cytotoxic pathway [6].

In this sense, LILT is employed to promote wound healing, tissue repair, and preventing the death of the tissue as well as relieving inflammation and edema in various areas of health [8]. Therefore, the aim of this study was to evaluate the effect of wavelengths and number of LILT sessions in a murine model of acute lipopolysaccharide-induced inflammation. In particular the ability to prevent the damage to the articular surface as a long-term endpoint.

2. Materials and Methods

2.1. Selection of Animals

For this work, 48 male HLL mice, originating from the Vivarium of the Princess Margaret Cancer Center were assigned to one of 2 groups. Group 1 consisted of 24 mice in which acute arthritis was induced by Zymosan A and LILT treated with one of three lasers (subgroups A, B, C) 15 min after injection or left untreated (D) to test the ability to prevent inflammation and joint damage. Animals were euthanized 24 h after treatment by cervical dislocation under general anesthesia. Group 2 consisted of 24 mice affected by acute arthritis as above, but treated twice; first at 15 min and then 24 h after a Zymosan A injection. Mice were also sacrificed 24 h after the 2nd treatment. This group tests the repeat treatment. All animals were housed with 4 mice per cage, with access to water and food ad libitum, in environmentally controlled temperature (23 ± 1 °C) and 12/12 hours light/dark cycle condition. This study was conducted by international ethical standards for the use and handling of animals following review and approval by the Ethics Committee on Animal Studies, University Health Network, Toronto, Canada, under protocol numbers 939 and 2415.

The two groups were divided into the following subgroups containing six animals each: A - continuous wave 660 nm LILT; B - continuous wave 808 nm LILT; C - LILT employing a 905 nm pulsed laser; and D – no LILT treatment as a control.

2.2. Lasers and Illumination Parameters

The 660 nm and 808 nm fiber optic coupled lasers equipped with a micro-lense were assembled at the Princess Margaret Cancer Centre provides both > 300 mW to the tissue surface. The 905 nm laser was manufactured by Theralase Inc. (Toronto, Ontario, Canada) and delivered an average power of 60 mW as 200 ns pulses at 10,000 Hz repetition rate. The emitter was placed 2.5 mm from the tissue surface. In all cases, the average irradiance on the skin was 25 mW cm^{-2} over 200 s for a radiant exposure of 5 J cm^{-2}.

2.3. Animal Handling

Animals were anesthetized by inhalation of 2% isoflurane in the air and when a deep anesthetic plane is attained a 0.3 mm, a skin incision is made at the level of the left knee for better visualization of the articular region. Injection of 10 µL Zymosan A (Sigma, Germany) into the synovial space was made with the aid of a Hamilton syringe (Hamilton Co, Sigma, Germany). The solution was composed by 30 mg of Zymosan A in 1 mL of saline solution. The right knee served as control. The skin incision was sutured before LILT. Following the predetermined time delay of 15 min and 24 h, for group 2, post-Zymosan An administration, LILT exposure commenced. After euthanasia, the left and right knees were retrieved and prepared for histology. Histological slides were used for quantitative and qualitative morphometric analysis and immunohistochemistry, to evaluate the presence and extends of inflammatory infiltrate and to analyze whether the LILT treatment was effective in preventing or reducing articular decay.

2.4. Histological Processing

After collection of the lower limbs, the articular region was extracted and the soft tissues carefully separated from the bone. The bone fragments were immersed in 10% formalin for 24 h followed by decalcification of the specimens in 0.5 M EDTA, exchanging the solutions every two days for a total of 15 to 30 days. The remaining acid was neutralized for 24 h in 5% sodium sulfate solution, and subsequently, specimens were dehydrated in an ascending alcohol concentration series. Once completed the bone specimens were placed in equal parts of alcohol and xylene (overnight) and diaphanized in xylene with three 2 hourly changes and embedded in paraffin.

2.5. Microscopic Analysis

For microscopic analysis, paraffin embedded samples were cut in a semi-serial fashion (5 cuts per slide), of 5 µm thickness covering the entire joint. Sections were stained with hematoxylin-eosin and Masson trichrome for the quantification of articular surface resorption using an AxioImager Z2 optical microscope (Zeiss, Germany) equipped with a digital camera (Zeiss, Germany).

2.6. Articular Surface Quantity

For quantification of the resorptive region at articular surface, ten histological sections were selected randomly. These histological cuts were analyzed under 5× lenses (AxioImager Z2, Zeiss, Germany). These measurements were done using StereoInvestigator (MBF, USA) stereological software using Cavalieri method. Results were expressed as percentual of resorption at the articular surface [9].