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Low level laser therapy modulates kinin receptors mRNA expression in the subplantar muscle of rat paw subjected to carrageenan-induced inflammation

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Abstract

Low level laser therapy (LLLT) has been used clinically in order to treat inflammatory processes. In this work, we evaluated if LLLT alters kinin receptors mRNA expression in the carrageenan-induced rat paw edema. Experimental groups were designed as followed: A_1 (Control-saline), A_2 (Carrageenan-only), A_3 (Carrageenan + laser 660 nm) and A_4 (Carrageenan + laser 684 nm). Edema was measured by a plethysmometer. Subplantar tissue was collected for kinin receptors mRNA quantification by Real time-PCR. LLLT of both 660 and 684 nm wavelengths administrated 1 h after carrageenan injection was able to promote the reduction of edema produced by carrageenan. In the A_2 group, B1 receptor expression presented a significantly increase when compared to control group. Kinin B1 receptor mRNA expression significantly decreased after LLLT's 660 or 684 nm wavelength. Kinin B2 receptor mRNA expression also diminished after both laser irradiations. Our results suggest that expression of both kinin receptors is modulated by LLLT, possibly contributing to its anti-inflammatory effect.

1. Introduction

Low level laser therapy (LLLT) is a novel therapy involving the application of low power monochromatic and coherent light to injuries and lesions [1]. LLLT has been used to treat muscular pain, although the biological mechanisms of the beneficial results observed in clinical trials remain unclear

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[2]. The ability of LLLT to reduce the duration of acute inflammation [3] and accelerate tissue repair in tendon and muscle injuries was proposed [4]. Lubart et al. suggested that LLLT may promote changes in the cellular redox state, playing a pivotal role in sustaining cellular activities, and promoting photobiostimulative processes [5]. For many years it has been proposed that low level laser therapy (LLLT) is able to ameliorate the pain, swelling, and inflammation associated with various forms of arthritis. Recently, Castano et al. demonstrated that LLLT was highly effective in treating inflammatory arthritis in rats that had zymosan injected into their knee joints in order to induce inflammatory arthritis [6]. The mechanisms behind the observed clinical pain relief after LLLT are poorly understood, and may involve an anti-inflammatory action [7,8]. Bjordal and colleagues demonstrated that LLLT is able to modulate inflammatory processes in a dose-dependent manner and can be used to significantly reduce acute inflammatory pain in clinical settings [8].

Carrageenan is a polysaccharide widely used to induce acute inflammation response in experimental animals, since it induces the release of different inflammatory mediators, such as histamine, bradykinin, prostaglandin, and superoxide anions [9]. The use of carrageenan as an irritant agent to induce rat paw inflammation was introduced by Winter and colleagues, becoming one of the most popular methods for drug testing and evaluation of new anti-inflammatory therapies [10,11].

Albertini et al. using the carrageenan-induced inflammation model, demonstrated the decrease in edema evolution at 4 h after the injection of carrageenan in subplantar muscle by LLLT [12]. These authors have shown that laser irradiation failed to inhibit the edema in adrenalectomized animals, indicating that LLLT possibly exerts its anti-inflammatory effects by stimulating the release of adrenal corticosteroid hormones.

Recently, we demonstrated a diminished COX-2 mRNA expression in subplantar tissue taken from rats treated with carrageenan followed by LLLT 1 h after carrageenan injection [13]. In these animals, COX-2 mRNA expression and edema volume were reduced by LLLT at 4 h after the injection of carrageenan. Moreover, Albertini et al. demonstrated the reduction (30–40%) in the mRNA content of cytokines TNF-α, IL-1β and IL-6 3 h after laser irradiation in paw muscle treated with carrageenan. This diminished effect in mRNA expression was observed using either LLLT's 660 nm or 684 nm red wavelengths 4 h after acute inflammation induction [14]. These results demonstrate that LLLT present an anti-inflammatory action which possibly modulates transcription factors linked to COX-2 and pro-inflammatory cytokines mRNA expression.

Kinin B1 and B2 receptors play a central role in the pathophysiology of inflammation [15–17]. Kinin B2 receptor is broadly and constitutively expressed, whereas B1 receptor is weakly expressed in most tissues under basal conditions but strongly upregulated following inflammation. Constitutive B2 receptor is thought to be preferentially involved in the early phase and the inducible B1 receptor in later phases of inflammation [18,19].

The involvement of B1 receptor on the carrageenan-induced inflammation model has been demonstrated. Costa and colleagues demonstrated the anti-inflammatory effects of the novel selective non-peptide kinin B(1) receptor antagonist, SSR240612 on the carrageenan-induced mouse pleurisy, reducing total cell and neutrophil counts [20].

The relation between carrageenan-induced inflammation and kinin B2 receptor was also suggested. The antinociceptive and the edema inhibition properties of the non-peptide kinin B2 receptor antagonist, NPC 18884, on the carrageenan-induced hyperalgesia has been shown [21]. Using the mouse pleurisy model induced by carrageenan, Saleh et al. demonstrated the dose-dependent inhibition of the exudation and total and differential cell content caused by intrapleural injection of carrageenan [22].

To our knowledge there are few, if any, studies investigating whether LLLT affects kinin receptors expression *in vivo*. Within this context, the present study was therefore designed to evaluate the kinin involvement in the anti-inflammatory properties of LLLT applied at different wavelengths (660 and 684 nm) on the carrageenan-induced rat paw edema, a classical model for acute inflammatory response. In order to study the anti-inflammatory mechanisms by LLLT, we determined the volume edema and the kinin receptors mRNA expression.

2. Materials and methods

2.1. Animals

All experiments were carried out in accordance with the guidelines of Vale do Paraíba University for animal care (protocol number: A034/2006/CEP). Experiments were performed using male Wistar rats (150–200 g) with food and water *ad libitum* provided by the Central Animal House of the Research and Development Department of Vale do Paraíba University (UNIVAP). All rats were placed in common cages and randomly divided into group of eight.

2.2. Laser irradiation

Diode lasers with mean output power of 30 mW and wavelengths of either 660 nm (model: laser unit, Kondortech) or 684 nm (model: Theralase) were used. Spectroscopic measurements carried out on both lasers showed no thermal drift for the 660 nm laser. The 684 nm laser showed a 0.4 nm wavelength drift from the cold to warm operation conditions. Stabilization at the 684 nm was achieved in a time period shorter than 30 s after turning on with the diode laser device at room temperature. The optical power was calibrated by using a Newport Multifunction Optical Meter (model 1835C). The laser beam illuminated an area of 0.785 cm^2 resulting in an energy dosage of 7.5 J/cm^2.

2.3. Experimental groups

Experimental group consisted of 32 male Wistar rats divided into 4 groups: A1 (Control-saline), A2 (Carrageenan alone), A3 (Carrageenan + laser 660 nm) and A4 (Carrageenan + laser 684 nm). Rats were given a subplantar injection (0,5 mg/paw) of either carrageenan (Sigma) or saline in the subplantar region from left paw, under anesthesia with halothane. The animals from A3 and A4 groups were irradiated at 1 h after the inflammation induced by carrageenan.

2.4. Experimental procedure

The animals were sacrificed by decapitation 4 h after each treatment and the subplantar muscles were quickly dissected,