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Differing energy densities with laser 670nm InGaP controls inflammation and collagen reorganization in burns

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ABSTRACT

Purpose: This study compared different energy densities of laser on second degrees burns in rats aiming to determine the most effective dosimetry in stimulation of the healing process.
Methods: Burns were induced in the dorsal skin of 54 animals divided into three groups (n: 18): **1**-without treatment; **2**-irradiated lesions by the Indium Gallium Phosphide (InGaP) 670nm (4.93J/cm^2) laser; **3**-irradiated lesions by the InGaP–670nm (9.86J/cm^2) laser. Samples were collected on the 2, 10 and 18 days after injury for structural, morphometry, biochemical analysis and Western blotting.
Results: The energy densities examined were effective in significantly increasing the total number of fibroblasts and blood vessels and reduce the number of inflammatory cells particularly in irradiated lesions with 9.86J/cm^2. This same energy density significantly increased the amount of GAGs (Glycosaminoglycans), decreased the TGF-β1 (Transforming Growth Factor β1) and increased the VEGF (Vascular and Endothelial Growth Factor) during the experimental period. This energy density also significantly increased the Collagen type I and decreased Collagen type III and the active isoform of metalloproteinase 9 (MMP-9).
Conclusions: The energy density of 9.86J/cm^2 was more effective in promoting cellular responses related to neoangiogenesis, decreasing inflammation and collagen fibers reorganization.

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1. Introduction

Burns produce large local and systemic physiological damage [1] such as the destruction of vascular and capillary integrity, edema formation [2]. The severity of a burn depends on the thermal agent and the contact time with the tissue and it can be evaluated according its depth in the Boyer's rating [3]. Burns result in functional limitations such as decreased body and environment control, as well as aesthetic problems that promote decreased self-esteem, social avoidance, and anxiety about the future [4]. The focus of therapy for burn patients has been of physical and social functionalities [5].

Tissue repair is a process characterized by interrelated events, into sequential phases: inflammatory, proliferative and remodeling under the action of specific cells, cytokines and various growth factors [6]. The TGF-β1 (Transforming Growth Factor), especially released in the early stages of this process, is secreted by keratinocytes, fibroblasts and platelets, acting as chemotactic for neutrophils and monocytes at the wound site [7,8]. Another important growth factor in tissue repair is VEGF (Vascular and Endothelial Growth Factor) whose secretion is induced by hypoxia and low pH, conditions that stimulate angiogenesis [9]. Metalloproteinases are also involved in the modulation of tissue integrity and modulate the half-life of the molecules of ECM (Extracellular Matrix) by means of selective degradation [10]. GAG (Glycosaminoglycan) and (PGs) Proteoglycans; on the other hand, are involved in proliferation, migration and cell differentiation during tissue repair [11].

The LLLT (low-level laser therapy) is involved in the healing process triggering the photoactivation of cellular mechanisms. Chromophore located in the mitochondria seem to absorb the laser's red and infrared light triggering increased protein synthesis, ATP production, cell proliferation, and reorganization of collagen [12]. The LLLT has stood out among the many therapeutic methods [6,13–15] and has demonstrated in various studies that improves tissue repair as it promotes collagen synthesis and deposition, increased vascularity, inflammation reduction, accelerating the wound remodeling. In this way, it is a promising work tool in the burn wounds therapy [1,8,15–20]. However, its effects are dependent on the different irradiation parameters used such as energy density, wavelength, laser irradiation frequency and duration of treatment [21].

The wavelength of the laser is critical in its absorption by the tissues and their produced physiological effects. Among the therapeutic lasers also known as low-intensity lasers are Helium–Neon (He–Ne), Gallium Arsenide (AsGa), Aluminum–Gallium–Indium–Phosphorus (AlGaInP) and Arsenide–Gallium–Aluminum (AsGaAl). The wavelengths between 632.8 and 904 nm are widely used in tissue repair [2,15,22]. Two different types of laser (670-InGaP and 830-nm GaAlAs) are used for Chiarotto et al. [8] in the treatment of second-degree burns in rats and it was observed that the laser 670-nm InGaP was more effective in increasing the numbers fibroblast at the injury site. Other authors have also demonstrated the beneficial effects of low intensity laser in tissue repair using 670 nm [6,23,24].

Thus, this study compared the action of different energy densities of Indium Gallium Phosphide (InGaP) 670 nm laser in second degree burns repair in Wistar rats to identify the best dosimetry to be used in treating this type of injury.

2. Methods

2.1. Animal model

The experimental procedure was developed using 54 male Wistar rats obtained from Hermínio Ometto University Center (UNIARARAS) — Center of Animal Experimentation. These animals with 120 days with ±300 g were maintained in individual cages under a 12/12-h light/dark cycle at a constant temperature (23±2°C) and humidity (55%).

2.2. Experimental procedure

In dorsal skin of all animals the burn lesions were induced according the protocol developed by Chiarotto et al. [8]. These were divided into three groups of 18 animals: group 1, untreated; group 2, lesions irradiated with an InGaP laser at 670 nm, 4,93 J/cm^2; group 3, lesions irradiated with an InGaP laser at 670 nm, 9,86 J/cm^2. Through anesthetic overdose and cervical dislocation, six animals per group were euthanized in two, ten and eighteen days after experimental injuries for collection of tissue samples and analysis morphometric analysis (n=3), Western blotting, quantitative analysis of glycosaminoglycans, hydroxyproline and zymography to metalloproteinases (n=3). The surgical and experimental procedures received approval by Ethics Committee of the Hermínio Ometto University Center (UNIARARAS) (protocol no. 022/2013) and conducted according with the Guide for the Care and Use of Laboratory Animals [25]. The animals were healthy and procedure did not promote stress.

2.3. Laser irradiation

The treatments occurred daily for 18 days according to protocol Chiarotto et al. [8]. For laser therapy was used a Physiolux Dual Bioset® InGaP laser (Indústria de Tecnologia Eletrônica Ltda., Rio Claro, São Paulo, Brazil) at a wavelength of 670 nm (visible red), selected in the continuous mode, with an output power of 30 mW, with the beam covering an area of 0.073 cm^2, applied for 12 s, energy density of 4,93 J/cm^2 (energy per point of 0.09 J, total energy of 0,36 J); and for 24 s, energy density of 9,86 J/cm^2 (energy per point of 0.18 J, total energy of 0,72 J). The utilization of two energy densities was based on studies performed by Ezzati et al. [16,17], Novaes et al. [26] and Fiório et al. [27] and the different doses and wavelength used in this protocol were chosen according to previous studies used by our research group in burns [6,14,24]. For irradiation the apparatus calibration was performed by the manufacturer.

2.4. Structural and morphometric analysis

The sample collection (25 mm in diameter) was made following different phases of tissue repair in skin.

After removing, the fragments of tissue were immediately fixed in 10% formaldehyde in Millonig buffer, pH 7.4, for 24 h at room temperature. After that, they were washed in buffer