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LIGHT-EMITTING DIODE THERAPY REDUCES PERSISTENT INFLAMMATORY PAIN: ROLE OF INTERLEUKIN 10 AND ANTIOXIDANT ENZYMES

D. F. MARTINS, [a,b*†] **B. L. TURNES,** [a,c†] **F. J. CIDRAL-FILHO,** [a] **F. BOBINSKI,** [d#] **R. F. ROSAS,** [a,b] **L. G. DANIELSKI,** [b,e] **F. PETRONILHO** [b,e] **AND A. R. S. SANTOS** [d]

[a] *Experimental Neuroscience Laboratory (LaNEx), University of Southern Santa Catarina at Palhoça, Santa Catarina, Brazil*

[b] *Postgraduate Program in Health Sciences, University of Southern Santa Catarina, Santa Catarina, Brazil*

[c] *Graduate Program in Neuroscience, Center of Biological Sciences, Federal University of Santa Catarina, Trindade, Florianópolis, Santa Catarina, Brazil*

[d] *Laboratory of Neurobiology of Pain and Inflammation, Department of Physiological Sciences, Centre of Biological Sciences, University Federal of Santa Catarina, Florianópolis, Santa Catarina, Brazil*

[e] *Laboratory of Clinical and Experimental Pathophysiology, University of Southern Santa Catarina at Tubarão, Santa Catarina, Brazil*

Abstract—*Background:* During the last decades, the use of light-emitting diode therapy (LEDT) has increased significantly for the treatment of wound healing, analgesia and inflammatory processes. Nevertheless, scientific data on the mechanisms responsible for the therapeutic effect of LEDT are still insufficient. Thus, this study investigated the analgesic, anti-inflammatory and anti-oxidative effect of LEDT in the model of chronic inflammatory hyperalgesia. *Experimental procedures:* Mice injected with Complete Freund's Adjuvant (CFA) underwent behavioral, i.e. mechanical and hot hyperalgesia; determination of cytokine levels (tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), IL-10), oxidative stress markers (protein carbonyls and thiobarbituric acid reactive species (TBARS)) and antioxidant enzymes (catalase (CAT) and superoxide dismutase (SOD)). Additionally, mice were pretreated with either naloxone or fucoidin and mechanical hyperalgesia was assessed. *Results:* LEDT inhibited mechanical and thermal hyperalgesia induced by CFA injection. LEDT did not reduce paw edema, neither influenced the levels of TNF-α and IL1-β; although it increased the levels of IL-10. LEDT significantly prevented TBARS increase in both acute and chronic phases post-CFA injection; whereas protein carbonyl levels were reduced only in the acute phase. LEDT induced an increase in both SOD and CAT activity, with effects observable in the acute but not in the chronic. And finally, pre-administration of naloxone or fucoidin prevented LEDT analgesic effect. *Conclusions:* These data contribute to the understanding of the neurobiological mechanisms involved in the therapeutic effect of LEDT as well as provides additional support for its use in the treatment of painful conditions of inflammatory etiology.

Key words: low-level light therapy, inflammation, persistent pain, mice.

INTRODUCTION

Persistent or chronic pain is a common chronic health problem which incurs high health care cost as it is associated with functional impairment leading to decreased quality of life (Willman et al., 2013). Inflammatory pain is one of the most common symptoms of the inflammatory disease and represents an important health problem. It is principally caused by the sensitization of primary nociceptive neurons by the direct action of inflammatory mediators (Ferreira, 1972; Ferreira, 1980).

Inflammatory cytokines (interleukin-1 beta [IL-1β], IL-6 and tumor necrosis factor-alpha [TNF-α]) as well as reactive oxygen species (ROS) are inflammatory mediators that sensitize and/or activate primary nociceptive neurons (Verri et al., 2006; Verri et al., 2007; Salvemini et al., 2011). During the inflammatory response, ROS and reactive nitrogen species (RNS) modulate phagocytosis, gene expression, and apoptosis (Verri et al., 2006). Oxidative stress results in activation of redox transcription factors, such as nuclear factor-kB (NF-kB) and AP-1, which play a crucial role in the induction of inflammatory cytokines and intercellular adhesion molecule-1 (ICAM-1) (Chen et al., 2004). However, in order to avoid the oxidative damage induced by ROS,

*Correspondence to: D. F. Martins, Experimental Neuroscience Laboratory (LaNEx), Postgraduate Program in Health Sciences, University of Southern Santa Catarina at Palhoça, Santa Catarina, Brazil. Tel: +55-48-3279-1167.
E-mail addresses: danielmartinsfisio@hotmail.com, daniel.martins4@unisul.br (D. F. Martins).
† These authors contributed equally to this study.
# Present Address: Graduate Program in Physical Therapy, Physical Therapy Department, Experimental Research Laboratory (LAPEx), College of Health and Sport Science, Santa Catarina State University (UDESC), Florianopolis, Brazil.

Abbreviations: ANOVA, analysis of variance; CAT, catalase; CFA, Complete Freund's Adjuvant; DNPH, dinitrophenylhydrazine; EDTA, ethylenediaminetetraacetic acid; IL-1β, interleukin-1 beta; LEDT, light-emitting diode therapy; NADPH oxidase, nicotinamide adenine dinucleotide phosphate oxidase; NF-kB, nuclear factor-kB; NO, nitric oxide; PN, peroxynitrite; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive species; TNF-α, tumor necrosis factor-alpha.

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the body is equipped with several lines of antioxidant defense, in which the enzymatic line consists of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) (Luchese et al., 2009).

The inflammatory pain induced by Complete Freund's Adjuvant (CFA) is a well-characterized model of experimental chronic pain, which allows the quantification of mediators involved in cell changes during the inflammatory process (Ren and Dubner, 1999; Rezazadeh et al., 2009; Martins et al., 2015). This model is characterized by infiltration of neutrophils, followed by paw (peripheral tissue – skin and muscle) injury by ROS and RNS as well as the release of other chemical mediators derived from active neutrophils such as inflammatory cytokines (Ren and Dubner, 1999; Rezazadeh et al., 2009; Martins et al., 2015). Cytokines and ROS are inflammatory mediators that lead to increased sensitivity to painful stimuli, and their inhibition represents a therapeutic approach in controlling acute and chronic pain.

Low-level light therapy (LLLT) uses visible or near-infrared light to produce photobiomodulation in biological tissues. In recent years an increasing number of studies support the analgesic effect of LED irradiation, e.g., preclinical studies (*in vivo* models) demonstrated that LED treatment (1) induced a dose–response analgesic effect in the model of postoperative pain in mice through activation of peripheral opioid receptors and activation of the L-arginine/nitric oxide (NO) pathway (Cidral-Filho et al., 2014); (2) reduced mechanical hypersensitivity and induced anti-inflammatory effects (TNF-α inhibition) in a mouse model of nerve injury (Cidral-Filho et al., 2013); (3) was effective in reducing the severity of oral mucositis on chemotherapy-induced mucositis in hamsters (Sacono et al., 2008). These data suggest a possible pain relief mechanism and anti-inflammatory activity for light-emitting diode therapy (LEDT). Moreover, clinical trials demonstrate that LEDT (1) combined with superpulsed laser induces analgesia and improves quality of life in patients with knee pain (Leal-Junior et al., 2014); and (2) is more effective than laser therapy on the management of oral mucositis in oncologic patients (Freitas et al., 2014). In addition, the use of a LED toothbrush resulted in decreased dentin hypersensitivity after 4 weeks in comparison to a placebo toothbrush in a double-blind randomized clinical trial (Ko et al., 2014).

Although the biological mechanisms involved in LED stimulation are described in the literature as similar to those of LASER, no study has examined the neurobiological mechanisms underlying LED stimulation in an animal model of chronic inflammatory pain. Thus, the present study investigated the analgesic, anti-inflammatory and anti-oxidative effects of LEDT in the mouse model of CFA paw injection, as well examined some of the possible mechanisms involved in this effect.

EXPERIMENTAL PROCEDURES

Animals

The experiments were conducted with adult male Swiss mice (25–35 g), obtained from Biotério or Universidade Federal de Santa Catarina (UFSC, Florianópolis, Santa Catarina, Brazil). All animals were housed in collective cages at 22 °C under a 12-h light/12-h dark cycle, with access to food and water *ad libitum*. The animals were acclimatized to the laboratory for 1 h prior to testing and the experiments were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983). Experiments were performed after approval of the protocol (13.007.4.08.IV) by the Ethics Committee of the University of Southern Santa Catarina and by the Ethics Committee of the Federal University of Santa Catarina (#PP00745). All efforts were made to demonstrate consistent effects of the LED treatments and to minimise both the number of animals used and their suffering.

LEDT

LEDT was performed with a MOLIMEDpen© (MDT Bioelectronics, Bettwiesen, Switzerland) device with the following specifications: 950-nm wavelength, irradiance of 80 mW/cm^2 and energy density of 1, 2 or 4 J/cm^2 (13, 25 and 50 s of irradiation time, respectively). During LEDT the animals were contained in a plastic tube with the tail and hind limbs protruding to facilitate access to the irradiation area (1 cm^2 – plantar aspect of the right hind limb). During irradiation LED probe was kept in contact with the irradiation area (Cidral-Filho et al., 2013; Cidral-Filho et al., 2014). LEDT treatments were initiated 24 h after CFA paw injection.

CFA paw injection

The mice in control and LEDT groups were injected with 20 μl of an 80% Complete Freund's Adjuvant (CFA – Mycobacterium tuberculosis) solution (diluted in phosphate-buffered saline) as described by Meotti et al. (2006) with minor modifications. The sham group was injected with 20 μl of pure phosphate-buffered saline instead.

Evaluation of mechanical hyperalgesia

Mechanical hyperalgesia was assessed as the percentage of paw withdrawals in response to a series of ten non-consecutive applications (at 1-min intervals) of a 0.4 g von Frey hair monofilament (VFH, Stoelting, Chicago, IL, USA) through the mesh floor to the plantar aspect of the animals' right hindpaw as described by Martins et al. (2015) with minor modifications: (1) stimulus was applied perpendicularly to the plantar surface; (2) the pressure applied was sufficient to curve the filament, thereby obtaining total pressure; (3) the animals were evaluated only when all four paws were accommodated on the screen; and (4) paw withdrawal response was considered only when the animal fully removed the paw from the screen.

To assess the effects of LEDT on CFA-induced persistent inflammatory pain, animals were treated with LEDT of different energy densities (1, 2 or 4 J/cm^2) 24 h after CFA i.pl. injection. Development of mechanical