Lasers in Surgery and Medicine 41:227–231 (2009)

In Vivo Effects of Low Level Laser Therapy on Inducible Nitric Oxide Synthase

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Background and Objective: Low level laser therapy (LLLT) has been demonstrated to modulate inflammatory processes with evidence suggesting that treatment protocol, such as wavelength, total energy, and number of treatments determine the clinical efficacy. In this study, the effects of LLLT mediated by different wavelengths and continuous versus pulsed delivery mode were quantified in a transgenic murine model with the luciferase gene under control of the inducible nitric oxide synthase (iNOS) expression.

Study Design/Materials and Methods: LLLT modulated iNOS gene expressed in the acute Zymosan-induced inflammation model is quantified using transgenic mice (FVB/N-Tg(iNOS-luc)). Here an energy density of 5 J cm^{-2} at either 635, 660, 690, and 905 nm in continuous wave mode and at 905 nm for short pulse delivery were evaluated. Age of the animals was determined as additional modulating the inflammatory response and the LLLT efficacy for some treatment protocols.

Results: Animals younger than 15 weeks showed mostly reduction of iNOS expression, while older animals showed increased iNOS expression for some LLLT protocols. Intensity and time course of inducible nitric oxide expression was found to not only depend on wavelength, but also on the mode of delivery, continuous, or pulsed irradiation.

Conclusion: LLLT exhibit different effects in induced inflammatory process according to different wavelengths and wave mode. Upregulation of iNOS gene following 905 nm pulsed wave suggests a different mechanism in activating the inflammatory pathway response when compared to the continuous wave. Lasers Surg. Med. 41:227–231, 2009. © 2009 Wiley-Liss, Inc.

Key words: bioluminescence imaging; biostimulation; inflammation; pulsed laser; Zymosan A

INTRODUCTION

Low level laser therapy (LLLT) has been demonstrated to promote photobiomodulation, including stimulation and inhibition effects and is used clinically for various conditions, including treatment of wounds, chronic pain, inflammation, and infections [1–6]. One of the proposed mechanisms of laser photobiomodulation involves the absorption of photons by intracellular chromophores and the production of reactive oxygen species (ROS), which in concentrations below the cytotoxic level has positive stimulatory effects on the cell [7].

For the continuous mode of light delivery, the wavelength-dependent ability to alter cellular mechanism in the absence of significant heating has been demonstrated through action spectra, suggesting a direct photochemical basis for the LLLT efficacy. A variety of potential photochemical targets have been suggested to give rise to the action spectra such as cytochromes within the red part of the optical spectrum, and temporary increase in the cell membranes to calcium ions [8]. Thus, the red and infrared portion of the optical spectrum opens possible therapeutic modulations in living tissues so their effects are dependent on the physiological state of the tissue at the moment of irradiation [9]. Studies show that stimulation and inhibition due to light irradiation can occur via the same photoacceptors, and therefore as the light dose increases, the photoacceptors are damaged and the effect decreases, showing a bi-phasic LLLT responses as function of irradiance (W cm^{-2}) [10]. Similar studies have been executed at 50% duty cycle in frequency modulation up to the kHz range [11]. Pulse laser radiation with low duty cycle [12] suggested transient heating possibly inhibiting NADPH oxidase.

While previously LLLT or laser biostimulation research was predominantly based on subjective evaluation of clinical or semi-quantitative pre-clinical studies, recently quantifiable pre-clinical models have been exploited to access the molecular basis for LLLT efficacy [13]. In this study, transgenic animals carrying the reporter luciferase were used to quantify iNOS gene expression following induction of acute inflammation and immediate single LLLT irradiation mediated by various wavelengths in continuous and pulsed mode.

Zymosan A has been used in several studies as a model system for inflammation induction in vivo [3,5,6,14,15].

Contract grant sponsor: Ontario Centres of Excellence; Contract grant sponsor: Theralase, Inc.
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Accepted 1 January 2009
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20745