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Photobiomodulatory effects of superpulsed 904 nm laser therapy on bioenergetics status in burn wound healing

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ABSTRACT

Burn wounds exhibit impaired healing as the progression through the normal sequential stages of tissue repair gets hampered by epidermal barrier disruption, compromised blood circulation, abrogated defence mechanism, pathologic inflammation, and septicemia. Our earlier results reported that superpulsed 904 nm LLLT enhanced healing and attenuated inflammatory response in burn wounds. The present study investigated the effect of superpulsed 904 nm LLLT (200 ns pulse width; 100 Hz; 0.7 mW mean output power; 0.4 mW/cm^2 average irradiance) on biochemical and molecular markers pertaining to bioenergetics and redox homeostasis on full-thickness burn wounds in experimental rats. Results indicated that superpulsed laser irradiation for 7 days post-wounding propelled the cellular milieu towards aerobic energy metabolism as evidenced by significantly enhanced activities of key energy regulatory enzymes viz. HK, PFK, CS and G6PD, whereas LDH showed reduced activity as compared to the non-irradiated controls. LLLT showed a significant increased CCO activity and ATP level. Moreover, LLLT also regulated redox homeostasis as evidenced by enhanced NADPH levels and decreased NADP/NADPH ratio. Western blot analysis demonstrated that LLLT produced an up-regulation of GLUT1, pAMPKα and down-regulation of glycogen synthase1 (GS1). Our findings suggest that superpulsed 904 nm LLLT augments burn wound healing by enhancing intracellular energy contents through modulation of aerobic metabolism for maximum energy output. Bioenergetic activation and maintenance of redox homeostasis could be one of the noteworthy mechanisms responsible for the beneficial NIR photobiomodulatory effect mediated through superpulsed 904 nm LLLT in burn wound healing.

1. Introduction

The high susceptibility of burn wounds to infections and the intricacies associated with their impaired healing has made the development of novel therapeutic interventions for burn injury a fertile area of innovation for biomedical research. For the last few decades, low-level laser (light) therapy (LLLT) has quite been successful in gaining major attention as a potential biophysical modality for treatment of various pathophysiological conditions. Research endeavors to determine novel applications of LLLT are continuously under progress. A plethora of scientific evidences pointed that the application of LLLT, better known as photobiomodulation has continuously been evolving from being a therapeutic modality for wound repair, regeneration, dentistry, neuronal repair and mitigation of pain and inflammation to its current progression as a pre-conditioning measure for neuronal pain, muscle injury, myocardial infarction, etc. [1,2].

LLLT uses light typically of narrow spectral width in the red and near-infrared (NIR) range at a non-thermal irradiance, absorbed by photoreceptors located in the mitochondria, and perhaps also by chromophores in the plasma membrane of cells, altering the activity of one or more endogenous enzymes and electron transport, which could initiate cell signaling pathways and alter cellular metabolism as well as proliferation [3]. More recently, a number of studies also reported that the photobiomodulatory effects are induced by athermic physiological processes producing photochemical and photobiological changes at cellular and molecular levels [1,4,5]. Moreover, it has been suggested that radiation of visible and NIR activates retrograde light-sensitive cellular signaling events to transport the light signal from mitochondria to the nucleus and alter the cell metabolism and functions [4].

In LLLT, successful therapeutic outcomes require selection of optimum optical exposure parameters comprising wavelength, pulse frequency, irradiation time, pulse width/on time, pulse interval/off time, peak power and total fluence delivered at the time of irradiation. A thorough evaluation of the studies carried out to examine the contribution of continuous wave (CW) and pulsed wave (PW)-mode LLLT in the last few decades divulges their immense biomedical applications. The studies have shown beneficial bio-effects of CW-mode of LLLT [6,7] however, some recent studies demonstrated PW-mode to be advantageous over the CW mode, particularly, in the context of wound healing

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and stroke management [8–10]. It has been reported that pulsed light is more effective than CW, since there are quench periods (pulse-off times) of longer duration than the on-timings which reduces tissue heating. Kymplova et al. demonstrated that effects of PW were better than CW in the treatment of episiotomies [9]. Joensen et al. reported that the penetration effect of superpulsed 904 nm laser was more than that of 810 CW [10]. Another study done by Ando et al. on traumatic brain injury reported that therapeutic effect of LLLT with 810 nm laser was more pronounced at PW 10 Hz pulse frequency than PW 100 Hz and CW [11]. All these studies accomplished on a good note substantiated the potential of PW over CW showing its higher penetration capability, which can reach greater treatment depths at very high peak powers without any detrimental thermal effects [8].

Superpulsed (Ga-As, 904 nm) LLLT emits tremendously short pulses in the order of billionth of a second (10^{-9} s), and this unique feature entails it with a predominant mechanistic advantage of administering extremely high peak powers followed by accumulation of more energy in the tissue undergoing repair. These extremely small pulses allow quick absorption at the cellular level as well as the period between pulses promotes a better environment for enhanced cell communication leading to an optimum form of augmented healing. These numerous benefits of superpulsed 904 nm LLLT stimulated us to explore its therapeutic potential, particularly in the context of impaired wound healing. Recently, our study has shown that superpulsed 904 nm LLLT attenuated the inflammatory response and augmented healing in burn wounds [12].

Burn-induced physical trauma represents one of the most devastating challenges to modern biomedical sciences. The tissue repair process of burn wounds deviates considerably from the normal course of healing due to the associated complications such as prolonged inflammation, oxidative stress, free radical-induced damage, delayed granulation tissue formation, reduced angiogenesis and septicemia [12]. The incompetence of the cells of the wound bed to respond to healing stimuli is one of the hitches with burn wounds which exacerbate the healing process. In aggregate, such pathophysiological insults result in the failure of these wounds to heal. Energy regulation is very much essential during the repair and restoration process, since the injured tissue undergoes massive destruction. It is well recognized that cells adjacent to the site of injury get involved in increased mitotic and biosynthetic activities in order to carry out the repair process. It has been demonstrated that the proliferating epithelial cells as well as wounded skin are primarily dependent on the carbohydrate metabolism for energy requirement [13]. Therefore, glucose utilization gets significantly altered during tissue injury which definitely affects the progression of healing.

It has been shown that LLLT stimulates tissue repair to the normal maximal level and consequently at a rate that is generally faster than that found in non-irradiated tissues. Although the role of cellular energy metabolism in the healing process has been implicated in both normal and chronic wounds [13], the effect of superpulsed LLLT on bioenergetics status in burn wound healing remains unknown. In view of the aforementioned information, it was hypothesized that superpulsed 904 nm LLLT can modulate the activities and expression of key regulatory enzymes and bioenergetics molecular markers involved in cellular energy metabolism, which in turn may accelerate burn wound healing. Thus, the present study aimed to investigate the impact of superpulsed 904 nm LLLT (0.7 mW, 100 Hz, 0.4 J) on modulation of bioenergetics and redox homeostasis in full-thickness burn wounds in experimental rats.

2. Material and Methods

2.1. Experimental Animals

The animal experiments were performed in accordance with the regulations specified by the Institute's Animal Ethical Committee (IAEC-02/DIPAS/2013) and conform to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Government of India. Male adult Sprague-Dawley rats (180 ± 20 g; animal colony of DIPAS, Delhi) were used in this study. The animals were maintained under controlled environment at the Institute's animal house at 25 ± 1 °C and 12 h:12 h light:dark cycle. The animals were housed one per cage with access to food and water ad libitum.

2.2. Burn Wound Model

The animals were anesthetized by an intraperitoneal (i.p.) injection of a ketamine-xylazine cocktail (90 mg/kg ketamine and 10 mg/kg xylazine) before burn induction and during follow-up treatment. The dorsal surface of the rat was shaved using electric fur clipper, and the underlying skin cleaned with 70% ethanol. To create a full-thickness thermal burn injury, an aluminum metal rod (1.5 cm diameter) was heated to 85 °C and applied to the shaved skin for 20 s as previously described [12]. The temperature of the metal rod was monitored with a fabricated digital computerized multimeter. Hot rod was resting on its own weight of 30 g. No additional pressure was applied on the hand leaded metal rod. Single burn wound was created on the dorsal side of each rat. After 24 h, dead tissues were excised using sterile surgical blade and scissors. The wound was left uncovered during the whole period of experiment.

2.3. Experimental Design

Our results previously reported that superpulsed 904 nm LLLT enhanced healing and attenuated inflammatory response in burn wounds [12]. The present study investigated the influence of low-level superpulsed 904 nm laser on biochemical and molecular markers pertaining to cellular energy metabolism and bioenergetics using previously optimized superpulsed 904 nm laser optical exposure parameters (200 ns pulse width; 100 Hz; 0.7 mW mean output power; 0.4 mW/cm^2 average irradiance; 0.23 J/cm^2 total fluence) on full-thickness burn wounds in experimental rats.

A total of 24 animals were randomly divided into two groups of 12 animals each (non-irradiated burn control and superpulsed 904 nm laser irradiated experimental). Further, control and experimental animals were divided in two sub-groups of 6 animals each. One sub-group comprising of both control and experimental animals was used for analysis of key regulatory enzyme activities associated with cellular energy metabolism. Another sub-group of control and experimental animals was used to investigate the cytochrome *c* oxidase (CCO) activity, ATP, NADP/NADPH levels and protein expression levels of glucose transporter1 (GLUT1), glycogen synthase1 (GS1) and phosphorylated AMP-activated protein kinase-α (pAMPKα).

2.4. Low-level Superpulsed Laser Irradiation

LLLT was performed using a Ga-As diode superpulsed laser (904 nm, NIR) with 90 W peak power (Physiolaser Olympic Basic, RJ Laser, Germany) to deliver a light spot centered on the dorsal surface of the rat with full-thickness burn. The irradiance was measured using a 3A-ROHS with Nova II laser power/energy meter (Ophir Optronics Solutions Ltd., Israel). The distal tip of the laser probe was set for laser irradiation onto the burn wound at a power density of 0.4 mW/cm^2. The duration of laser irradiation was 10 min, and the total fluence delivered was 0.23 J/cm^2. The complete laser parameters were the same as used in the previous experiment [12]. LLLT was applied daily on the burn wounds at the same time for 7 consecutive post-wounding days with the first application being 1 h after creation of wound. Non-illuminated control rats were kept anesthetized for the same time period as the laser illuminated rats. Animals were sacrificed after 7 days post-wounding.