Journal of Photochemistry & Photobiology, B: Biology 186 (2018) 152–159

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Combination of medicinal honey and 904 nm superpulsed laser-mediated photobiomodulation promotes healing and impedes inflammation, pain in full-thickness burn

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ARTICLE INFO

Keywords:
904 nm superpulsed laser
Burn wound healing
Combination therapy
Medicinal honey
Photobiomodulation

ABSTRACT

Burn wound is a complex multi-factorial pathophysiology producing excruciating pain and psychological discomfort among patients, which imposes a major burden on the healthcare system. Multi-target therapy focuses on augmented healing by regulating different phases of tissue repair. Recently, photobiomodulation (PBM)-induced wound healing has achieved profound impetus as a non-invasive, drug-free biophysical therapeutic approach. On the other hand, medicinal honey known to possess antibacterial and immunomodulatory properties and is being used as an effective treatment option for infected wounds. The present study aimed to determine whether the combination of medicinal honey and PBM using superpulsed 904 nm laser treatment could additively accelerate full-thickness burn wound repair in rats. Animals were randomly allocated into 4 experimental groups: control (C), PBM superpulsed 904 nm laser treated (PBMT), honey treated (HT) and combined treatment (CT). The dual treatment exhibited an enhanced wound area contraction and hexosamine content as compared to the other groups. Histopathological analysis revealed increased cellular proliferation, extracellular matrix accumulation and decreased inflammation in the CT group. Further, the CT group demonstrated synergistically attenuated inflammation, pain and enhanced cell adhesion, migration as evidenced by significantly reduced protein expression of TNF-α, NF-κB, IL-1β, COX-2, substance-P receptor and up-regulation of fibronectin, respectively as compared with the other groups. Thus, the findings of present study signify that the combination of medicinal honey and PBMT accelerates the repair process of burn wounds. The study showed that therapeutic efficacy of 904 nm superpulsed laser-mediated PBM augments in the presence of medicinal honey by enhancing cellular proliferation and attenuation of inflammation and pain in burn wound healing.

1. Introduction

Thermal burns are one of the most prevalent and debilitating types of trauma, which show impaired repair as the normal sequential phases of healing becomes vulnerable by disruption of epidermal barrier, reduced angiogenesis, sustained inflammation, oxidative stress, enhanced proteolysis and septicemia [1]. These difficulties subsequently lead to intractable healing process that may require life-long rehabilitation, incur profound medical, social and financial encumbrance to the patients. Moreover, the multifaceted environment of the burn wound milieu limits the number of therapeutic interventions that could facilitate repair and hence, the field of burn wound healing has remained a remarkable source of innovative therapeutic interventions.

In the last few decades, rigorous efforts are constantly being aimed to search the optimum healing modalities to overcome the impediments associated with burn injury, either by novel medications or alternative medicine, or combination of both. In this context, recent advances in light-based dermatologic therapies have re-invigorated interest in the potential of photobiomodulation (PBM)-induced wound repair and regeneration to offer drug-free promising biophysical healing intervention [2, 3]. PBM could be described as a non-invasive form of phototherapy that make use of light emitted from coherent (laser) or quasi-coherent (filtered lamp, LED) source mostly in the red and near-infrared (NIR) spectrum at non-thermal irradiance to augment healing process, attenuate inflammation, ameliorate pain and restore functions.[3] The mechanistic explanation of PBM is still not clearly understood, however, the knowledge gained so far state that photon energy gets absorbed by intracellular endogenous chromophores (primarily by the mitochondrial complex-IV, i.e. cytochrome *c* oxidase, CCO), which elicits photobiological events by increasing ATP production, brief burst

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https://doi.org/10.1016/j.jphotobiol.2018.07.008
Received 15 March 2018; Received in revised form 5 June 2018; Accepted 7 July 2018
Available online 18 July 2018
1011-1344/

of ROS, photodissociation of nitric oxide, modulation of calcium and cAMP levels, which eventually regulate different downstream effectors molecules and transcription factors, activating retrograde mitochondrial signaling cascade and light sensitive/ heat-gated ion channels. All these events lead to enhanced cell proliferation, migration, protein synthesis and improved cell survival [3, 4]. There are ample scientific shreds of evidence demonstrating that PBM augments chronic non-healing wounds [5–9].

Honey, an ancient remedy in wound care, is gaining renewed popularity as alternative therapy for multi-drug resistant bacteria. The medicinal honey possesses anti-inflammatory, immunomodulatory, anti-microbial and wound healing activities [10, 11]. It is considered useful as topical antimicrobial agent against bacteria and fungi, due to its high osmolarity and hydrogen peroxide production by glucose oxidase. The clinical observations suggest that medicinal honey plays an important role in healing of infected wounds [12]. In spite of the multifactorial benefits of medicinal honey in the management of impaired wounds, the widespread acceptability is likely to be slow at best. Therefore, to find a combination that has both potential therapeutic healing and antimicrobial effects on the chronic wounds would be of great significance.

Findings from our previous investigations revealed that superpulsed 904 nm laser-mediated PBM significantly attenuated inflammatory response, enhanced cellular proliferation, granulation tissue formation and facilitated activation of bioenergetics, which eventually led to accelerate burn wound repair [5, 6]. However, the effects of PBM therapy on elimination of microbial infection have not been clearly elucidated. In chronic non-healing wounds, microbial infection hinders the normal pace of the healing process. The limited efficacy of single healing agent or modality to treat the multifactorial pathophysiology of burn wounds prompted us to explore the effect of multi-modality combination therapy. As per existing literature, no previous studies have been reported that could show the wound healing efficacy of the combination treatment of medicinal honey and PBM. Thus, the present study intends to investigate whether the combination of medicinal honey and PBM using superpulsed 904 nm laser could act synergistically in their action in augmenting full-thickness burn wound healing in rats.

2. Materials and Methods

2.1. Animals

Male Sprague-Dawley rats (180 ± 20 g) were taken from DIPAS animal colony. Animal experimental procedures were approved by the institute animal ethical committee (IAEC/DIPAS/2015–03). The animals were kept at 25 ± 1 °C with 12 h light: 12 h dark cycle and housed in a separate cage under aseptic conditions and provided ad libitum sterile rodent diet and water.

2.2. Burn Wound Induction

Hair was shaved from the dorsal skin of rat by means of electric fur clipper followed by surface cleaning using 70% ethanol. Burn wound induction on animal was carried out under a cocktail anesthesia of ketamine and xylazine (90 mg/kg and 10 mg/kg body weight) i.p., as per the previously described protocol [6]. Animals were allowed to recover and the excision of dead tissues was done post-24 h of burn induction. The wound was kept open throughout the study.

2.3. Study Design and Treatment Protocol

The present study was undertaken to evaluate the combined effects of photon interaction with biological system of healing process using 904 nm superpulsed laser [5, 6] and topical application of medicinal honey on full-thickness burns in rats. Forty-eight rats were randomly selected and allocated into two sets of twenty-four animals each. Both experimental sets comprise four groups of six rats each as follows:

Control (C) group: non-treated wounds; PBM treated (PBMT) group: wound irradiated with a 904 nm superpulsed laser (Ga—As diode) (Physiolaser Olympic Basic, RJ-Laser, Germany), the complete optical exposure parameters delivered were the same as previously described (100 Hz, 200 ns pulse-width, 0.2 J/cm^2 total energy density, 0.4 mW/cm^2 average power density, 10 min treatment) [5], PBMT was performed once daily for seven consecutive post-wounding days; medicinal honey treated (HT) group: the medical-grade Manuka *Leptospermum* honey (Medihoney, Derma Sciences Inc. Toronto, Canada, #31505) was applied topically (200 µl) over the wound surface with the help of sterilized applicator, 1 h post-creation of burn wound, once daily and followed for seven consecutive post-wounding days; combined treatment (CT) group: initially medicinal honey was applied topically over the burn wound surface followed by superpulsed 904 nm laser irradiation, post-3 h of honey application, this treatment regimen continued for seven consecutive post-wounding days, following which the animals of all 4 groups were euthanized.

2.4. Collection of Wound Tissue

The wound healing parameters were assessed after seven days post-wounding. In the earlier studies it has been shown that maximal changes in various parameters associated with biophysical, biochemical, cellular and molecular healing profiles pertaining to wound repair occur prominently during first week of post-wounding [5, 13]. Wound tissues obtained from all 4 groups were used to measure the biophysical, biochemical, histopathological and molecular markers for the assessment of healing (n = 6 samples for each parameter). The animals of the first set were used to measure pro-healing parameters viz. wound area contraction, DNA, total protein, hexosamine (HA, ECM marker), hydroxyproline (HP, collagen marker) and histopathological analysis. The animals of the second set were used to measure cytochrome *c* oxidase (CCO) activity, ATP content, transforming growth factor- β (TGF-β1) level, and protein expression levels of tumor necrosis factor-α (TNF-α), nuclear factor-kappa B (NF-κB), interleukin (IL)-1β, cyclooxygenase-2 (COX-2), substance-P receptor, fibronectin and Na, K-ATPase.

2.5. Pro-healing Marker Analysis

Photo-recording was performed on the day of burn induction (zero-day), fourth and eighth day post-wounding using Nikon Coolpix S3400 and wound area contraction was examined in digital pictures. The rate of wound contraction was analyzed using ImageJ software (NIH, USA) and values were represented in square millimeters, as described earlier [14]. The wound tissues excised after seven days treatment were used to assess the pro-healing markers like DNA, protein, HA and HP contents, according to the methods described previously [15–18].

2.6. Histopathological Analysis

Wound tissues were fixed in buffered formalin (10%). Four-µm thick tissue section was stained with H&E, and monitored for the histomorphometric changes under the microscope, and images were captured.

2.7. Tissue Processing for Molecular Analysis

Wound tissue homogenate was prepared in radioimmunoprecipitation assay (RIPA) buffer (20%, *w/v*) using Polytron homogenizer (PT3100, Switzerland) and centrifuged at 4000 x g for 30 min at 4 °C. The supernatant was used for differential protein expression analysis using immunoblotting, TGF-β1 protein level quantification using ELISA, CCO enzyme activity and ATP content analysis. The protein concentrations were measured followed by the method of Lowry et al. [16].