Journal of Photochemistry & Photobiology, B: Biology 169 (2017) 63–69

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

The effect of combined photobiomodulation and metformin on open skin wound healing in a non-genetic model of type II diabetes

Mohammadali Asghari [a], Ali Kanonisabet [a], Mandanad Safakhah [a], Zahra Azimzadeh [a], Ataroalsadat Mostafavinia [b], Soudabeh Taheri [c], Abdollah Amini [b], Seyed Kamran Ghorishi [d], Reza JalaliFiroozkohi [a], Sahar Bayat [e], Mohammad Bayat [f,*]

[a] School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c] Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Department of Statistics, Qom University, Qom, Iran
[e] Nicholas Senn High School, Chicago IL, USA
[f] Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 16 January 2017
Accepted 2 March 2017
Available online 6 March 2017

Keywords:
Photobiomodulation
Low-level laser therapy
Type II diabetes mellitus
Non-genetic model of type II diabetes mellitus
Wound healing
Biomechanical properties
Microbial flora
Rat

ABSTRACT

This study intended to examine the combined influences of photobiomodulation (PBM) and metformin on the microbial flora and biomechanical parameters of wounds in a non-genetic model of type II diabetes mellitus (TII DM).

We induced a non-genetic model of TII DM in 20 rats by feeding them a 10% fructose solution for 2 weeks followed by an injection of streptozotocin (STZ, 40 mg/kg). After 21 days from the injection of STZ, we induced one full-thickness skin wound in each of the diabetic rats. We randomly divided the rats into four groups: i) placebo; ii) pulsed wave laser (890 nm, 80 Hz, 0.324 J/cm^2); iii) metformin; and iv) laser + metformin. Rats received daily intraperitoneal injections of metformin (50 mg/kg). On days 7and 15 we inspected the microbial flora of each wound. On day 15 we obtained a standard sample from each healing wound for biomechanical analyses.

PBM significantly decreased colony-forming units (CFUs) 7 days after wound infliction compared to the placebo group (LSD test, $p = 0.012$). Metformin significantly enhanced the biomechanical property (stress high load) of the wounds compared to the placebo group (LSD test, $p = 0.028$). We observed the same significant result for PBM compared to the placebo group (LSD test, $p = 0.047$).

PBM significantly accelerated the wound healing process and significantly reduced CFUs of bacteria in a non-genetic rat model of TII DM.

1. Introduction

Type II diabetes mellitus (TII DM) is a complex heterogeneous group of metabolic disorders that include hyperglycemia and impaired insulin action and/or insulin secretion. TII DM causes dysfunctions in multiple organs and tissues. Globally, TII DM affects a large population of individuals [1], accounting for over 90% of DM cases [2]. Diabetic foot ulcers (DFUs) are a common complication of DM and present a significant health risk to patients, as well as impose a large economic burden [3]. Approximately 15% of patients who have DM will experience a DFU during their lifetimes, with an annual incidence of 1%–4% [3]. DFUs typically develop in patients that have neuropathy which affects approximately 85% of patients with DFUs, peripheral vascular disease (15%), or both (neuroischemic foot ulcer). Patients with neuropathy may have sensory peripheral neuropathy, motor sensory neuropathy, or autonomic neuropathy, which lead to DFUs [3–5]. Diabetic patients with ischemia are at higher risk for amputation, which is considerably enhanced in the presence of an infection. Amputation is proceeded by DFU in 80% of cases [6]. Subsequently, chronic DFUs present a clinical challenge for physicians who treat DM patients [7].

The biological process of wound healing in humans comprises four accurately and highly programmed phases: hemostasis, inflammation, proliferation, and remodeling. All four phases must occur in the appropriate sequence and time frame for a wound to successfully heal. When

* Corresponding author at: Cellular and Molecular Biology Research Centre, Shahid Beheshti University of Medical Sciences, 1985717443, 19395/4719, Tehran, Iran.
E-mail addresses: mohammad.a.asghari@gmail.com (M. Asghari), alikanoonisabet@yahoo.com (A. Kanonisabet), mandanasafakhahmd@gmail.com (M. Safakhah), zahahraazimzadehmd@gmail.com (Z. Azimzadeh), a.mostafavinia@gmail.com (A. Mostafavinia), so_taheri@yahoo.co.uk (S. Taheri), d.amini2008@yahoo.com (A. Amini), atty_ghoreishi@yahoo.com (S.K. Ghorishi), reza.jalali.7082@gmail.com (R. JalaliFiroozkohi), sbayat1@cps.edu (S. Bayat), mohbayat@sbmu.ac.ir (M. Bayat).

http://dx.doi.org/10.1016/j.jphotobiol.2017.03.002
1011-1344/

the tissue is damaged, the normal healing response commences. At the time of blood leakage into the injury site, platelets make contact with exposed collagen and release clotting factors, Platelet Derived Growth Factors (PDGF), and Transforming growth factor beta (TGF-β). Following hemostasis, neutrophils and macrophages enter the wound site. The process of phagocytosis, as part of this inflammatory phase, is continued by macrophages which continue to release additional PDGF and TGF-β. The proliferative phase is initiated by fibroblasts which migrate to the wound site once it is cleared out. The new collagen matrix is deposited by fibroblasts and then, at the final phase of remodeling, becomes cross-linked and organized. In order for this efficient, highly controlled healing process to occur, numerous cell-signaling events are necessary [8]. Numerous factors can interfere with one or more phases of this process and cause improper or defective wound healing [8,9]. Severely impaired wound healing occurs in DM [10–14]. The lack of epithelial migration (absence of the epithelial tongue) at the nonhealing wound edge occurs in chronic ulcers [10]. Fibroblasts show phenotypical alterations as well as reduced migration and proliferation. These cells have the ability to respond to administration of additional growth factors or cellular therapy.

Despite the existence of consistent protocols in standardized care, physiological impairments can result in DFU complications and delayed healing [10,11]. A high glucose environment is accompanied by changes in cellular morphology, reduction of proliferation, and abnormal keratinocyte differentiation [12]. Stress high load (maximum stress) and bending stiffness (modulus) of both murine and human diabetic skin wounds have shown significant reductions in comparison with nondiabetic skin [13]. Defective proliferation and migration of dermal fibroblasts takes place because of glycation [14,15].

Photobiomodulation (PBM) is used in the clinic to stimulate open skin wound healing in diabetic animals. Despite a few studies that have used animal models of TII DM, the majority of research that has examined the influences of in wound healing was performed in animal models of TIDM. These studies have used genetic diabetic models [16–22]. There may be considerable differences in wound healing response among inbred animal strains [23]. However when using such strains, variance falls to levels that can yield significant findings with small group sizes [24]. The speed of wound closure [16,17,19,22], histological evaluation [16–20,22], blood glucose and glycated hemoglobin (hemoglobin A1c) [21], and growth factor expression (17, 22) have been used to analyze the experimental results [16–22]. Histological slides have assessed wound epithelialization, granulation tissue formation, cellular content, and collagen deposition. These results showed that PBM improved wound healing in genetic diabetic models mainly from a histological point of view.

Metformin, a biguanide, is currently a first line medication for initial treatment of TII DM that works by suppressing glucose production by the liver via activation of AMP-activated protein kinase (AMPK) [23]. Although one of the earliest medications prescribed to patients with TII DM, few studies have investigated the influence of metformin on wound healing [24].

The results of two investigations clearly show reduced collagen synthesis and enhanced catabolism of collagen associated with accelerated conversion of soluble to insoluble collagen in experimentally induced DM [25,26]. Decreased wound tensile strength was associated with decreased amounts of collagen synthesis in diabetic animals. When compared with the control group, skin from diabetic animals had 30% less collagen [27]. Wounded animals with uncontrolled DM had decreased wound strength [28]. The biomechanical test has previously been utilized to evaluate wounds in studies on laser-treated animals [29–31].

Recently, a fructose-fed streptozotocin (STZ)-induced TII DM rat model was developed to study TII DM or test anti-diabetic agents [32,33].

Currently, no solid data has been reported that determined the effect of combined PBM and metformin on the healing process of full-thickness skin incisions in a fructose-fed STZ-induced TII DM rat model. Results from two recent studies showed that pulsed wave laser with an 890 nm wavelength, 80 Hz frequency, and 0.2 J/cm^2 energy density significantly accelerated the healing process in TI DM rats [31,34]. The main objective in this investigation focused on the combined effect of PBM in addition to the use of metformin on the healing process of incisions in TII DM rats. The combined use of PBM and metformin could improve severe DFUs in patients. We evaluated tensiometric (biomechanical) properties of healing skin incisions by a materials testing machine and examined microbial flora from the wounds by routine microbiological tests.

2. Materials and Methods

2.1. Animals and Study Design

We purchased 20 adult male Wistar rats (4 months of age) that weighed ~220 g from Pasteur Institute (Tehran, Iran). Rats were individually housed in standard rat cages in a standard animal house on a 12-h light/12-h dark schedule at approximately 24 °C. Animals received water and food ad libitum. TII DM was induced in all rats. We made an incision on the skin of each animal's back. The rats were randomly divided into 4 groups of 5 animals per group. Group 1 was the placebo (control) group that received vehicle control medium and a switched off laser. The second group received laser. The third group received metformin and the fourth group received laser + metformin. On days 7 and 15, we examined the microbial flora from the wounds. On day 15, a sample from each healing wound was submitted for biomechanical examination. The Medical Ethics Committee at Shahid Beheshti University, Tehran, Iran approved all procedures of the current study (protocol no. 5849).

2.2. Induction of Type II Diabetes Mellitus (TII DM)

TII DM is a heterogeneous disorder characterized by insulin resistance and partial pancreatic β-cell dysfunction. During the first 14 days of the study, the animals received a 10% fructose (Biobasic, Canada) solution to induce insulin resistance instead of drinking water. After the 14-day period, the animals received intraperitoneal injections of STZ (40 mg/kg; Enzo Life Sciences, Inc., USA) dissolved in sterile distilled water to induce partial pancreatic β-cell dysfunction. One week after the STZ injection, we used a portable glucometer (Glucoplus, GM300, Biomince, GMH, Heerbrugy, Switzerland) to measure nonfasting blood glucose (NFBG) levels in blood collected from the animals' tail veins. Animals with a NFBG level >200 mg/dl were considered diabetic. Animals with a NFBG level <200 mg/dl were excluded from the study [32].

2.3. Body Weight and Blood Glucose

We evaluated body weight changes and blood glucose levels weekly during the experimental period.

2.4. Surgery

On the 21st day after receipt of the STZ injection, we anesthetized the diabetic rats with ketamine hydrochloride (i.m., 50 mg/kg body weight) along with diazepam (i.m., 5 mg/kg body weight). Subsequently, the fur on the back of rats was shaved and the shaved area was sterilized with alcohol and povidone/iodine. One longitudinal, full thickness 12-mm incision was made on the upper thoracic region with a No. 15 blade and scalpel.

2.5. Photobiomodulation and Metformin Administration

Wounds on the animals from the second and fourth groups were exposed to pulsed wave laser at 0.324 J/cm^2 [MUSTANG 2000 with LO7