Journal of Photochemistry & Photobiology, B: Biology 186 (2018) 81–87

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

Photobiomodulation improved stereological parameters and sperm analysis factors in streptozotocin-induced type 1 diabetes mellitus

Sara Dadras[a], Mohammad-Amin Abdollahifar[a], Hamid Nazarian[a], Seyed Kamran Ghoreishi[b], Somaye Fallahnezhad[a], Parvaneh Naserzadeh[c], Vahid Jajarmi[d], Sufan Chien[e,1], Mohammad Bayat[a,*]

[a] Department of Biology and Anatomical Sciences, Shahid Beheshti University of Medical Science, Tehran, Iran
[b] Department of Statistics, University of Qom, Qom, Iran
[c] Department of Pharmacology and Toxicology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[e] Price Institute of Surgical Research, University of Louisville, and Noveratech LLC of Louisville, Louisville, KY, USA

ARTICLE INFO

Keywords:
Photobiomodulation
Diabetes mellitus
Testicle
Stereological parameters
Sperm analysis factors
Low-level laser therapy

ABSTRACT

The aim of this study was to evaluate the effect of photobiomodulation (PBM) on testicular tissues and fresh sperm analysis factors in streptozotocin (STZ)-induced type one diabetes mellitus (T1DM) mice.

T1DM was induced in 15 male Syrian mice by injection of 200 mg/kg STZ. After one month, mice were divided randomly into three groups, harboring 5 mice each: 1, control group; 2, first laser group (890 nm, 80 Hz, 0.03 J/cm^2) and 3, second laser group (0.2 J/cm^2). Then the mice were euthanized and testicles were dissected for stereological studies, and both epididymis and vas deferens were removed for fresh sperm analysis. Data were analyzed by statistical methods.

A significant increase was observed in the Sertoli cell count in both PBM groups, compared to the control group. In addition, the second PBM group shows a significant increase in the Sertoli cell count, compared to the first PBM group. Both PBM groups show significant increase in the Leydig cell count, compared to the control group. There were significant increases of the length in the seminiferous tubules in both PBM groups, compared to the control group. In addition, the second PBM group showed a significant increase of the length in the seminiferous tubules, compared to the first PBM group. The second PBM group showed a significant increase in the sperm count, compared to the control, and first PBM groups. The first PBM group showed a significant increase in sperm count, compared to the control group. The sperm motility and count were significantly increased in the second PBM group, compared to the control and first PBM groups. The sperm motility was significantly increased in the first PBM group, compared to the control group.

PBM with 0.2 J/cm^2 and 0.03 J/cm^2 energy densities significantly improved the stereological parameters and fresh sperm analysis factors, compared to the control group in STZ-induced T1DM in mice. Moreover, the PBM with 0.2 J/cm^2 energy density was statistically more effective, compared to the 0.03 J/cm^2.

1. Introduction

In 2014,422 million adults were living with diabetes mellitus (DM) worldwide [1]. DM affects male reproductive organ and leads to infertility [2]. More than 124 million patients with DM have also sexuality and fertility complications. It has been stated that about 50% of diabetic males have sexual problems [3]. Testicular disorders, and declined fertility potential are problems that have been reported in male patients with DM. Reduced semen volume, decreased sperm motility, sperm count, normal morphology, and reduced serum testosterone have been reported in male with DM [3].

Photobiomodulation (PBM) stimulates growth and cellular proliferation in many cells and it has been presented as a beneficial method [4]. Several experiments have been performed to define the positive effects of PBM on the testicle or spermatogenesis both in animal models and cell culture systems [5–17]. In these studies PBM enhanced Ca2+ uptake by the mitochondria, improved Ca2+ binding to sperm cell membranes, increased the velocity of the fresh sperm of dogs, and

* Corresponding author.
E-mail addresses: bayat_m@yahoo.com, mohbayat@sbmu.ac.ir (M. Bayat).
[1] Supported in part by NIH grant DK105692.

https://doi.org/10.1016/j.jphotobiol.2018.06.018
Received 30 March 2018; Received in revised form 26 June 2018; Accepted 27 June 2018
Available online 30 June 2018
1011-1344/

healthy and oligospermic and asthenospermic patients, improved spermatogenesis, reduced paroxetine-induced penile anesthesia, restored testicular degeneration in rams, and improved the amount of cells in seminiferous tubule [5–17].

Pulsed wave (PW) laser suggests many probable advantages [18]. Because there are pulse OFF times following the pulse ON times, PW lasers could create fewer tissue heat than continuous wave (CW) lasers [18].

To date, there is no evidence regarding the effects of PBM on male reproductive organ in diabetic subjects. Here, we evaluated the effect of PWPBM on testicular tissues in streptozotocin (STZ)-induced type one DM (T1DM), in mice by the stereological parameters, including numbers of spermatogonia, primary spermatocytes, spermatid, Sertoli and, Leydig cells, seminiferous tubules, and testicle volume and interstitial testicle volume. We also analyzed fresh sperm analysis factors, including sperm motility, sperm count, and sperms with abnormal morphology.

2. Materials and Methods

2.1. Animals and Study Design

We used 15 male Syrian mice which were obtained from Pasteur Institute of Iran the Production and Research Complex, Tehran, Iran. All mice were housed individually in standard mice cages. A standard mice and rat diet was provided, and the lighting was maintained below 25 Lux. In order to enable operators in the rat rooms to perform visual tasks, light/dark cycles of 12 h/12 h were employed, and dimmers were used to create two light periods between the light and dark cycles. Animals were kept at room temperature (20–22 °C, and 24–26 °C during surgery) and the humidity was maintained at 40%–70%. The room ventilation rate was kept at of approximately 15–20 air changes per hour.

T1DM was induced in mice by injection of 200 mg/kg STZ. All animals were kept for one month after administration of STZ, in order to establish DM. Mice were divided randomly into 3 groups, harboring 5 mice each: 1, control group; 2, first laser group (890 nm, 80 Hz, 0.03 J/cm^2, 3 times per week, 3 weeks)and 3, second laser group (0.2 J/cm^2).

In our study, the sample size for each group was determined based on a simple linear mono-gram approach, introduced by Day and Graham [19] with a conservative size of 5. Here, the approximate power and level of the corresponding test were 0.9 and 0.05, respectively. After 3 weeks, mice were euthanized and one testicle was dissected for the stereological studies, and both epididymis and vas deferens were removed for fresh sperm analysis. Data were analyzed by statistical methods.

2.2. Induction of T1DM

All methods were accepted by the Medical Ethics Committee of Shahid Beheshti University of Medical Sciences with IRB approval number (IR.SBMU.MSP/REC.1395.583). Mice were housed in a standard animal home. T1DM was induced in all mice by dissolving STZ in distilled water (200 mg/kg, single dose, I.P.) [20]. T1DM has been defined as a blood glucose level > 250 mg/dl, measured using the blood samples withdrawn seven days after the STZ injection. The mice, blood glucose levels and body weights were recorded throughout the experiment. All animals were kept for one month after administration of STZ, in order to establish DM [21].

2.3. PBM

The both testicles of groups 2 and 3 were subjected to an infrared laser [MUSTANG 2000, LO7 pen, Technica Co., Moscow, Russia], using the following parameters:

- Peak power output: 75 W
- Average power: 1.08 mW
- Power density: 1.08 mW/cm^2
- Wavelength: 890 nm
- Pulse frequency: 80 Hz
- Sport size: 1 cm^2
- Pulsed duration: 180 μs
- Duration of exposure of each testicle for first and second laser groups was 30 s and 200 s, respectively.
- Energy density for first and second laser groups was 0.03 J/cm^2 and 0.2 J/cm^2, respectively.

The PBM protocol was initiated after 30 days of STZ administration, and performed three sessions per week, for a period of three weeks. Each testicle was received one shooting of laser. During the experiment, the first group was submitted to the PBM with the laser machine being switched off, and was considered the placebo (control) group. Finally, the entire group of mice was euthanized and all testicles were dissected. Both testicles were submitted for stereological examinations, and bilateral epididymis and vas deferens were used for sperm morphology, motility, and viability tests.

2.4. Pathology and Stereology

The testicles were fixed in Bouin's Solution overnight, and submitted for light microscopy examination. Ten sections with 5 and 20 μm thicknesses were stained with Hematoxylin & Eosin method, and 10 microscopic fields with magnification of 40× were studied for each case.

2.4.1. Estimation of the Number of Cells

By using the optical dissector method and systematic uniform random sampling (SURS), the total number of spermatogenic cells (numbers of spermatogonia, primary spermatocytes, spermatid) and Sertoli and, Leydig cells) were determined. The microscopic field position was adopted by an equal interval of moving the stage and systematic uniform random sampling [22, 23]. Microcator was used for measurement of *Z*-axis movement of the microscope stage. An unbiased counting frame with exclusion and inclusion borders was superimposed, according to the sections' images, observed on the monitor. A cell was counted, if it was placed completely or partially within the counting frame and did not reach the exclusion line. The formula for calculation of numerical density (Nv) is as follows:

$$Nv = [\Sigma Q/(h \times a/f \times \Sigma p)] \times (t/BA)$$

In which “h” has been recognized as the height of the dissector. “ΣQ-”, “ΣP” and “a/f” have been recognized as the number of the nucleus, the total number of the unbiased counting frame in all fields and the frame area, respectively. BA has been recognized as the block advance of the microtome, set at 20 μm. Eventually, “t” has been recognized as the real section thickness measured in every field, using the microcator. The total number of the spermatogenic cells was evaluated by multiplying the numerical density (Nv) and the total volume (V) (Figs. 1 and 2) [24].

$$N\ (total) = NVv \times V\ (final)$$

2.4.2. Estimation of the Length Density of the Seminiferous Tubules

The following formula was used for measuring length density of the seminiferous tubules (Fig. 3):

$$LV = 2\Sigma Q/(\Sigma P \times a/f)$$

In this formula, “ΣP” and “a/f” were recognized as the total number of the counted frame. “ΣQ” was denoted as the total number of the vasa deference counted per sample and a/f means the area of the counting [24].