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Therapeutic Effects of 10-HzPulsed Wave Lasers in Rat Depression Model: A Comparison Between Near-Infrared and Red Wavelengths

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Background and Objective: The application of transcranial low-level light/laser therapy (tLLLT) in the range of red to near-infrared (NIR) spectrum for psychological disorders is a new area that is attracting growing interest in recent years. The photomodulation effects of NIR and red coherent lights on the activity of cytochrome c oxidase in neuronal cells of brain have been recently introduced. This study, therefore, sought to compare the therapeutic effects of 10-Hz pulsed wave NIR (810 nm) laser with red (630 nm) laser using the same delivered energy density and Citalopram in rat chronic mild stress (CMS) model of depression and anxiety.

Materials and Methods: CMS procedures (for 4 weeks) were used to induce stress. GaAlAs diode laser with red and NIR wavelengths on 10-Hz pulsed wave (50% duty cycle) were used to perform tLLLT treatment for three weeks. An energy density of about 1.2 J/cm^2 per each session was delivered through a light spot with a diameter of 3-mm to the prefrontal cortex for both wavelengths. Citalopram (10 mg/kg, Intraperitoneal) was administered for twenty-one consecutive days to the drug group.

Results: The findings of the present study showed an increase in swimming and decrease in immobility time, for both NIR laser and Citalopram groups compared to the stress group in forced swimming test. Anxiety-like behaviors showed insignificant decrease in all treatment groups in elevated plus maze test. The induction of stress significantly increased serum cortisol levels and treatments with both red laser and Citalopram decreased it. Hyperglycemia induced by CMS returned to normal levels in all treatment groups. The assessment of body weight also showed a significant increase in NIR laser group compared to the stress group by the end of the experiment.

Conclusions: This study showed that non-invasive tLLLT using 10-Hz pulsed NIR laser light was as effective as Citalopram and more effective than red laser in the treatment of depressive-like behaviors and may help improve tLLLT as an alternative non-pharmacological treatments of psychological disorders such as depression. Lasers Surg. Med.48:695–705, 2016. © 2016 Wiley Periodicals, Inc.

Key words: transcranial low-level laser therapy; near-infrared light; chronic mild stress; cortisol; depressive-like behavior; anxiety-like behavior

INTRODUCTION

Depression is a common and severe mood disorder which would always needs urgent treatment. The prevalence of major depression disorder (MDD) amounts to about 16.6% of adults at some point in their lifetime [1]. It has been predicted that, by 2020, MDD will rank as the second leading cause of global disability [2]. Despite the fact that pharmacotherapy is a common and effective method for the treatment of depression, factors like delayed onset, dedication to the treatment procedures, and side effects influence its efficacy [3]. Moreover, about 30% of patients do not respond to the normal dose of antidepressants [4]. When pharmacotherapy fails to ease the symptoms of major depression, electroconvulsive therapy is an alternative psychiatric treatment [5]. However, memory impairment caused by this therapy has a direct relation to the frequency of treatment [6]. Based on the evidence, these two conventional therapies provide insufficient treatment for depression. Therefore, alternative or complementary non-pharmaceutical methods receive close attention in psychological therapies.

In recent years, transcranial low-level light/laser therapy (tLLLT) has been introduced as a brain photoneuromodulation method which uses red to near-infrared (NIR)

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lights spectrum (600–1,100 nm) to induce transform biochemical changes within neuronal cells by delivering energy through the skull [7–10]. The electromagnetic spectrum at wavelengths between 600 and 830 nm have been introduced as a safe method [11,12], being quite applicable to neurological disorders [8,13,14]. The non-ionizing energy levels in this spectrum are between 2.06 and 1.49 eV/photon (according to Plank's equation: $E(eV) = 1{,}240/\lambda(\text{nm})$ energy can be calculated for each photon based on its wavelength) which lead to appropriate electronic excitation of some organic molecules and photochemical reactions [15]. Several studies have indicated that cellular activation could be triggered by the interaction between this light spectrum and some mitochondrial photoacceptors and would lead to an increase in adenosine triphosphate (ATP) [16–18]. Cytochrome c oxidase is a primary mitochondrial chromophore for photomodulation that absorbs energy in this safe spectrum [19]. In addition, some studies have shown that photomodulation by LLLT has neuroprotective effects in neuronal cells [20] and improves some functions in cells such as modulation of reactive oxygen species, efficiency in the flow of electrons through the mitochondrial respiratory chain, and increases in intracellular calcium ions [21].

Using non-invasive tLLLT to improve emotional and psychological functions has been proposed by scientists as a promising hypothesis. The first study in this regard studied beneficial therapeutic effects of photoneuromodulation, conducted by Schiffer et al. [22] in patients with major depression and anxiety. It was designed to deliver NIR Light emitting diodes (LEDs) energy to the forehead of patients. Some studies in recent years have been also carried out using transcranial NIR LEDs and laser lights that have pointed to the safety of the methods used, as well as reduced levels of depression in the patients with MDD [23–25]. In addition, the efficiency of the tLLLT using NIR pulsed wave (PW) in reducing depressive-like behaviors in animal models have been among other findings [26,27]. However, effective tLLLT treatment parameters for depression and anxiety disorders remain largely unexplored.

Two wavelengths (630 nm and 810 nm) of laser lights on 10-Hz PW mode were used to compare the photoneuromodulation effects of tLLLT with those of Citalopram in rat chronic mild stress (CMS) model. The CMS is the most widely used animal model to investigate depressive-like and anxiety-like behaviors when the animal is exposed to a series of mild and unpredictable stressors over several weeks [28].

The aim of this study was to determine antidepressant and anti-anxiety effects of 10-Hz PW red and NIR tLLLT in CMS rat model. Also, transmission factor and optical characteristics of the skull and brain tissue were measured in male Wistar rats for 630 and 810 nm wavelengths, which are generally used in the neurological disorders.

MATERIALS AND METHODS

Subjects

Fifty Wistar male at an average age of twelve weeks weighing between 180–200 g provided from the animal house of Tabriz University of Medical Sciences (TUMS) were used in this study. Upon admission, all animals were evaluated for general health and mobility. Rats were kept on a 12-hour light/12-hour dark cycle and room temperature of 23–25°C with food and water freely available except during experimental procedures. Prior to the experiments, rats were allowed ten days to adapt to the environmental conditions. During the experiment, all animals were weighed weekly for body weight. The research protocol of this study was approved by Ethics and Research Committee of TUMS (Code number 93/1-10/10).

CMS Procedure

The rats were randomly divided into two groups: one control group ($n = 10$), and one CMS group ($n = 40$). The control group was housed in a different room and had no contact with the stressed animals. These animals were not subjected to any kind of manipulation except for weighing and box cleaning (three times a week). Forty rats in CMS group were exposed to the CMS procedure for one week and the procedure was repeated during the following four weeks. The CMS procedure of this study was consistent with methods reported by Lewitus et al. [29]. Each week of stress regime consisted of eight different stress situations such as: two periods of stroboscopic illumination (300 flashes/min), one period of soil bedding, two periods of white noise (80 dB), two periods of 45° cage tilt, one period of paired housing, periods of food and/or water deprivation where water deprivation was followed by exposure to an empty water bottle and food deprivation was followed by restricted food. At the end of CMS induction, the CMS group was divided into stress, Citalopram, red laser and NIR laser groups ($n = 10$ each). Details of the study design, including procedures, time periods, and behavioral tests are shown in (Fig. 1).

Laser Treatment

Twenty rats from CMS group were randomly divided into two groups that received red and NIR laser a day after CMS procedures were implemented. A couple of red ($\lambda = 630$ nm) and NIR ($\lambda = 810$ nm) probes derived by GaAlAs diode laser (Mustang 2000+, Moscow, Russia), with the output power of 35 ± 2mw and 240 ± 5mw, respectively, were used in this study. The laser mode was set on 10-Hz PW with 50% duty cycle and 2-millisecond duration time for both probes. Rats were placed in a Plexiglas restrainer for the duration of the treatment. The laser probe was placed on the midline of the dorsal surface of the shaved scalp overlying the skull in the prefrontal region (Fig. 2). The probe was in contact with scalp through drop of water as coupling media. Laser irradiation at the tip of the acupuncture nuzzle, 3 mm in diameter, was set to give a power of 6.2 ± 0.1 mw for red and 39.3 ± 1.1 mw for NIR probes. The laser beam area was 0.07 cm^2 and the power densities were 89 mw/cm^2 and 562 mw/cm^2 for red and NIR beams, respectively. An average energy density of 1.18 ± 0.01 J/cm^2 per each

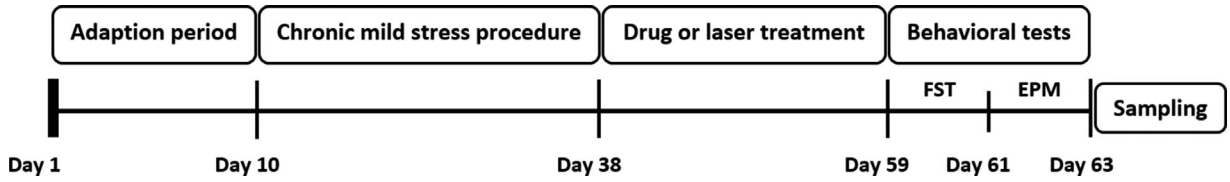

Fig. 1. Timeline diagram of the experiments. Animals exposed to CMS procedures for four weeks. A day after of the CMS procedures three treatment groups received Citalopram, red and NIR laser treatments for three weeks. Stress group did not receive any treatment during these weeks. Rats subjected to forced swimming test (FST), and elevated plus maze (EPM) the days after the last treatment sessions. Sampling was done following the experiment of the EPM for measuring the serum cortisol and glucose levels. CMS, chronic mild stress; NIR, near-infrared.

session and about 14.4 J/cm^2 over entire treatments were delivered to the animal's prefrontal cortex with both red and NIR lasers. This proposed energy density was determined based on our preliminary measurements on rats' skull and scalp to estimate the amount of delivered dose on the prefrontal cortical surface. The details of this measurement are explained in the "Laser Beam Transmission Measurements" session. Transcranial LLLT treatments were done four times a week for three weeks.

Citalopram Treatment

Ten rats from CMS group were randomly chosen. Animals in this group received Citalopram (Mahban-Daru, Pharmaceutical Company Tehran, Iran) with a daily dose of 10 mg/kg by Intraperitoneal (ip) route a day after the implementation of CMS procedures for twenty-one consecutive days. The Citalopram dose was chosen based on previously published protocol [30]. The control group received saline instead of Citalopram.

Fig. 2. The schematic view of the laser beam location over the rat head. The laser probe was held over prefrontal region of the skull in direct contact with the shaved scalp.

Forced Swimming Test

The FST is a behavioral model and one of the most widely used tests to evaluate antidepressant activity in rats [31]. The FST procedure used in this study was the same as that previously applied by Kawaura et al. [32]. After the treatment period, all animals were subjected sequentially to this test. This procedure was conducted by placing rats in a transparent glass cylinder (Height: 50 cm, Diameter: 25 cm) containing water to a height of 30 cm at 25 $\pm$ 1°C. The water was replaced by fresh water after every trial. The test duration consisted of a ten minutes period and was conducted between 09:00 and 13:00 hours. The test session was digitally recorded for each animal and subsequently scored manually. The parameters of interest were time spent swimming (showing active swimming motions, more than necessary to merely maintain its head above water, for example, moving around in the cylinder), climbing (making intense movements with its forepaws in and out of the water, usually directed against the walls), and immobility (floating without struggling and making only those movements necessary to keep the head above the water).

Elevated Plus Maze

The EPM is one of the commonly used and well-known tests of anxiety-like behaviors in rats [33]. The EPM of the present study was a plus shaped apparatus and consisted of two (50 $\times$ 10 cm) open arms, and two (50 $\times$ 10 $\times$ 50 cm) enclosed arms and a central platform (10 $\times$ 10 cm), elevated to a height of 50 cm. The apparatus was located in the center of room and testing was carried out in a quiet, dimly lit room, between 13:00 and 15:00 hours and was recorded by a camera positioned above the apparatus. The duration of the test was five minutes. Rats were placed individually in the central zone, facing one of the open arms. An animal was considered to be on the open or closed arms (spent time) whenever all four paws were in the respective arm. The EPM was cleaned with a 70% ethanol solution between each test. The parameters of interest