Neuroscience Letters 553 (2013) 99–103

Contents lists available at ScienceDirect

Neuroscience Letters

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

Thermal effects of transcranial near-infrared laser irradiation on rabbit cortex

Yongmei Chen [a], Luis De Taboada [b], Michael O'Connor [a], Scott Delapp [b], Justin A. Zivin [a,*]

[a] University of California San Diego, Department of Neuroscience, 9500 Gilman Drive MTF321, La Jolla, CA 92093-0624, USA
[b] PhotoThera, Inc., 6190 Corte Del Cedro, Carlsbad, CA 92008, USA

HIGHLIGHTS

- We examined thermal effect and safety of transcranial near-infrared laser therapy.
- Laser powers utilized in animal stroke research do not cause cortical tissue heating.
- Repeated laser treatment is safe to treat stroke injury.

ARTICLE INFO

Article history:
Received 10 June 2013
Received in revised form 22 July 2013
Accepted 28 July 2013

Keywords:
Lower level laser therapy
Photothermal effect
Safety

ABSTRACT

Transcranial near-infrared laser therapy (TLT) improves stroke outcome in animal models. Adequate laser doses are necessary to exert therapeutic effects. However, applying higher laser energy may cause cortical tissue heating and exacerbate stroke injury. The objective of this study is to examine the thermal effect and safety of transcranial near-infrared laser therapy. Diode laser with a wavelength of 808 nm was used to deliver different power densities to the brain cortex of rabbits. Cortical temperature was monitored and measured using a thermal probe during the 2 min transcranial laser irradiation. Neuro-pathological changes were examined with histological staining 24 h after laser treatment. Transcranial laser irradiation for 2 min at cortical power densities of 22.2 and 55.6 mW/cm^2 with continuous wave (CW) did not increase cortical temperature in rabbits. With the same treatment regime, cortical power density at 111.1 mW/cm^2 increased brain temperature gradually by 0.5 °C over the 2 min exposure and returned to baseline values within 1–2 min post-irradiation. Separately, histological staining was evaluated after triple laser exposure of 22.2 mW/cm^2 CW and 111.1 mW/cm^2 pulse wave (PW) and showed normal neural cell morphology. The present study demonstrated that the TLT powers currently utilized in animal stroke studies do not cause cortical tissue heating and histopathological damage.

1. Introduction

Low-level laser therapy (LLLT) with wavelength in the near-infrared spectrum of light delivers low-energy laser light to induce photochemical reactions in tissues, a process described as photobiomodulation [1]. LLLT has been used to facilitate tissue repair and prevent cell death. Transcranial infrared laser therapy (TLT) is a form of LLLT that is able to penetrate the skull and is capable of photostimulating the brain tissue [2,3].

TLT has been shown to improve neurological functions in different animal stroke models [4–7]. In addition, clinical trial studies have shown safety and positive effects for single TLT treatments within the 24 h of the stroke onset [8]. NeuroThera Effectiveness and Safety Trials (NEST) 1 and 2 demonstrated safety for TLT to treat stroke patients [9]. The pooled analysis of the two trials showed that TLT improves long-term neurological functions after stroke injury [10,11]. However, NEST-3 was recently halted due to lack of efficacy, suggesting more work is needed to establish the most beneficial treatment parameters for TLT.

Different irradiation doses, mode, treatment duration and frequency of TLT have been used in stroke studies [4,5,7,12]. Several studies have shown benefits within the power range of 7.5–20 mW/cm^2 [4,5,7,12]. Therefore, the optimal irradiation energy dose and treatment modality for ischemic stroke have not yet been established. Some studies have reported a benefit of LLLT with higher irradiation power densities [12–14]. However, applying additional laser energy may have minimal or no effect on the target tissue because of the biphasic effects of TLT [15,16]. Further, irradiation at higher power densities could potentially be

* Corresponding author at: P.O. Box 676025, Rancho Santa Fe, CA 92067, USA. Tel.: +1 858 442 7116.
E-mail address: jzivin@ucsd.edu (J.A. Zivin).

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http://dx.doi.org/10.1016/j.neulet.2013.07.049

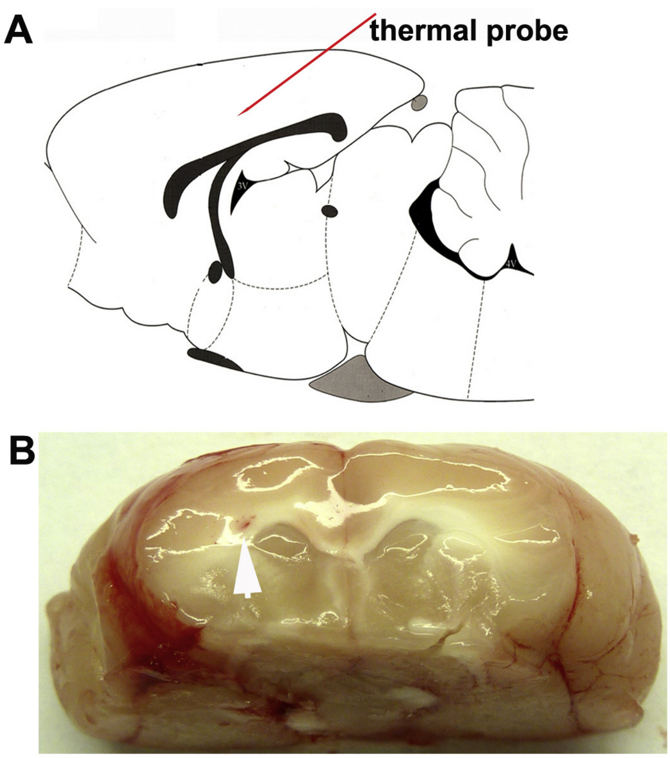

Fig. 1. (**A**) Schematic diagram of a rabbit brain saggital section showing the insertion angle and position of a thermal probe, depicted by a red line. (**B**) A representative rabbit brain coronal section shows the position and depth of a thermal probe tip, indicated by a white arrow. The probe tip is ~2–3 mm below cortical surface. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

detrimental to the brain tissue and aggravate stroke injury due to cortical tissue heating [17]. The purpose for this study was to determine the photothermal effects of transcranial near-infrared laser irradiation in rabbit cortex.

2. Materials and methods

All the animal procedures used in this study were approved by the Department of Veterans Affairs and the Veterans Administration San Diego Healthcare System and met the guidelines of the National Institutes of Health.

2.1. Cortical temperature measurement

Male New Zealand white rabbits (Western Oregon Rabbit Co., Philomath, OR) weighing 2 kg to 2.5 kg were used for the experiments. Rabbits were anesthetized with isoflurane through a facemask, 5% in 3 L/min at induction, and 3% in 3 L/min as a maintenance dose [18]. Body temperature was maintained with heating gel pad. Under anesthesia, a burr hole was drilled 2.5 mm lateral to the sagittal suture and 1 mm posterior to the coronal suture. A thermal probe was slowly inserted at around 45 °C angle into the cortex as illustrated in Fig. 1A (Luxtron 812 Fiber Optic Thermometer, Lumasense, Inc., Santa Clara, CA). Cortical temperature was measured continuously, before, during and after laser irradiation.

2.2. TLT

TLT was delivered using a GaAIAs diode laser with a wavelength of 808 ± 10 nm (PhotoThera, Inc., Carlsbad, CA) as described previously [4,5]. The laser was coupled to a female SMA-905-adapted, 5 mm in diameter, custom-designed metal-backed glass fiber optic probe via an OZ Optics Ltd. fiber optic patchcord cable (step index fiber with a 920 m core diameter and a Numerical Aperture of 0.22). The fiber optic probe utilized specially designed optics to generate a divergent (numerical aperture of 0.37), diffused 5 mm diameter beam at its distal tip. The distal tip of the fiber optic probe was in direct contact with the animal's shaved scalp posterior to bregma on the midline. For thermal effect measurement, rabbits were irradiated at cortical power densities of 11.1 mW/cm^2, 22.2 mW/cm^2, 55.6 mW/cm^2 and 111.1 mW/cm^2 in continuous or pulse mode for 2 min. These doses were chosen to represent several fold higher powers than required for neurological improvement in previous stroke studies [4,5,8,19].

2.3. Histological staining

Fully awake rabbits were placed in a Plexiglas restrainer for laser treatment. Rabbits were irradiated for 2 min at cortical power densities of 22.2 mW/cm^2 in CW or 111.1 mW/cm^2 with100 Hz pulsed wave at 20% duty cycle. Same laser power output was applied to rabbit skull at 1 h and 2 h time points after first irradiation treatment. As control group, laser probe was placed on rabbit shaved skull for 2 min without laser activation. Animals were euthanized 24 h after the triple TLT treatment with 1 to 1.5 mL of Beuthanasia-D through the marginal ear vein. Brain tissues were collected and fresh frozen with dry ice. Brain tissues were sectioned with cryostat and processed for standard Nissl and Hematoxylin and Eosin (H&E) staining as detailed in our previous publications [20,21]. Three rabbits were assigned to each experimental group. Six sections (three sections anterior and three sections posterior to the probe site) from each animal were sampled and analyzed.

3. Results

3.1. Brain temperature measurement

Fig. 1A illustrates a brain saggital section showing the insertion and position of a thermal probe in the cortical tissue. Fig. 1B is a representative rabbit brain coronal image showing that the thermal probe tip is around 2.5 mm below the cortical surface. Different laser power output, corresponding laser power densities and energies received in the cortical tissue in this study are outlined in Table 1. The average power density for the CW is the same as the peak power density. The average power density for the 100 Hz is 1/5 the peak power density. Accordingly, the average power density for 100 Hz with a 1000 mW peak power is 200 mW.

TLT was delivered by a diode laser with a wavelength 808 nm for 2 min. The rabbits remained anesthetized during the temperature recording and the scalp was closed at the site of probe insertion. Fig. 2 shows the real-time tracking of cortical

Table 1
TLT parameters.

Mode	Laser power output (mW)	Power density at the cortex[a] (mW/cm^2)	Energy density at the cortex (J/cm^2)
CW	200	22.2	2.7
CW	500	55.6	6.7
CW	1000	111.1	13.3
PW, 100 Hz[b]	1000	111.1	2.7

[a] Values for the cortical power density were calculated from the laser power output with a 5 mm beam and a 2% transmission of intensity through the scalp and skull.
[b] Peak power density is shown; the corresponding average power density is 22.2 mW/cm^2.