Transcranial Application of Low-Energy Laser Irradiation Improves Neurological Deficits in Rats Following Acute Stroke

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Background and Objectives: Low-level laser therapy (LLLT) has been shown to have beneficial effects on ischemic skeletal and heart muscles tissues. The aim of the present study was to approve the effectiveness of LLLT treatment at different locations on the brain in acute stroked rats.
Study Design/Materials and Methods: Stroke was induced in 169 rats that were divided into four groups: control non-laser and three laser-treated groups where laser was employed ipsilateral, contralateral, and both to the side of the induced stroke. Rats were tested for neurological function.
Results: In all three laser-treated groups, a marked and significant improvement in neurological deficits was evident at 14, 21, and 28 days post stroke relative to the non-treated group.
Conclusions: These observations suggest that LLLT applied at different locations in the skull and in a rather delayed-phase post stroke effectively improves neurological function after acute stroke in rats. Lasers Surg. Med. 38:70–73, 2006. © 2006 Wiley-Liss, Inc.

Key words: biostimulation; stroke; rats; acute; laser

INTRODUCTION

Treatment of active stroke in humans is still a major medical problem despite the extensive research studies [1]. The only therapy used currently in clinic is the thrombolytic one up to 3 hours post stroke. However, only about 5% of stroked patients in the US get this therapy due to side effects and late arrival to the hospital [1]. Several attempts to attenuate the ischemic process and improve functional outcome have been made recently in experimental animals. Erythropoietin (EPO) has been shown to increase angiogenesis, neurogenesis, and functional recovery in stroked rats [2]. Injection of bone marrow cells (MSCs) has also been shown to contribute to better functional outcome and to attenuate the response of reactive astrocyte to ischemia [3].

Low-level laser therapy (LLLT) has been found to modulate various biological processes [4,5]. In an experimental model of the infarcted heart it was previously demonstrated that LLLT had a profound cardioprotective effect, resulting in a 50–70% reduction in infarct size 4–6 weeks post left descending coronary artery chronic occlusion. This phenomenon was partially attributed to a significant increase in the number of undamaged mitochondria and ATP content, as well as inducible heat shock proteins and catalase (in the serum) in infarcted laser-irradiated rats and dogs as compared to non-irradiated ones [5–8]. LLLT has also been shown to biomodulate processes in the nervous system. Anders et al. [9] recently reviewed the beneficial effects of LLLT on functional recovery of injured peripheral nerves. The effect of LLLT on stroke has been investigated to a limited extent. Leung et al. [10] have shown that LLLT causes suppression of nitric oxide synthase activity and upregulation of TGF-β1, in stroked rats. It was also demonstrated that transcranial infrared laser therapy applied 6 hours post embolic stroke improved clinical rating scores in rabbits [11].

In the present study we explored the effect of LLLT applied to various regions of the brain on the neurological outcome post acute stroke in rats.

MATERIALS AND METHODS

Experimental Procedure

We used 169 mature Sprague–Dawley rats (295–315 g body weight) supplied by Zivic Laboratories (Zelienople, PA) that passed the screening phase (see below). All rats underwent induction (under halothane inhalation) of acute stroke using a filament that was introduced into the middle cerebral artery to create a permanent occlusion (MCAO). The experimental protocol was approved by the animal care committee of Zivic laboratories. Rats were examined at 24 hours (screening phase) for their minimal neurological deficit according to the modified neurological score (MNS) modified from Chen et al. [12]. Rats that were scored above five on the MNS (comprised to marked neurological deficit) were included in the study and divided into four groups

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each comprising a random combination reflecting approximately the entire range of neurological deficits (see Fig. 1) as follows: In three groups the laser was applied to irradiate either ipsilateral (38 rats), contralateral (39 rats), or both (40 rats) sides of the stroke. In the fourth (control) group (43 rats) the laser probe was placed on the skin of the rats as in the above groups but was not turned on.

Neurological Functional Tests

The MNS used to estimate neurological deficit in the rats was composed of six different behavioral examination tests as follows: Fore limb flexion (0 = both limbs splayed outwards and 1 = limbs crossed); torso twisting (0 = no twist and 2 = severe body movements when rat held by tail); hind limb placement (0 = replacing and 1 = limb remains down); gait disturbances (0 = normal straight walk, 2 = tight arc walk, 3 = severe impairment); 2″-wide beam walk test (0 = normal walk along the beam and 1 = two limbs hanging off beam); 3/4″-beam balance tests: (0 = walk normally, 2 = hugs beam but contralateral limb not fully grasping, and 4–6 = rat falls of beam within 60–20 seconds, respectively).

Laser Treatment

A Ga-As diode laser wavelength 808 nm (Photothera, Inc., San Diego, CA) was used in the study. The laser was employed transcranially on the shaved skin of the skull by placing the tip of the fiber optic (4 mm diameter) onto the skin (at approximately 45 degrees angle to the plan of the abdomen of the anesthetized rat) at two locations on the head (3 mm dorsal to the eye and 2 mm anterior to the ear) either ipsilateral, contralateral, or to both sides of the stroke. These locations had been determined from prior measurements to be sufficient to illuminate the entire one side lateral brain hemisphere due to dispersion of the laser beam by the skin and the skull at that side. The laser irradiation power density at the tip of the fiber optic was set to give a power density of 7.5 mW/cm^2 on the brain. This optimal power density was determined based on our previous studies on the effect of LLLT on the ischemic heart [8] and on preliminary experiments with stroked rats. The exact power density on the brain was set in preliminary experiments with fresh skulls, using a laser power meter (Ophir, Inc., Jerusalem, Israel) to measure transmission through the skull and an infrared viewer to measure the laser beam diameter after dispersion through the skull. The duration of laser irradiation was 2 minutes (energy density of 0.9 J/cm^2) at each point on the skull.

Statistical Analysis

For each time interval post stroke the means of each group were compared using analysis of variance test (ANOVA) and pairwise comparison using least significant difference (LSD). For each group and time interval post stroke, the categories were compared using non-parametric Kruskal–Wallis test for testing null hypothesis that samples had been drawn from the same identical population.

RESULTS

Figure 1 represents results of the neurological score of the control and three laser-treated groups. There was no statistical difference in the neurological score of one day post stroke between the four groups of rats, indicating that the severity of stroke was identical in all groups. In the control non-laser irradiated rats a 24% improvement in neurological score was achieved 14 days post stroke and then a mild improvement in the neurological deficit was evident up to 28 days post stroke. However, all laser-treated groups continued to gradually improve from 14 to 28 days post stroke exceeding a highly significant ($P<0.001$) average (of all three laser groups) reduction of 38% in MNS in comparison to the non-treated control group. At 28 days post stroke there was only a 32% reduction in neurological score (from 10.2 to 5.9 MNS) in the control group while in the three laser-treated groups a 63% [from 10.1 to 3.7 (averaged MNS)] and highly significant ($P<0.001$) reduction was noticed comprising

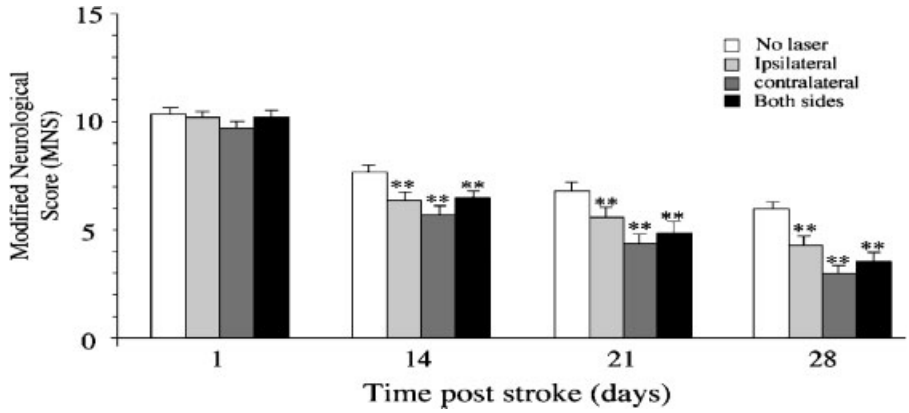

Fig. 1. Kinetics of modified neurological score post stroke in control (open columns), ipsilateral (light gray columns), contralateral (dark gray columns), and both sides to stroke (dark columns) application of laser to rat skull. Bars represent mean ± SEM of 38–43 rats in each group. **Highly statistically significant ($P<0.01$) over control non-laser group.

about a twofold increase in neurological function. At that time interval there were no significant differences in the neurological score between the groups of rats to which the laser was applied to different regions of the brain.

In each of the four groups the stroked rats were subdivided into three categories according to the severity of the stroke at the screening phase [1 day post stroke, as follows: low-medium (MNS 5–8), high-medium (MNS 8–11), and severe (MNS 11 and above)]. These rats were tested at the different time intervals for their neurological scores and comparison of the scores was performed between groups using the Kruskal–Wallis test. It was found that the differences in scores of the same group (control or laser) were highly significant at 1 day post stroke, when subdivision into the various categories was performed. At 14, 21, and 28 days post stroke the significance between categories within each group was significant (P value of 0.001, 0.009, and 0.02 for 4, 21, and 28 days, respectively) only in the group to which the laser was applied to both the ipsilateral and contralateral sides of the stroke. Thus, at 28 days post stroke the percentage improvement of the "low-medium" group category (relative to day 1 neurological score) was 86% (from 6.9 ± 0.7 at day 1 to 1.0 ± 0.35 at 28 days). In the rats assigned to the "low-medium" group and receiving LLLT, for example, only on the contralateral side, improvement was only 51% (from 6.8 ± 1.2 at day 1 to 3.3 ± 2.2 at 21 days) in the time interval between day 1 and day 28 post stroke.

DISCUSSION

The results of the present study clearly indicate for the first time that LLLT applied at 24 hours post stroke effectively improves neurological function in rats following acute stroke. The extent of improvement at 4 weeks post stroke in the laser-irradiated rats was highly significant and about twofold that of the non-treated rats. Another finding of this study is that there are no major differences in effect regarding the location of laser application relative to the stroke occurrence in the brain. Ipsilateral, contralateral, or even both sides laser treatment efficiently improved the neurological outcome of rats post stroke. Lapchak et al. [11] in his study on stroked rabbits applied LLLT transcranially to the center of the pariental region of the skull, which illuminated both ipsilateral and contralateral sides of the brain where the stroke had occurred. In accordance with the present study, they demonstrated significant improvement in neurological outcome of the rabbits up to 6 hours post stroke. At 24 hours post stroke the laser irradiation demonstrate no further neurological improvement in the rabbits. It may be postulated that the different findings are a consequence of the models used, which differ from each other both in animal species and the method of induction of stroke (via filament or craniotomy vs. injection of microbead through the carotid artery).

One interesting outcome of the present study is that of the beneficial effect of the laser on the rats in the group with relatively low neurological deficit, where laser was applied to both the ipsilateral and contralateral sides of the stroke relative to one-side treatment only. This may indicate that treatment of the entire brain with LLLT may also be preferential to the application to only one side of the brain (i.e., ipsilateral to the stroke) in patients with a relative low neurological deficit.

The results of this study also indicate that beneficial biostimulatory response in the ischemic zone in the brain takes place even when the laser is applied only to the contralateral side of the stroke, when the laser source effectively irradiates the intact brain hemisphere and the stroke zone is irradiated by a significantly lower laser power density. We have determined an estimated power density drop from 10 mW/cm^2 at the cortex of the rat brain to approximately 7.5 μW/cm^2 at 1.8 cm depth from the cortical surface (Photothera Inc., internal research report). Thus, it may be postulated that even when the contralateral side to the stroke is irradiated at an optimal power density of 7.5 mW/cm^2 and the ischemic zone to suboptimal energy, the overall beneficial biological effect is evident. Indeed it has been shown previously that LLLT applied to the ischemic heart was beneficial when the entire lateral part (and not only the ischemic infracted zone) of the heart was irradiated by the laser [6–8]. Furthermore, it has been shown in non-ischemic skeletal muscles that there was a significant upregulation of antioxidant and heat shock proteins (neuroprotective molecules) by LLLT at similar power density and wavelength to the laser used in the present study [13].

The mechanisms associated with the marked beneficial effect of the laser on the stroked brain are not clearly understood. However, neuroprotective effects like elevation of heat shock proteins, preservation of mitochondria etc., as found for ischemic heart [6] and skeletal muscles [13], cannot be ruled out. The improvement in neurological functional performance with time by the LLLT may also suggest a positive effect on neurogenesis. Thus it may be postulated that beneficial effect is achieved by modulation of several processes in the brain. Indeed it was previously shown that LLLT can affect both nitric oxide synthase activity and upregulation of TGF-β1 simultaneously in stroked rats [10].

The present study also has clinical application. The treatment given at 24 hours post stroke resulted in a similar magnitude of efficiency to the thrombolytic therapy currently used in patients up to 3 hours post stroke [11]. The delivery of the laser transcranially to the human brain (unpublished data) is technically feasible and makes the LLLT a possible favorable treatment for acute stroked patients.

In conclusion, the present study clearly demonstrates that LLLT can significantly improve neurological deficit following acute stroke and address its optimal application. The mechanisms associated with this phenomenon will have to be elucidated in further studies.

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