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BRAIN RESEARCH

Research Report

Transcranial near infrared laser treatment (NILT) increases cortical adenosine-5′-triphosphate (ATP) content following embolic strokes in rabbits

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ARTICLE INFO

Article history:
Accepted 9 October 2009
Available online 23 October 2009

Keywords:
Ischemic stroke
Neuroprotection
Mechanism
Translational science
Light therapy

ABSTRACT

Transcranial near infrared laser therapy (NILT) improves behavioral outcome following embolic strokes in embolized rabbits and clinical rating scores in acute ischemic stroke (AIS) patients; however, the cellular mechanism(s) involved in NILT neuroprotection have not been elucidated. It has been proposed that mitochondrial energy production may underlie a response to NILT, but this has not been demonstrated using an *in vivo* embolic stroke model. Thus, we evaluated the effect of NILT on cortical ATP content using the rabbit small clot embolic stroke model (RSCEM), the model originally used to demonstrate NILT efficacy and initiate the NEST-1 clinical trial. Five minutes following embolization, rabbits were exposed to 2 min of NILT using an 808 nm laser source, which was driven to output either continuous wave (CW), or pulsed wave modes (PW). Three hours after embolization, the cerebral cortex was excised and processed for the measurement of ATP content using a standard luciferin-luciferase assay. NILT-treated rabbits were directly compared to sham-treated embolized rabbits and naïve control rabbits. Embolization decreased cortical ATP content in ischemic cortex by 45% compared to naive rabbits, a decrease that was attenuated by CW NILT which resulted in a 41% increase in cortical ATP content compared to the sham embolized group ($p>0.05$). The absolute increase in ATP content was 22.5% compared to naive rabbits. Following PW NILT, which delivered 5 (PW1) and 35 (PW2) times more energy than CW, we measured a 157% (PW1 $p=0.0032$) and 221% (PW2 $p=0.0001$) increase in cortical ATP content, respectively, compared to the sham embolized group. That represented a 41% and 77% increase in ATP content compared to naive control rabbits. This is the first demonstration that embolization can decrease ATP content in rabbit cortex and that NILT significantly increases cortical ATP content in embolized rabbits, an effect that is correlated with cortical fluence and the mode of NILT delivery. The data provide new insight into the molecular mechanisms associated with clinical improvement following NILT.

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doi:10.1016/j.brainres.2009.10.022

1. Introduction

The photobiostimulation effects of infrared laser therapy or transcranial laser therapy (NILT) have been documented for *in vitro* and *in vivo* conditions (Ad and Oron, 2001; Byrnes et al., 2005; Desmet et al., 2006; Detaboada et al., 2006; Ilic et al., 2006; Lampl et al., 2007; Lapchak and Araujo, 2007; Nissan et al., 1986; Oron et al., 2001). The biological effects of infrared laser therapy are wavelength-specific, and are not due to thermal effects (Anders et al., 1993; Castro-e-Silva et al., 2003; Mochizuki-Oda et al., 2002). Energy in the infrared region (IR) of the electromagnetic spectrum is non-ionizing and, therefore, poses none of the hazards associated with UV light. It has been demonstrated that irradiation with specific infrared wavelengths, such as the 800–830 nm wavelength is able to both penetrate the skull and brain (Detaboada et al., 2006; Ilic et al., 2006; Lapchak et al., 2004b; Zhang et al., 2000).

There is evidence that suggests that a primary mitochondrial chromophore or acceptor molecule for photobiostimulation is cytochrome c oxidase (COX)(Desmet et al., 2006; Eells et al., 2003). The COX enzyme complex contains two copper centers, Cu_A and Cu_B with the Cu_A center having a broad wavelength absorption peak between 800 and 830 nm in its oxidized form. COX, a terminal enzyme in the cellular respiratory chain, which is located in the inner mitochondrial membrane plays a central role in the bioenergetics of eukaryotic cells by delivering protons across the inner membrane, thereby driving the formation of ATP by oxidative phosphorylation (Medeiros and Jennings, 2002).

In previous studies (Lapchak et al., 2004b, 2007b, 2008), we have shown that NILT when administered at a wavelength of 808 nm can produce significant behavioral improvement in small-clot embolized rabbits, when administered either as a continuous wave (CW) or a pulsed wave (PW). Moreover, we have shown that NILT has long therapeutic window, up to 6 h in the preclinical setting (Lapchak et al., 2004b, 2007b) and a durable effect, up to 21 days post-treatment (Lapchak et al., 2004b, 2007b) The recent clinical trials of NILT using a CW delivery mode have also documented efficacy of NILT when patients were treated within 24 h of a stroke (Lampl et al., 2007; Zivin et al., 2009). Based upon the science of light and photobiostimulation (Ad and Oron, 2001; Byrnes et al., 2005; Desmet et al., 2006; Detaboada et al., 2006; Ilic et al., 2006; Lampl et al., 2007; Lapchak and Araujo, 2007; Nissan et al., 1986; Oron et al., 2001), we hypothesize that the efficacy of NILT may be mediated by enhanced mitochondrial function, but this has not been conclusively demonstrated. Therefore, the goal of the present study is to evaluate the effect of NILT on cellular ATP content using an *in vivo* stroke model. For this study, we studied the effects of both CW and PW NILT on cortical ATP content in a rabbit small clot embolic stroke model (RSCEM) (Lapchak et al., 2002, 2004b, 2007a), where multiple infarct ischemia is produced by the injection of blood clots into the cerebral vasculature via an indwelling carotid catheter.

2. Results

In this study, we measured the effects of embolization with small-sized blood clots on cortical ATP content. In this study, cortical tissue was used for this study for 2 reasons: first, ischemic cortical tissue (parietal/occipital cortex) can be accurately and rapidly excised from rabbits following euthanasia. Ischemic cortical tissue was white/beige, soft and spongy in nature compared to normal tissue which is pink and firm. Second, we can quantitatively calculate the cortical fluence (in J/cm^2) delivered using the specific CW and PW modes chosen for this study. Embolized rabbits with behavioral deficits presented with one or more of the following behavioral signs associated with encephalopathy: ataxia, leaning, circling, nystagmus, loss of balance, loss of limb/facial sensation and occasionally, paraplegia.

2.1. *Effect of CW NILT on cortical ATP content*

Five minutes post-embolization rabbits were treated with NILT. We found that by 3 h post-embolization there was a significant ($p<0.05$) decrease of ATP content within the ischemic cortex compared to the same cortical region excised from control naive rabbits. In naive control rabbits, baseline cortical ATP content were 830±43 nmol/mg tissue ($n=8$), compared to 456±77 nmol/mg tissue ($n=8$) in sham-treated embolized rabbits (Fig. 1). Following embolization, there was a measurable decrease in cortical ATP content.

In Fig. 1, we also present the effects of CW NILT (2 min, cortical irradiance of 7.5 mW/cm^2; cortical fluence of 0.9 J/cm^2) on cortical ATP content. In previous studies (Lapchak et al., 2004b, 2007b), we have found that CW NILT using the same settings used for these biochemical studies, significantly improved behavior when rabbits were treated post-embolization. In this study, we found that CW NILT increased cortical ATP content by 41%, and this resulting net tissue content of ATP increase was not statistically different from either the sham-treated embolized control group or the naïve control group ($p=0.1357$ and 0.0826, respectively).

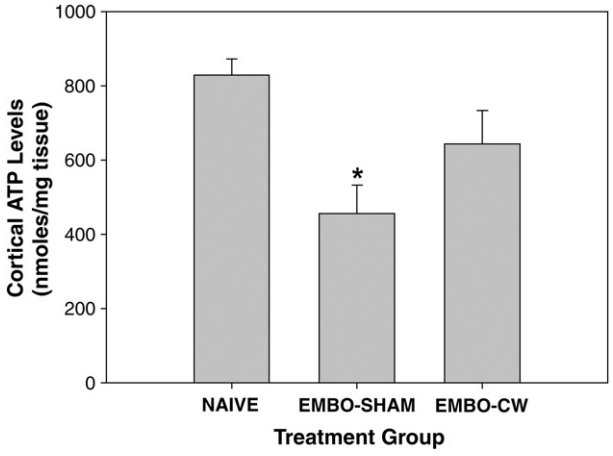

Fig. 1 – Effects of embolization and CW NILT on cortical ATP content. Embolization (EMBO-SHAM) resulted in a 46% decrease of cortical ATP (*$p<0.05$) compared to NAÏVE control rabbits, a decrease that was attenuated (41% increase) by CW NILT (EMBO-CW). There was no significant difference between the EMBO-CW group and either the EMBO-SHAM or the NAÏVE control group using an $n=8$ per group ($p=0.1357$ and 0.0826, respectively).