Brain Research 1646 (2016) 125–131

Contents lists available at ScienceDirect

Brain Research

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

Research report

A novel method to promote behavioral improvement and enhance mitochondrial function following an embolic stroke

Paul A. Lapchak*, Paul D. Boitano

Cedars-Sinai Medical Center Department of Neurology & Neurosurgery, Advanced Health Sciences Pavilion Suite 8305, 127 South San Vicente Blvd, Los Angeles 90048, United States

ARTICLE INFO

Article history:
Received 1 February 2016
Received in revised form
5 April 2016
Accepted 16 April 2016
Available online 11 May 2016

Keywords:
Disease of aging
Neurodegeneration
Ischemic stroke
Cerebral blood flow
Neuroprotection
Thrombolysis
Combination therapy
transcranial laser therapy
photobiostimulation

ABSTRACT

Tissue plasminogen activator (tPA) is the only FDA-approved treatment for stroke; tPA increases cerebral reperfusion, blood flow and improved behavior. Novel transcranial laser therapy (TLT) also enhances cerebral blood flow and activates mitochondrial function. Using the rabbit small clot embolic stroke model (RSCEM), we studied the effects of continuous wave TLT (7.5 mW/cm^2) alone or in combination with standardized intravenous (IV) tPA (3.3 mg/kg) applied 1 h post-embolization on 3 endpoints: 1) behavioral function measured 2 days [effective stroke dose (P_{50} in mg) producing neurological deficits in 50% of embolized rabbits], 2) intracerebral hemorrhage (ICH) rate, and 3) cortical adenosine-5′-triphosphate (ATP) content was measured 6 h following embolization. TLT and tPA significantly ($p < 0.05$) increased P_{50} values by 95% and 56% ($p < 0.05$), respectively over control. TLT-tPA increased P_{50} by 136% over control ($p < 0.05$). Embolization reduced cortical ATP content by 39%; decreases that were attenuated by either TLT or tPA treatment ($p < 0.05$). TLT-tPA further enhanced cortical ATP levels 22% above that measured in naïve control. TLT and tPA both effectively and safely, without affecting ICH rate, improved behavioral outcome in embolized rabbits; and there was a trend ($p > 0.05$) for the TLT-tPA combination to further increase P_{50}. TLT and tPA both attenuated stroke-induced ATP deficits, and the combination of tPA and TLT produced an additive effect on ATP levels. This study demonstrates that the combination of TLT-tPA enhances ATP production, and suggests that tPA-induced reperfusion in combination with TLT neuroprotection therapy may optimally protect viable cells in the cortex measured using ATP levels as a marker.

1. Introduction

Transcranial near-infrared laser therapy (TLT) has been formally studied in 3 clinical trials as a potentially useful method to treat stroke victims (Hacke et al., 2014; Kasner et al., 2013; Lampl et al., 2007; Zivin et al., 2009), but demonstrating reproducible efficacy in a diverse patient population has been problematic (NEST-3 trial ended due to futility). Prior to initiating the NEST clinical trials, TLT was tested and studied in stroke models using a variety of small animals including mice, rats and rabbits [reviewed in Lampl (2007) and Lapchak (2010b, 2012a)]. While statistically significant efficacy was noted, TLT was never optimized with respect to effective power density requirements, transmission characteristics and behavioral or biochemical effect relationships prior to initiating clinical trials. Numerous study reports have been demonstrated that laser irradiation using an 800 nm wavelength device is able to penetrate the skull and brain (Detaboada et al., 2006; Ilic et al., 2006; Lapchak et al., 2004c; Zhang et al., 2000), but recent information suggests that insufficient levels of laser light penetrate the thick human skull (Lapchak et al., 2015a; Tedford et al., 2015) at the power density used for clinical stroke studies. The dramatic decrease in laser penetration has also been emphasized by Yaroslavsky et al., (Yaroslavsky et al., 2002) and Pitzschke et al. for (Pitzschke et al., 2015b) for human brain and (Pitzschke et al., 2015a) for rabbit brain. Moreover, importantly, the NEST trials excluded patients receiving standard-of-care therapy, tissue plasminogen activator (tPA), even though we previously showed that TLT in combination with tPA did not exacerbate ICH (Lapchak et al., 2008a) in middle cerebral artery clot-occluded rabbits (i. e.: rabbit large clot embolic stroke model). Until this report, much needed combination therapy data was missing from the TLT development profile.

For translational stroke studies, there is utility in using smaller animals that have thin skulls which allows for laser light transmission, which penetrates through the complete thickness of the

* Corresponding author.
E-mail addresses: Paul.Lapchak@cshs.org (P.A. Lapchak), paul.boitano@cshs.org (P.D. Boitano).

http://dx.doi.org/10.1016/j.brainres.2016.04.039
0006-8993/

brain allowing for effective activation of neuroprotective and neurorestorative pathways (Chung et al., 2012; Huang et al., 2012; Naeser and Hamblin, 2011; Streeter et al., 2004; Xuan et al., 2013, 2014). For TLT to be efficacious, it has been hypothesized that the primary mitochondrial chromophore or acceptor molecule for laser photobiostimuation, cytochrome c oxidase (COX) (Desmet et al., 2006; Eells et al., 2003) must be activated. The COX enzyme copper centers, Cu_A and Cu_B can absorb light energy transmitted through the skull in the 800–830 nm range. COX, a terminal enzyme in the cellular respiratory chain, thereby drives the formation of adenosine-5′-triphosphate (ATP) by oxidative phosphorylation (Medeiros and Jennings, 2002). Simply stated, TLT may prevent extensive cell death and penumbral involvement, (expansion and loss) due to ATP depletion during the initial stroke phase (Dirnagl et al., 1999; Li et al., 2016; Moskowitz et al., 2010).

In previous studies (Lapchak et al., 2004c; Lapchak et al., 2008a; Lapchak and De Taboada, 2010), we demonstrated that TLT when administered at a wavelength of 808 nm can produce significant behavioral improvement in small-clot embolized rabbits, and attenuate cortical ATP losses (Lapchak and De Taboada, 2010). Moreover, tPA can also attenuate behavioral deficits induced by embolization (Lapchak et al., 2004a; Zivin et al., 1985; Zivin et al., 1988), and tPA is currently the only FDA-approved pharmaceutical treatment for ischemic stroke patients. tPA promotes clot lysis, which increases cerebral blood flow to the formerly clot-affected core and penumbra (del Zoppo, 1988; Hacke et al., 1998, 2004, 2014; Lapchak, 2002; Lapchak et al., 2004a; Lyden et al., 1989, 2006; Lyden, 2006), resulting in clinical and/or behavioral improvement.

Based upon the observation that both TLT and tPA can attenuate behavioral deficits following, we hypothesize that a combination therapy may be beneficial and possibly be more effective than individual therapies and that efficacy may be mediated by enhancing mitochondrial function. Therefore, the goal of the present study is to evaluate the effect of combined TLT and tPA therapy on behavioral outcome and cellular ATP content using the rabbit embolic stroke model. We have previously shown that the combination of TLT and tPA is safe and does not increase mortality or ICH incidence (Lapchak et al., 2008a). In this study, we examined the effects of TLT and tPA on behavior, safety (ICH incidence) and cortical ATP content using the rabbit small clot embolic stroke model (RSCEM) (Lapchak et al., 2004a, 2004c; Lapchak, 2015a).

2. Results

In this systematic study, we compared the effects of TLT, tPA and TLT-tPA administration to a single designated saline-treated control group to minimize the use of this larger animal species. Multiple control groups were not included because we have previously shown that baseline control group P_{50} values do not vary significantly (Lapchak et al., 2008b). In this study, therapies were administered 1 h post-embolization because a previously published comprehensive correlative analysis (Lapchak, 2010d, 2012b) for diverse "neuroprotective" strategies suggested that 1 h post-embolization in the rabbit represents approximately 2.43–3 h in stroke patients, a clinically relevant time considering that tPA is FDA-approved for use within 3 h in stroke patients and has shown efficacy up to 4.5 h after a stroke (Alper et al., 2015; Hacke et al., 2008; Lansberg et al., 2009) in clinical trials.

2.1. Behavioral analysis

As shown in Fig. 1, the P_{50} value in the control group was 1.18 ± 0.15 mg (n=20). Treatment with TLT significantly (Fig. 1**A**) increased the P_{50} value to 2.30 ± 0.47 mg (n=19; p=0.026) whereas

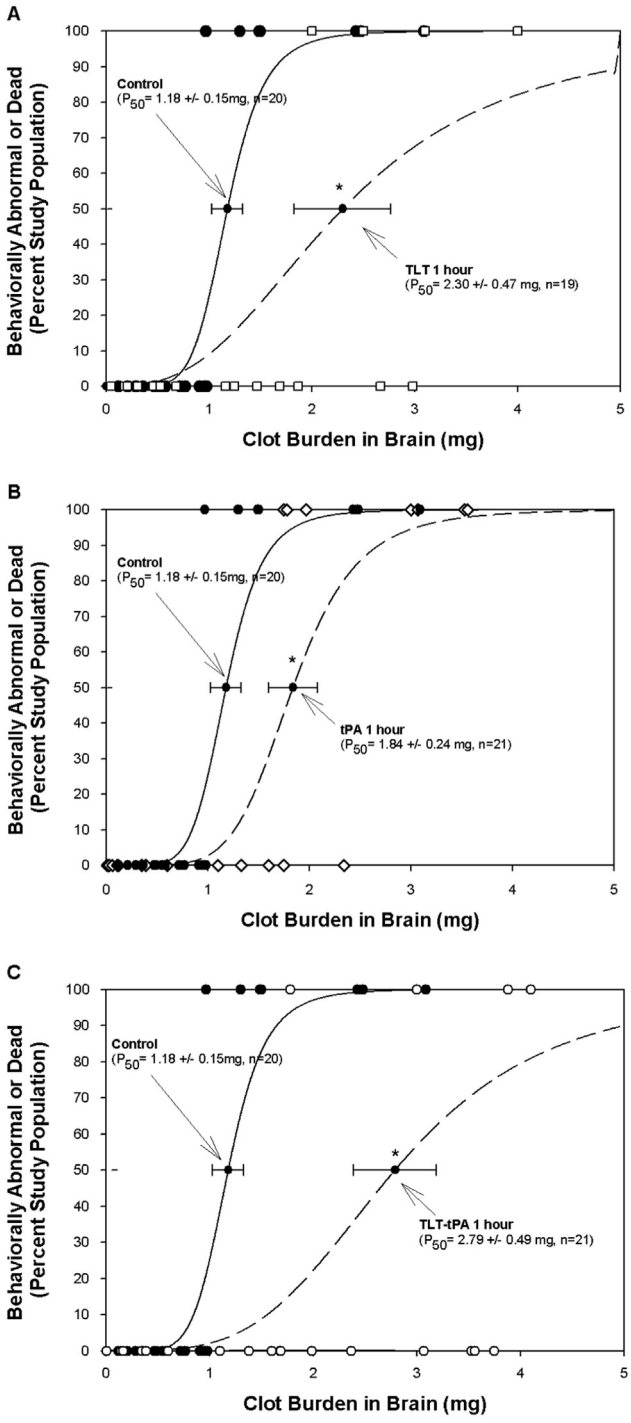

Fig. 1. Effect of (A) TLT, (B) tPA or TLT-tPA (C) on behavioral outcome. For the superimposed graphs, behaviorally normal animals are plotted on the y-axis at 0 and abnormal animals are plotted at 100. The figure shows that there is positive correlation between the raw data and the statistically fit sigmoidal quantal curves. (A) (black circles- control; white squares TLT); (B) (black circles- control; white triangles tPA) (C) (black circles- control; white circles TLT-tPA). The slope of each quantal curve is defined by the statistical quantal analysis program and is a direct reflection of the behavioral response of the population around the P_{50} point. TTL vs control ($p < 0.05$); tPA vs control ($p < 0.05$); TLT vs tPA, TLT-tPa vs tPA, and TLT-tPA vs TLT ($p > 0.05$).

tPA treatment (Fig. 1**B**) increased the P_{50} value to 1.84 ± 0.24 mg (n=21; p=0.026) compared to control. When embolized rabbits were treated with a combination of IV tPA and TLT, the P_{50} value was increased to 2.79 ± 0.49 mg (n=21, p=0.0038) compared to control (Fig. 1**C**), and $p > 0.05$ compared to either TLT or tPA alone.