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Research article

Dose-finding study of phototherapy on stroke outcome in a rabbit model of ischemic stroke

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ABSTRACT

Goal: While transcranial laser therapy (TLT) has been shown to improve clinical outcome in a preclinical model of ischemic stroke, optimal timing and dosing has yet to be tested adequately. The purpose of this study was to assess clinical stroke outcome in the Rabbit Small Clot Embolic Model (RSCEM) with dose escalating TLT.

Methods: We utilized the rabbit small clot embolic stroke model (RSCEM) using dose-escalating regimens. Behavioral analysis was conducted at 24 h post-embolization, allowing for the determination of the effective stroke dose (ES_{50}) or clot amount (mg) that produces neurological deficits in 50% of a group of rabbits. Using the RSCEM, a treatment is considered beneficial if it significantly increases the ES_{50} compared with the control group.

Findings: A significant behavioral benefit was seen at triple TLT of 111 mW treatment of 2 min at 2 h post-embolization (6.47 ± 1.06, $n = 17$; $p = 0.03$), compared with the previously used regimen (3.09 ± 0.51, $n = 15$).

Conclusion: TLT results in significant behavioral improvement when administered 2 h post-embolization. Studies are warranted to evaluate this therapy in combination with thrombolysis.

1. Introduction

Low-energy laser irradiation (LELI) uses low-powered laser energy, at wavelengths from approximately 600–1100 nm to induce a photochemical reaction in the cell, without generating heat, by a process referred to as biostimulation or photobiomodulation [1]. Transcranial infrared laser therapy (TLT) is a form of laser therapy that is able to penetrate the skull and is capable of photostimulating the brain tissue located at least a few centimeters below the skull [2]

The effects of TLT in acute ischemic stroke have been widely studied. Lapchak et al. used the rabbit small clot embolic stroke model (RSCEM) to demonstrate long-term behavioral improvement with TLT when treating animals at 3 and 6 h after cerebral embolization [3]. TLT applied at 24 h after the ischemic injury using a middle cerebral artery occlusion (MCAO) rat model produced a significant improvement of the neurological severity score as compared with the controls when measured 14 days after the stroke [4]. DeTaboada et al. replicated these results in the same rat MCAO model [5]. Human studies have showed safety and favorable effects when TLT is applied as a single treatment within 24 h of the stroke onset [6]. The NeuroThera Effectiveness and Safety Trials (NEST) 1 and 2 demonstrated safety for human treatment in acute ischemic stroke [6,7], and the pooled analysis of both trials showed efficacy of TLT treatment for reduction in long-term disability measured by the modified Rankin Scale [8]. Unfortunately, NEST 3 failed to confirm the previous two clinical trials for unknown reasons [9]

The different treatment times, irradiation doses, and energy levels of TLT tested in acute ischemic stroke models have been frequently successful in reaching good outcomes [3–6,10,11]. However, the optimal timing and the irradiation dose for acute ischemic stroke have not yet been established. Some studies [12] have shown an incremental benefit of TLT with higher irradiation power and energy levels. But applying additional TLT energy can have minimal or no effect on the target tissue due to possible heating of the tissue [13]. Further irradiation can even be detrimental to the tissue itself due to its thermal effect [14].

Most of the neuroprotective therapies tested in animals have shown better efficacy when used early in the ischemic injury process, but eventually losing their efficacy as time from onset to initiation of therapy increases. In contrast TLT appears to be equally effective when applied at different times within the first 24 h of the

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Table 1
RSCEM Rating.

Normal (No abnormal behavior symptoms)
Abnormal (place a check next to the abnormal behavior exhibited)
ataxia
leaning
circling
coma
death
lethargy
nystagmus
loss of balance
loss of limb or facial sensation
paraplegia

ischemic brain injury although further work needs to be done in this area. It is possible, therefore, that TLT has the potential to photomodulate at different stages of the brain ischemic cascade. The purpose of this study was to assess clinical stroke outcome in the Rabbit Small Clot Embolic Model (RSCEM) with dose escalating TLT.

2. Materials and methods

2.1. Design and sample

This was a blinded, randomized, controlled study in the rabbit small clot embolic model (RSCEM). The sample consisted of male, 2–4 kilogram (kg), one year old, New Zealand white rabbits. Animals were purchased from Rabbit Source, Ramona, Calif. Rabbits and supplied food (alfalfa cubes) and water ad libitum while under quarantine in an enriched environment for at least 3 days before experimental use. The University of California San Diego and Veterans Administration San Diego Health System (VASDHS) Institutional Animal Care and Use Committees (IACUC) approved the surgical and treatment procedures used in this study (Protocol #07-043).

In this model, a catheter is placed in the right internal carotid artery under anesthesia then animals are allowed to recover to baseline function. When animals are fully recovered from anesthesia and are neurologically normal, small embolic clots are then injected into the catheter to produce infarction (embolization). Treatment is then given at an allotted time post-embolization and behavior is assessed at 24 h via the RSCEM clinical rating scale (Table 1). Animals were randomized to a variety of progressively more powerful TLT therapeutic regimens starting 2 h post-embolization for up to 6 min of therapy (see Table 2 for treatment regimens). The investigator was blinded to treatment groups and responsible for administering the experimental treatment; a trained laboratory technician blinded to treatment assessed the primary outcome measures. Care was used throughout the study to minimize pain and discomfort. Rabbits were euthanized if they showed extreme discomfort, or unable to reach food or water.

2.2. Procedures

2.2.1. Surgical procedures

Surgery was performed in a sterile controlled environment with a room temperature between 22.8 °C and 23.2 °C. All surgical, embolization, and histological procedures were done based on the techniques of Zivin and Lapchak [15–17]. Rabbits were anesthetized with isoflourane (5% induction, 3% maintenance) by face mask, the bifurcation of the right carotid artery was exposed, and the external carotid was ligated just distal to the bifurcation. Sterile technique was used to implant a catheter into the right common carotid artery. This catheter was advanced to the internal carotid artery, secured with ligatures, and the distal end left accessible outside the neck. The rabbits were allowed to recover from anesthesia for a minimum of 2 h until they were awake and behaving normally as evidenced by no identified persistent behaviors from the RSCEM behavior scale prior to embolization. Animals that died before embolization administration were replaced in the sample. Animals that died or were euthanized after embolization and treatment were included in the study.

2.2.2. Preparation and administration of small clot embolism

Standard animal models of ischemic stroke involve anesthetized, intubated subjects [18], thus potential neuroprotection provided by the anesthetic agent may interfere with stroke outcome [19]. The RSCEM rabbit remains conscious during clot administration and treatment to more accurately mimic the human ischemic stroke condition.

Blood drawn from a donor rabbit was allowed to clot at 37 °C and suspended in Dulbecco's Phosphate Buffered Saline (PBS) solution containing 0.1% bovine serum albumin and emulsified with a Polytron small particle cutter. Clots were sized by sequential filtration through a 240-μm^2 screen and a 105-μm^2 nylon net; those retained were washed with PBS and allowed to settle. This supernatant was then removed and the clot particles labeled with tracer quantities of 15-μm radiolabeled microspheres (Cobalt-57) to enable quantification of clot weight after sacrifice. PBS solution was then added to the clot particles so that clot particles were suspended in 1 mL, which was drawn into a syringe for administration.

Clot particles were rapidly administered through the intra-arterial injection catheter, and the system was flushed with normal saline ensuring that no air bubbles were present that may cause air embolism.

2.2.3. Laser administration

For the TLT, rabbits were placed in a Plexiglas restrainer for the duration of the treatment, and the rabbits' heads were shaved. The laser probe was placed in direct contact with the skin overlying the skull. An AcuLaser low-energy laser, with wavelength of 808.5 nm and fitted with an OZ Optics Ltd fiber optic cable (PhotoThera, San Diego, CA) and laser probe measuring 2 cm in diameter, was used.

2.3. Outcome measures

2.3.1. Behavioral outcome-quantal analysis

The behavior of each animal was rated as either normal or abnormal/dead using a dichotomized behavioral score by an examiner blinded to the original treatment assignment and the quantity of clots in the brain was measured. The behaviorally normal rabbits did not have any signs of impairment; whereas behaviorally abnormal rabbits had loss of balance, head leans, circling, seizure-type activity, or limb paralysis, coma or death (Table 1). The validated behavioral outcome in this model is the ES_{50} (the weight of clots (mg) that produces neurologic dysfunction in 50% of a group of animals). The ES_{50} evaluates the quantitative relationship between the amount of clot vs. the number of neurologically abnormal animals in a group (as assessed by the RSCEM clinical rating scale). Logistic (S-shaped) curves were fitted to reflect an x axis of clot weight (mg) vs. a y axis of RSCEM outcome scored as either normal (no symptoms) or abnormal ($\geq$1 symptom). The more effective the treatment, the higher the ES_{50} given that effective treatments lead to an increase in the clot weight necessary to produce behavioral abnormality [20,21]. The RSCEM has been utilized successfully in stroke research as it has been used to test pharmacologic interventions such as recombinant tissue plasminogen activator (rt-PA), NXY-059, Tenecteplase (TNK), and Microplasmin [3,22–24]. The RSCEM clinical rating scale has been shown to have a <5% inter-rater variability providing a consistent and easy measurement of