31P MRS indicates possible effects of transcranial laser therapy in an animal model

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INTRODUCTION: Transcranial Laser Therapy (TLT) is a novel noninvasive technology showing promise in clinical trials for the treatment of ischemic stroke [1-2]. TLT also has shown some efficacy in animal models of major depression (MD), Alzheimer's Disease (Alz), and traumatic brain injury (TBI) [3-5]. TLT administered at 808 nm wavelength is thought to improve mitochondrial function and cerebral metabolism by stimulating cytochrome C oxidase (CCO) protein [4]. To begin to investigate effects of TLT on brain metabolism, we used phosphorus (31P) magnetic resonance spectroscopy (MRS) in a healthy older adult beagle dogs (canis lupus familiaris). We hypothesized that TLT would both acutely and chronically enhance brain bioenergetics, as reflected by increased phosphocreatine/ATP ratios.

MATERIALS AND METHODS: ***Animals:*** Four healthy adult female beagles were subjects in these studies, which were conducted under institutional animal care and use committee (IACUC) approvals at McLean Hospital and at Toxikon Corporation (Bedford, MA), where dogs were housed during the study and where subchronic TLT treatments occurred. ***Magnetic Resonance Imaging and Spectroscopy:*** Scanning of anesthetized dogs was performed at 9.4T on a 29 cm horizontal bore Varian/Agilent scanner with a 10 G/cm gradient system and a half-volume surface coil of 10cm active length. Images were acquired in 3 planes for voxel localization. The voxel (8 mm × 10 mm × 12 mm) was positioned over the anterior cingulate cortex. 31P MRS was carried out using ISIS (image-selected in vivo spectroscopy) single-voxel spectroscopy [6]. MRS parameters were, TR=3000 ms, TM=10 ms, excitation: 150 μs 90° hard pulse (8 kHz excitation bandwidth) centered on the ©NTP peak, 8 kHz acquisition bandwidth, 8192 complex points, 128 averages (16 phase cycles); total acquisition time ~6½ min. Hyperbolic secant 180° pulses (3000 μs) were used for localization. Dogs were sedated with acepromazine (0.025 mg/kg) followed by butorphanol (0.5–1 mg/kg), then anesthetized with propofol (5 mg/kg), intubated, and then maintained initially with a constant rate of propofol infusion (0.1–0.4 mg/kg/min) during scan setup and switched over to isoflurane (1-2%) and breathable air (1L/min). ***TLT:*** The PhotoThera Research Laser System (RD0010) was used to deliver continuous wave laser output (808±10 nm, near-infrared wavelength) between 4.2-5.8 watts, with 9.5 watts pulsed peak dose. TLT was administered for 2 minutes at each of 2 partially overlapping anterior and posterior cranial locations, covering the cerebral cortex. ***Research design:*** Dogs were imaged before receiving TLT (baseline), then were removed from the scanner and subjected to TLT, and repositioned in the scanner to undergo serial 31P MRS for up to 2 hrs post-TLT. Care was taken to reposition dogs in the same location for all scans. Following scanning, dogs were recovered from anesthesia and then transported to Toxikon Corporation where they were housed. During housing, each dog underwent subchronic TLT treatments while fully awake (3 times/week for 2 weeks), using the same TLT treatment parameters as described above, and then transported to McLean Hospital for follow up 31P MRS. Again, a baseline scan was acquired followed by TLT treatment, followed by serial post-TLT MRS scans. ***MRS processing:*** 31P spectra were phased, baselined, and fitted in MestreNova using a Levenberg-Marquardt algorithm. ***Statistical Analysis:*** Data were analyzed with 2-way ANOVA with session (scan 1 versus 2) and and within-session (pre-TLT, post-TLT) MRS acquisition time treated as factors. The dependent variable was the ratio phosphocreatine (PCr)/beta-nucleoside triphosphate (β-NTP). Statistical analyses were carried out in STATA 12.

RESULTS: The PCr/ β-NTP ratio significantly differed as a function of session, was increased at scan 2 (P=0.0007, F(1,24)=15.30). Interaction effects were not significant and were not included in the final model (from which the p-values are reported). Figure 3 illustrates time courses of these data via a factorial plot.

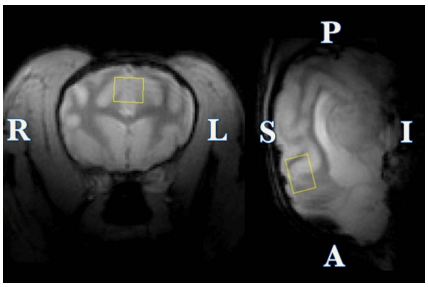

Fig. 1. Representative MRS voxel placement at the level of the cerebral cortex and the anterior cingulate

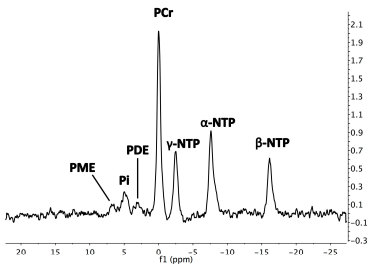

Fig 2. Representative 31P spectrum

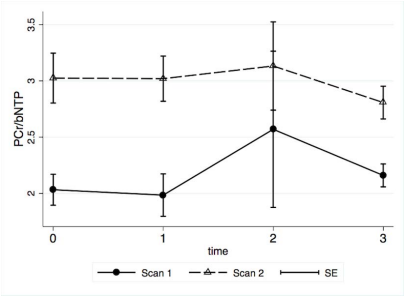

Fig 3. PCr/ β-NTP ratios (time points grouped factorially and averaged for easier visualization)

DISCUSSION: We found that subchronic TLT increased PCr/ β-NTP ratios, paralleling similar effects detected in humans after short-term oral creatine supplementation [8-9]. Increased PCr/ β-NTP ratios have been interpreted to reflect increased bioenergetic efficiency or a shift in the creatine kinase equilibrium favoring PCr production, resulting in more high energy phosphorus equivalents available to convert to PCr upon demand [8-9]. Such effects could be especially beneficial in brain disorders involving compromised bioenergetics, such as stroke, MD Alz, and TBI. Our observation of a significant TLT effect only after subchronic administration could indicate that TLT effects develop slowly. It is known that the expression of CCO protein, a target for TLT, increases upon neuronal stimulation via transcriptional events [10]. If the TLT-induced increase we detected in PCr/β-NTP ratios is in part mediated by CCO protein upregulation, that could explain why we only observed effects after subchronic TLT. One caveat worth noting is the fact that our baseline PCr/ β-NTP ratios averaged 2.03±0.28, higher than ratios of about 1.2 in human brain [8-9]. Although similar PCr/ β-NTP ratios have been reported in beagle brain [11], muscle PCr/ β-NTP ratios tend to be closer to 3.0 [12] and we believe that there may be a muscle contribution to our measurements. Interestingly, TLT also increases muscle mitochondrial respiration [13], so we may be detecting muscle and brain effects of TLT. Notwithstanding our study limitations, we believe our data warrant additional studies to more fully characterize the effects of TLT on cerebral energy metabolism, to determine whether TLT may be therapeutically beneficial in brain disorders associated with abnormal cerebral metabolism.

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