

MRI Techniques to Measure rCBF in Experimental Cortical Impact Injury in Mice

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Introduction: Traumatic brain injury (TBI) remains a leading cause of death and disability worldwide, hospitalizing upwards of 500,000 people per year in the United States alone. Neuroprotective mechanisms have been the subject of great interest for their ability to limit the post-injury and secondary injury affects associated with, and often exacerbating, the initial trauma. TBI results in potentially severe damage to the cerebral vasculature and a reduction in regional cerebral blood flow (rCBF). Arginase, an enzyme formed during the final step of the urea cycle, catalyzes the hydrolysis of L-arginine to yield L-ornithine and urea. Studies have identified arginase in the vasculature and have implicated the upregulation of this enzyme in the development of several conditions, including vascular disease, endothelial dysfunction, and experimental asthma and hypertension. Furthermore, arginase is pivotal in the regulation of nitric oxide (NO) synthesis via competition with endothelial nitric oxide synthase (eNOS) for the substrate L-arginine.

Cerebral hypoperfusion in the contused brain following traumatic brain injury is associated with substantially decreased NO levels resulting from limited substrate availability. We hypothesize that transgenic mice deficient in the enzyme arginase will display improved blood flow when observed with MRI. Ultimately, this mechanism may provide a new therapeutic intervention to restore CBF and improve neurological outcome after TBI.

Materials and Methods:

TBI: Transgenic arginase II deficient mice and WT counterparts were tested. Prior to surgical procedure for administration of TBI, mice were imaged for baseline rCBF and undamaged anatomy. Surgical intubation was performed by exposing the trachea through a midline neck incision and directly observing the entry of the endotracheal tube into the trachea. The tube was sutured to the bottom lip and used to maintain the animal on 2-2 1/2 % Isoflurane in oxygen for the remainder of the surgery. Mice were then placed in a stereotaxic frame and secured with ear bars and an incisor bar. A midline cranial incision was made, followed by a 5mm craniectomy above the right parietal cortex, being careful not to damage the dura mater. Mice were subjected to controlled cortical impact injury (3m/s; 1.5mm deformation) and mechanically ventilated in the MRI for the remainder of the study. Respiration and temperature were monitored with a respiratory pad and rectal probe, respectively.

MRI: Mice were imaged on a 9.4 T Bruker Avance Biospec Spectrometer, 21-cm bore horizontal scanner with a 35 mm volume resonator (Bruker BioSpin, Billerica, MA). A pilot scan was conducted and anatomical scans were utilized to align the slice to just in front of the hippocampus (anterior to the injury). A series of images were collected to assess relative cerebral blood flow (rCBF), injury site utilizing arterial spin labeled (ASL), and the apparent diffusion coefficient (ADC). The imaging parameters for the ASL perfusion scan were as follows: TR=7555.4; TE=16.73; FOV=1.50 cm; slice thickness=5.0 mm, matrix=64 x 64; NEX=2. Each selective and non-selective scan took approximately 2 min to acquire using Paravision software (Bruker BioSpin, Billerica, MA). rCBF was calculated from changes in signal intensity between selective and non-selective scans using the following equation: $rCBF = \lambda((1/T_{1(selective)}) - (1/T_{1(non-selective)})$. ROIs for the scans were from the cortex and striatum ipsilateral and contralateral to the injury site.

Results: Blood flow deficits are known to exist following TBI. The rCBF calculations indicate a marked improvement in most of the brain regions selected in arginase deficient mice compared with their wildtype counterparts. These preliminary data suggest that mice lacking arginase either experience less blunting of their blood flow or recover their perfusion more rapidly after trauma.

Conclusions and future directions: The arginase enzyme competes with the important vasoregulatory enzyme eNOS for the substrate L-arginine. Mice that are deficient or lack arginase activity appear to have more favorable rCBF following TBI than WT mice indicating that L-arginine may be a limiting factor in blood flow regulation. Additional studies will be conducted to verify these data and also assess the mechanism.

References:

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2. Hlatky R, Lui H, Cherian L, Goodman JC, O'Brien WE, Constant CF, Roberston CS : The role of nitric oxide synthase in the cerebral hemodynamics after controlled cortical impact injury in mice. *J Neurotrauma* **20**: 995-1006, 2003.

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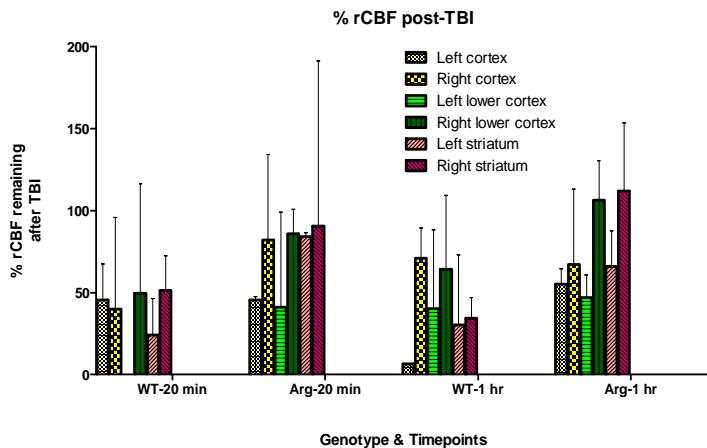
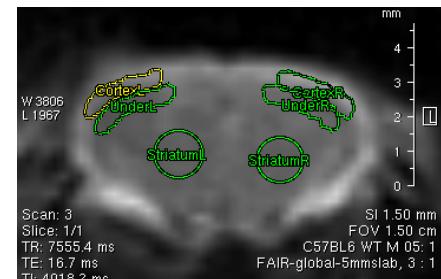


Figure 1: (Top) Perfusion scan image with regions of interest (ROI).

Figure 2: (Bottom) Percent of normalized rCBF remaining post-TBI.

Normalized rCBF for two timepoints (20 min. and 1 hr. post-TBI) for wildtype (WT, n=3) and arginase^{-/-} (Arg, n=4) mice.