

# NAA as a non-invasive biomarker in traumatic brain injury: neuroprotective effects of cyclosporine A

J. L. Harris<sup>1</sup>, H. Yeh<sup>2</sup>, N. E. Berman<sup>3</sup>, and W. M. Brooks<sup>1</sup>

<sup>1</sup>Hoglund Brain Imaging Center, University of Kansas Medical Center, Kansas City, KS, United States, <sup>2</sup>Department of Biostatistics, University of Kansas Medical Center, Kansas City, KS, United States, <sup>3</sup>Department of Anatomy & Cell Biology, University of Kansas Medical Center, Kansas City, KS, United States

## Introduction

Traumatic brain injury (TBI) is a major public health concern. At least 1.5 million injuries occur each year in the United States alone, with annual costs approaching \$US60 billion. Despite success in animal models of TBI, clinical trials in humans have not resulted in improved outcomes. The initial physical impact of TBI unleashes numerous processes - including neuronal mitochondrial dysfunction, inflammation, oxidative stress, neuronal necrosis, apoptosis, excitotoxicity, and edema - that each contribute to ultimate outcome. One reason for the failure of trials is this complexity of TBI pathology, and the absence of robust biomarkers to reflect specific pathophysiological mechanisms. Non-invasive biomarkers sensitive to specific TBI mechanisms would have considerable value in designing and implementing clinical trials and for monitoring individual treatment efficacy.

N-acetylaspartate (NAA), a metabolite synthesized in neuronal mitochondria and detected by proton magnetic resonance spectroscopy (MRS), might serve as a non-invasive biomarker of mitochondrial integrity after TBI [1]. Previous studies in human survivors of TBI have linked NAA with cognitive recovery, although the specific mechanism has not been elucidated [2]. In the present study we examine a time course of changes in NAA and behavioral impairment after TBI in a well-characterized animal model. We then investigate whether these NAA changes are sensitive to manipulation of mitochondrial status by cyclosporine A (CsA), an experimental neuroprotective agent that inhibits mitochondrial permeability transition after TBI.

## Methods

Adult male Fischer 344 rats (n=10) were subjected to unilateral controlled cortical impact (CCI) of the sensorimotor cortex. Injury parameters were: impact tip size = 5mm; velocity = 1.5m/s; depth = 2.0mm; contact time = 300ms. In a subset of animals, CsA was injected i.p. (20 mg/kg) immediately following injury and again on Day 1 (D1). A Varian 9.4T system was used to collect MR spectra from a 3 x 2.5 x 3mm voxel adjacent to the injury contusion site using a water-suppressed STEAM sequence (TE=2ms, TR=4000ms;

[3]). First and second order shims were adjusted using FASTMAP [4]. Spectra were analyzed using LCModel [5]. Baseline NAA levels were quantified prior to injury and post-injury on D0 (1-2 hrs post-injury), D1, D3, D7, and D14. Results were expressed as a percent of pre-injury baseline. To test behavioral recovery after CCI, an accelerating rotarod paradigm (0-30 rpm over 300 sec) was used. Animals were trained on the rotarod for 5 days prior to injury, and tested on D1, D3, D7, and D14. Student's t-tests were used to analyze differences in metabolite levels and behavioral performance.

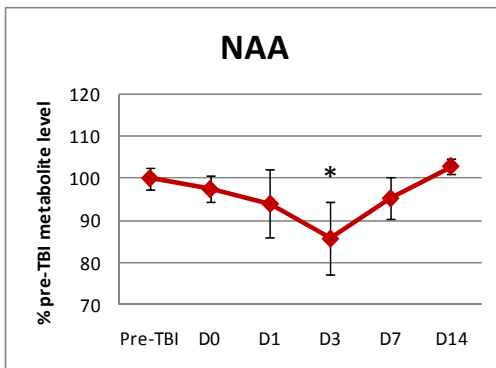


Figure 1. MRS detects changes in NAA over time after TBI (\*p<0.05).

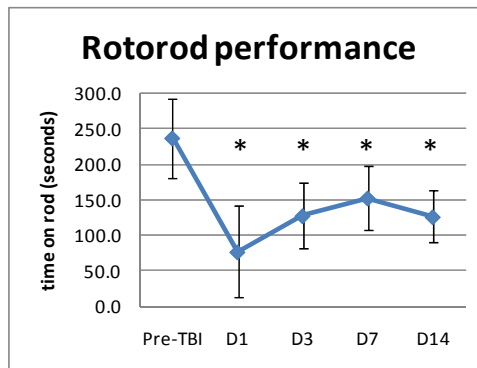


Figure 2. The rotarod behavioral test demonstrates impaired motor coordination after TBI (p<0.05).

## Results

In untreated rats, NAA levels reached their lowest value 3 days after TBI, followed by recovery to pre-injury levels (Fig. 1). We also observed a significant behavioral deficit on the rotarod which persisted out to 14 days after TBI (Fig. 2). At 3 days, when NAA was lowest in untreated TBI animals, CsA treated animals showed attenuated NAA reduction and a corresponding improvement in behavioral performance (Fig. 3).

## Discussion

These results demonstrate that NAA is sensitive to modulation by CsA, an agent that protects mitochondrial function after TBI. In a previous study, Signoretti *et al* used invasive techniques to show that CsA restores whole brain NAA concentration 6 hours after TBI in rats [1]. Our study is the first to use MRS *in vivo* to demonstrate CsA-mediated neuroprotection in intact animals. These results (i) establish the feasibility of longitudinal studies that quantify both biomarkers and behavior within the same animal and (ii) link NAA evidence of neuroprotection to improved cognitive recovery following TBI. Our findings support the hypothesis that NAA may be used as a non-invasive biomarker of neuronal mitochondrial integrity following brain injury. This interpretation takes on particular importance since NAA can be measured non-invasively in human TBI patients with existing clinical MRI scanners and sequences.

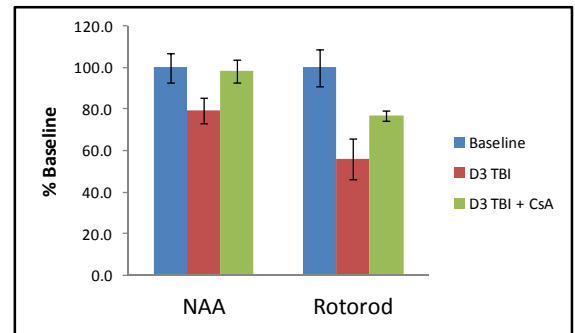


Figure 3. At 3 days post TBI, CsA preserves NAA levels (left) and improves rotarod behavioral performance (right).

**References:** [1] Signoretti, S., et al., J Neurotrauma, 2004. 21(9): p. 1154-67. [2] Friedman, S.D., et al., Neurology, 1999. 52(7): p. 1384-91. [3] Tkac, I., et al., Magn Reson Med, 1999. 41(4): p. 649-56. [4] Gruetter, R., Magn Reson Med, 1993. 29(6): p. 804-11. [5] Provencher, S.W., Magn Reson Med, 1993. 30(6): p. 672-9.