Salivary Biomarkers of Traumatic Brain Injury

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Abstract

Traumatic brain injury (TBI) may or may not be clinically evident in the acute period after cranial impact or blast pressure exposure, and the magnitude of delayed cognitive deficit resulting from a mild-moderate TBI is often difficult to estimate in the hours following the event. Also, the cumulative effect of multiple TBIs upon brain health remains difficult to gauge. Together these problems limit prudent deployment of personnel and detrimentally affect long-term health management of military personnel. Similar challenges are manifest in certain civilian endeavors such as sports medicine where repeated mild TBIs may be commonplace. We have been attempting to identify convenient protein biomarkers for TBI that can be measured non-invasively, in small quantities of saliva. We have identified a 60 kDa proteolytic fragment of the brain microtubule-associated protein, collapsin response mediator protein-2 (CRMP2) that shows promise as a salivary biomarker for TBI. Western blots readily detected the low-molecular weight CRMP2a fragment (s-CRMP2) in both human and rodent saliva as well as human and rodent brain tissue. In mice exposed to experimental blast pressure-TBI, s-CRMP2 increased 10-fold within 12 hours and remained elevated for at least 3 days. A small-molecule therapeutic, lanthionine ketimine-ethyl ester (LKE) that functionally enhances CRMP2, is being developed and has been shown to improve cognitive function in rodent models of Alzheimer’s disease and in a fluid percussion model of TBI. The combination of CRMP2 biomarker-based TBI assessment and CRMP2-directed pharmacotherapy represents a promising avenue for future personalized medical advancements. Supported in part by an AFOSR grant FA9550-13-1-0187.

CRMP2 has properties favorable for a biomarker of brain injury

- Neuronal microtubule-associated protein with high expression
- Proteolytically processed after neural injury
- Needful for neuron structure and function
- Adapts cargo proteins and vesicles to kinesin and dynein for microtubule-directed transport
- Hensley lab has uncovered roles in autophagy
- Loss of function → axonal deterioration
- Binds the novel sulfur amino acid metabolite lanthionine ketimine (LK). Patented brain-penetrating LK derivatives functionally enhance CRMP2

A CRMP2 fragment is present in saliva and responds to blast injury

Human saliva was collected by salivary swab from the parotid region. Swab tips were boiled in 100 μL reducing SDS-PAGE buffer and proteins electrophoresed across 4-20% gradient polyacrylamide gels then wet blotted to PVDF membranes and probed with a commercial antibody to CRMP2. Positive control is human brain cortex lysate.

A blast protocol was developed using a gas-driven devicer. A 0.01 inch plastic membrane was ruptured using high-pressure helium gas, creating a shockwave that traveled through the tube, striking the animal’s skull, which was situated 2cm from the edge of the tube. The total pressure of the blast sustained by each animal was about 200 psi. All animals were monitored after the blasts to ensure that their weight, posture, locomotion, and eating/drinking were not severely compromised. Some of the animals did experience hind limb deficits post-blast, and we assume that this occurred due to a disruption of the corticospinal tract.

After the blast protocol was complete, post-blast collections were taken at four time points: 6, 12, 24 and 48 hours after the time of the “trauma”. Baseline (BL) comparisons were also made to the “control” or rats that did not undergo the trauma. The animals were given 80 mg/kg ketamine/xylazine as an anesthetic and 0.5 mg/kg pilocarpine as a salivary stimulant via IP injection.

Salivary CRMP2 in blast injury

Findings: Transient increase in one CRMP2 proteolysis fragment at 12 h, decreased at 24h, perhaps beginning a secondary phase increase at 48h post-blast.

References


