Inflammation in the pain pathway in a model of mild closed head injury: Implications for post-concussion headache

Macolino CM¹, Daiutolo BV¹, Tyburski AL¹, Elliott MB¹,²

¹Department of Neurological Surgery; ²Department of Neuroscience, Thomas Jefferson University, Philadelphia, PA

Introduction

Headache is a hallmark feature of post-concussion syndrome. Post-concussion headache (PCH) is highly prevalent in the military with as high as 97.8% reporting having headaches¹ occurring in up to 85% of athletes following 2-4 concussive or mTBI. In the Military and sports, return to duty or play guidelines state that a soldier/player should be asymptomatic before returning to physical activity². However, headache following concussion is commonly dismissed.

PCH can be a new headache resulting from head trauma or worsening of pre-existing headache disorder. Incidence rates for concussion will continue to grow along with the increasing awareness and improvements in diagnosis. In many patients, PCH resolves in three months; however, in some cases, it persists for much longer³. Acute PCH is most likely due to acute inflammatory mechanisms. If headache after concussion is indicative of ongoing neuroinflammation, then headache is an important clinical sign that the neurological system is healing and there is a continued susceptibility to damage. Understanding the post – concussion symptomology, including headache, is important for concussion management, as well as preventing chronic disorders from developing.

Abnormalities within several areas of the trigeminovascular system are common in migraine and other headache disorders. In our previous studies, changes in the trigeminovascular system correlated with headache – like behavior (mechanical allodynia) in murine⁴ and rat⁵ models of focal traumatic brain injury, controlled cortical impact (CCI). The goal of this study was to compare changes in the trigeminal pain pathway and related behavior between a mild CCI injury (with focal diffuse features) and a mild closed head injury (CHI) injury (with diffuse injury only).

Methods

Injury: Male adolescent Sprague Dawley rats were randomized into CCI, single CHI (sCHI) or repeated (two injuries) CHI (rCHI) groups and compared to craniotomy (CR) or incision – only (INC) control groups.

Behavior: Baseline and post-operative neurological testing included periodical von Frey mechanical sensory testing to evaluate the presence of allodynia, accelerated Rotarod testing to evaluate changes in balance and motor function and Barnes maze to detect deficits in spatial learning and memory.

Immunohistochemistry: Changes for markers of injury/inflammation including β-Amyloid Precursor Protein (APP, Invitrogen #512700), Glial Fibrillary Acidic Protein (GFAP, Millipore #5804), Ionized Calcium-Binding Adaptor Molecule – 1 (IBA-1, Wako #019-1974), Calcitonin Gene Related Peptide (CGRP, Sigma #C8198) and Inducible Nitric Oxide Synthase (iNOS, Enzo #AD1-905-431) were evaluated in the ganglia and Ventral Posteriormedial Nucleus (VPM)/Ventral Posteriorlateral Nucleus (VPL) region of the thalamus using Nikon NIS Elements software.

Statistical Analyses: GraphPad Prism Software was used to analyze data via One-Way ANOVA followed by Bonferroni’s Post-Hoc test. p<0.05 was considered statistically significant.

Results

Figure 1 – Closed Head Injury Surgical Procedure: A 10mm modified impactor tip was aligned and centered 1mm lateral, 5mm anterior and posterior to Bregma on the right parietal-temporal region at a 20° angle. Using a stereotactic controlled cortical impact device (Leica), depth of injury was set to 2.0mm at a velocity of 5.0m/s. For repetitive closed head injury, injury was initiated on Day 0 (D0) and repeated on Day 3 (D3).

Figure 2 – Number of CGRP and iNOS positively stained trigeminal ganglia cells at seven days after injury: CGRP is significantly increased in both CCI and CHI injured rats compared to incision (*p<0.05, respectively) and craniotomy controls (p<0.05). iNOS is significantly increased in CCI, sCHI and rCHI injured rats compared to incision (**p<0.01, and ***p<0.001, respectively). One way ANOVA was statistically significant **p<0.01.

Figure 3 – Trigeminal Ganglia CGRP (A-D) and iNOS (E-H) immunohistochemistry seven days after injury: Incision control ganglia show a low level of CGRP (A) and iNOS (E) staining after injury while sCHI, rCHI and CCI injured rat ganglia have significantly increased staining (B-D, F-H) in the V1 region of the trigeminal nerve. Scale bar = 100 µm

Figure 4 – Periorbital von Frey thresholds seven days after injury: In the ipsilateral region, rCHI rat thresholds were significantly reduced compared to incision, sCHI and CCI thresholds (**p<0.01, and ***p<0.001, respectively) and CCI thresholds were significantly reduced compared to incision and craniotomy (CR) thresholds (**p<0.01 and ***p<0.001, respectively). In the contralateral region, rCHI thresholds were significantly reduced compared to incision and CCI thresholds (**p<0.01 and ***p<0.001, respectively) while CCI thresholds were significantly reduced compared to incision thresholds (**p<0.01). One way ANOVA was statistically significant **p<0.01.

Conclusion

Increased CGRP and iNOS positive ganglia cells in the trigeminal ganglia compared to incision and craniotomy controls indicate that peripheral sensitization is independent of the type of injury.

Group differences in mechanical allodynia (reduced periorbital von Frey thresholds) after rCHI and CCI injuries indicate that central sensitization is present for both types of injuries, while rCHI showed the greatest reduction in threshold. Results indicate that the type of injury results in differences in central pain mechanisms.

Rotarod performance was performed to determine if motor deficits would result in a lack of response during von Frey testing and to compare the effects between models. Cutaneous hypersensitivity was found in the presence of rotarod deficits indicating motor deficits do not confound sensory testing. A return to baseline after sCHI along with values for rCHI indicate CHI is a mild TBI compared to CCI.

Both rCHI and CCI injury induce sensitization of the trigeminal ganglia, while the degree of central sensitization may be dependent on the type of injury. Astrogliosis and microgliosis in thalamic pain regions (VPM/VPL) in the absence of β-APP accumulation indicates inflammation is present without direct axonal injury in this region.

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Contacts:

Corresponding PI: melanie.elliott@jefferson.edu
First author: christine.macolino@jefferson.edu

References:

¹Theiler et al., 2010;50:1262-1272; ²Seifert and Evans, 2010;14:292-2; ³Hoffman et al., 2011; ⁴Seifert TD, 2013; ⁵Lucas et al., 2011; ⁶Elliott et al., 2012; ⁷Macolino et al., 2014.