Abstract
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Brain cathepsin B is elevated following trauma in both mild-closed and severe-penetrating traumatic brain injury models.

Brittany Abbatiello[1], Shonnette Grant[1], Gregory Hook[2], Vivian Hook[3], Frank C. Tortella[1], Deborah A. Shear[1], Angela M. Boutte[1]

[1] Brain Trauma Neuroprotection and Neurorestoration Branch, Walter Reed Army Institute of Research, Silver Spring, MD
[2] American Life Science Pharmaceuticals, Inc., San Diego, CA
[3] Skaggs School of Pharmacy and Pharmaceutical Sciences, Dept. of Neurosciences, University of California, San Diego, La Jolla, CA

Comprehensive analysis of key mediators involved in traumatic brain injury (TBI) is tantamount to understanding mechanisms involved in injury progression. Cathepsin B is a cysteine protease implicated in several neurodegeneration and TBI models, such as controlled cortical impact. The purpose of this preliminary study was to determine if brain cathepsin B is also up-regulated in penetrating ballistic-like brain injury (PBBI) or repeated projectile concussive impact (rPCI) in rodent models of severe or mild TBIs.

For PBBI and sham/craniotomy controls, coronal brain tissue sections were isolated at various time-points post-injury. Repeated (r)PCI was conducted once daily for 4 consecutive days (d); control groups received anesthesia alone. Righting-reflex (RR) was determined immediately and select brain tissue regions were collected 1d after the last incident. Both pro- (~37-43kDa) and mature (~20-25kDa) Cathepsin B protein levels were determined by western blotting and densitometry (mean+/-SEM arbitrary units (AU)). Enzymatic activity was determined by generation of amino-methyl coumarin (AMC) in a fluorescent micro-plate assay. Comparisons between injured and control groups are discussed (2-tailed, t-Test, $p \leq 0.05$) and correlative analysis is indicated (1-way, Pearson *r*).

Pro-cathepsin B upregulation in brain slices was monophasic and peaked 2-3d after PBBI (13.3+/-1.2 and 15.2+/-2.3 AU) compared to Sham (1.2+/-0.1 to 3.1+/-1.3 AU). Interestingly, mature cathepsin B was maximally increased 7d after PBBI (384.1+/-39.7 AU), and doubled compared to Sham (174.2+/-25.4 AU). In the prefrontal cortex, pro-cathepsin B was not detectable in sham/anesthesia controls, but was increased to (1.7+/-0.5 AU) after rPCI. In this brain region, the mature form was nearly 7-fold greater after rPCI (14.2+/-3.6 AU) compared to controls (2.2+/-1.5 AU) and proteolytic activity was marginally increased. Surprisingly, cerebellar activity increased by nearly 3-fold to rPCI (3.0+/-0.6 μmoles) from anesthesia alone (1.4+/-0.2 μmoles) and was positively associated with RR ($r = +0.65$, $p=0.12$). Conversely, decreased cerebellar activity in anesthesia controls was negatively correlated with RR ($r = -0.98$, $p=0.008$).

Overall, Cathepsin B was upregulated in severe and mild injury models. Furthermore, activity correlated to the inability to regain consciousness after concussion. These findings suggest that brain cathepsin B has a role in multiple TBI models and is linked to neurological deficits.

Keywords: brain injury, cathepsin B, concussion/PCI, penetrating ballistic-like brain injury