Markers of Astrocyte Reactivity in *in vitro* Models of Blast-Induced Neurotrauma

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***Short Abstract* — Astrocyte reactivity is a response often to mechanical stimulation that is defined by biochemical changes and increased proliferation. There is a growing need to understand this reactive response in relation to insult associated with common mechanisms of traumatic brain injuries. This study aimed to characterize the response of astrocytes after exposure to overpressure using both two- and three-dimensional models. Structural and other related biomarkers were quantitatively assessed at acute time points after exposure. Results indicate that cytoskeletal structure of the cells was not compromised and some reactive markers indicate different time periods of activation of the two- and three-dimensional models.**

***Keywords* — astrocyte, reactivity, neurotrauma, cytoskeleton**

I. INTRODUCTION

Blast-induced neurotrauma is a growing concern in military personnel, with more than 73% of casualties in recent military endeavors involving explosives [1]. The prevalence and long-term impacts of these injuries dictate a need to better understand cellular responses to injury in this context in order to be able to design targeted therapeutics. Astrocytes play a critical role in the central nervous system's response to injury [2]. Moreover, they have a reactive response, termed astrogliosis, as a result of exposure to mechanical stimuli. Astrogliosis is characterized by increased proliferation as well as up-regulation of activation markers including glial fibrillary acidic protein (GFAP) [2-4]. The role of astrocyte activation in both neuroprotection and degeneration has been explored [2, 5-6], however, the response is still not completely understood. In this study, cells were exposed to overpressure profiles characteristic of blast exposure. This study aimed to compare two- and three-dimensional models of astrocyte reactivity to quantitatively assess the effect of both exposure and environment on gene expression for several structural proteins as well as a proliferation marker. Each target was chosen as a potential biomarker for activation in response to overpressure.

II. EXPERIMENTAL APPROACH

C6 astroglioma cells (ATCC, CCL-107) were used in two- and three-dimensional *in vitro* models to characterize acute astrocyte response and reactivity to an overpressure of 18-20 psi. A custom chamber was used to measure and record overpressure in real time. Quantitative assessment was conducted using reverse transcription, real-time polymerase chain reaction (RT-PCR). RNA was extracted from samples at 48 and 72 hours post exposure and was used to synthesize cDNA for RT-PCR. Fold changes for each target (Table 1) were calculated by using a delta-delta ($\Delta\Delta$) C_t method and by normalizing to a sham group.

Table 1. Biomarkers of interest for astrocyte reactivity.

Target	Classification/Function
Glial fibrillary acidic protein	intermediate filament, mechanical strength
β-actin	cytoskeletal protein, shape, integrity
Vinculin	cytoskeletal protein, anchors actin
Piezo2	transmembrane protein, cation channel, mechanosensor
Ezrin	peripheral membrane protein, adhesion, communication
Mitogen-activated protein kinase kinase 1	enzyme, stimulates MAP kinases pathways (proliferation)

III. RESULTS

In both models there were significant differences in fold change of GFAP expression from the sham groups ($p<0.05$), with opposite responses at 48 hours. For the two-dimensional model, GFAP expression was elevated to a fold change of 1.66, whereas it was decreased to 0.51 for the three-dimensional model. Both models showed a return to normal levels by 72 hours. This suggests different time periods of activation relative to environment. There were no significant differences from sham for other structural components at either time point, however, several targets had trending increases from the 48 to the 72-hour time point. While analysis suggests no significant structural damage to the cells, it does show potential for differential activation markers between the two- and three-dimensional *in vitro* models.

REFERENCES

[1] Cernak I, Noble-Haeusslein LJ (2010) Traumatic brain injury: an overview of pathobiology with emphasis on military populations. *J Cereb Blood Flow Metab* **30**, 255-266.

[2] Buffo A, Rolando C, Ceruti S (2010) Astrocytes in the damaged brain: molecular and cellular insights into their reactive response. *Biochem Pharmacol* **79 (2)**, 77-89.

[3] VandeVord PJ, et al. (2008) Up-regulation of reactivity and survival genes in astrocytes after exposure to short duration overpressure. *Neurosci Lett* **434 (3)**, 247-252.

[4] Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci* **32(12)**, 638-647.

[5] Myer DJ, et al. (2006) Essential protective roles of reactive astrocytes in traumatic brain injury. *Brain* **129**, 2761-2772.

[6] Pekny M, Wilhelmsson U, Pekna M (2014) The dual role of astrocyte activation and reactive gliosis. *Neurosci Lett* **565**, 30-38.

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