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Molecular mechanisms of increased cerebral vulnerability after repeated mild blast-induced traumatic brain injury☆

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ABSTRACT

The consequences of a mild traumatic brain injury can be especially severe if it is repeated within the period of increased cerebral vulnerability (ICV) that follows the initial insult. To better understand the molecular mechanisms that contribute to ICV, we exposed rats to different levels of mild blast overpressure (5 exposures; total pressure range: 15.54–19.41 psi or 107.14–133.83 kPa) at a rate of 1 per 30 min, monitored select physiological parameters, and assessed behavior. Two days post-injury or sham, we determined changes in protein biomarkers related to various pathologies in behaviorally relevant brain regions and in plasma. We found that oxygen saturation and heart rate were transiently depressed following mild blast exposure and that injured rats exhibited significantly increased anxiety- and depression-related behaviors. Proteomic analyses of the selected brain regions showed evidence of substantial oxidative stress and vascular changes, altered cell adhesion, and inflammation predominantly in the prefrontal cortex. Importantly, these pathological changes as well as indications of neuronal and glial cell loss/damage were also detected in the plasma of injured rats. Our findings illustrate some of the complex molecular changes that contribute to the period of ICV in repeated mild blast-induced traumatic brain injury. Further studies are needed to determine the functional and temporal

Abbreviations: AD, amygdala; BBB, blood brain barrier; bTBI, blast-induced TBI; CNS, central nervous system; DHC, dorsal hippocampus; ICV, increased cerebral vulnerability; mTBI, mild traumatic brain injury; OF, open field; PFC, prefrontal cortex; PTSD, post-traumatic stress disorder; rmTBI, repeated mild traumatic brain injury; TBI, traumatic brain injury; USU, Uniformed Services University; VHC, ventral hippocampus.

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relationship between the various pathomechanisms. The validation of these and other markers can help to diagnose individuals with ICV using a minimally invasive procedure and to develop evidence-based treatments for chronic neuropsychiatric conditions.

1. Introduction

Mild traumatic brain injuries (mTBIs) constitute approximately 80% of all traumatic brain injuries (TBIs) [1]. Among civilians, mTBIs (better known as concussions) affect ~1.3 million individuals in the US annually, mostly during contact sports such as boxing, hockey, and football [1,2]. In the military, ~266,000 service members suffered mTBIs between the years 2000 and 2012 with the overwhelming majority of injuries being blast-induced (http://www.dvbic.org/dod-worldwide-numbers-tbi). Because mTBIs typically result in transient and very mild symptoms, many injured individuals return to the same activity—sports and/or military duty—and significant numbers are re-injured, some multiple times causing repeated mild TBI (rmTBI).

A single mTBI can result in neurobehavioral problems such as increased anxiety and cognitive impairment, but the probability of short- as well as long-term impairment is significantly higher following rmTBI [3]. The exposure to a single mTBI triggers a complex cascade of pathological (as well as restorative) responses during what is known as the secondary injury process. Depending on the severity of the insult, some or most of these changes dissipate over time especially after mTBIs. However, re-injury during the ongoing secondary injury process can result in a disproportionally severe—even fatal—outcome and significantly increase the probability of long-term damage. While severe TBIs have unique symptoms (early malignant cerebral edema and delayed severe vasoconstriction), mTBI, symptoms and likely the components of its secondary injury process are shared with other forms of closed head TBIs. However, one important unknown is whether the onset and extent of the individual pathologies are shared among the different forms of mTBIs.

Clinical and experimental data have demonstrated that the interval between insults is a key factor in determining outcome severity after rmTBIs [4,5]. The interval after the initial insult during which re-injury causes disproportionately adverse effects is referred to as the period of increased cerebral vulnerability (ICV). While the exact pathomechanism of ICV is currently unknown, two key studies using different models of rmTBI have shown that decreased cerebral glucose metabolism and increased axonal and vascular vulnerability are major contributors to ICV [6–8]. The authors have concluded that these otherwise transient changes predispose the brain for additional damage if subsequent insults take place within the period of ICV. Whether or not other components of the secondary injury process contribute to ICV as well as the exact "window period" of vulnerability have yet to be determined [5,9].

Previous studies, including our own, have identified several of the pathobiologies involved in blast-induced TBI (bTBI), which may contribute to ICV. These include oxidative stress, metabolic and vascular changes, altered cellular adhesion, inflammation, as well as axonal, neuronal and glial cell damage [10,11]. Accordingly, we have selected protein biomarkers that are representative of the various functional clusters listed above and assayed them using reverse phase protein microarray (RPPM). While we have successfully utilized RPPM for the identification of blast-induced changes in brain tissue, cerebrospinal fluid, plasma and/or serum [10,12–16], the method does not provide absolute protein values like other antibody-based assays (e.g., ELISA) and its use is limited to the availability of specific antibodies. However, RPPM offers superior sensitivity, specificity, a dynamic range, and high throughput ideal for assaying large numbers of samples.

Our previous works using the rodent model of bTBI identified some of the functional deficits sustained following single and repeated mild blast overpressure exposure as well as several of the molecular and cellular changes associated with each type of injury [11]. We found that when rats were exposed to mild blast overpressure (~138 kPa) once per day for five consecutive days, the resultant damage (as indicated by specific protein biomarker levels in the plasma and in brain tissue) was only moderately greater in multiple blast-injured rats compared to single-injured rats at 2 h post-injury, 22 days, or even 42 days after the injury. Consistent with the observations from the abovementioned studies, our data indicated that the period of ICV after blast-induced mTBI is considerably shorter than 24 h in rats.

To better understand the biology of ICV, in this study we exposed rats to a total of five mild blasts at the rate of 1 per 30 min. As described herein, this increased frequency/shortened interval of insults resulted in significant alterations in physiological parameters, neurobehavioral deficits, and extensive molecular changes that can be detected in functionally relevant brain regions as well as in circulating blood. The detected changes in protein biomarker levels implicate oxidative stress, vascular pathologies, and inflammation in the cerebral vulnerability that follows blast-induced mTBI. These markers can be tested in humans who have suffered an mTBI and if validated they will aid in identifying individuals with ICV and in determining a "safe return to duty/play".

2. Materials and methods

2.1. Animals

A total of 22 male Sprague Dawley rats (weight at arrival: 280–300 g; approximate age in days: 63–67) (Charles River Laboratories, Wilmington, MA) were used in our study. Upon arrival at the Uniformed Services University (USU; Bethesda, MD), animals were housed in pairs in standard rat cages in a reverse 12 h-light 12 h-dark cycle with food and water ad lib. During the 5-day acclimation period, animals were handled for 5 min each day prior to undergoing baseline physiological monitoring and behavioral testing. Baseline horizontal activity results