

# Biomarkers of Blast-Induced Neurotrauma: Profiling Molecular and Cellular Mechanisms of Blast Brain Injury

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## Abstract

The nature of warfare in the 21st century has led to a significant increase in primary blast or over-pressurization injuries to the whole body and head, which manifest as a complex of neuro-somatic damage, including traumatic brain injury (TBI). Identifying relevant pathogenic pathways in reproducible experimental models of primary blast wave exposure is therefore vital to the development of biomarkers for diagnostics of blast brain injury. Comparative analysis of mechanisms and putative biomarkers of blast brain injury is complicated by a deficiency of experimental studies. In this article, we present an overview of current TBI biomarkers, as well as outline experimental strategies to investigate molecular signatures of blast neurotrauma and to develop a pathway network map for novel biomarker discovery. These biomarkers will be effective for triaging and managing both combat and civilian casualties.

**Key words:** biomarkers; blast; brain injury; molecular pathways; traumatic brain injury

## Introduction

**A**N UNDERSTANDING OF THE BIOLOGICAL MECHANISMS, and particularly the biomarkers of blast-induced neurotrauma, remain elusive. Symptoms of blast brain injury often do not manifest themselves until sometime after the injury has occurred (Cernak et al., 1999a; Yilmaz and Pekdemir, 2007). Mild or moderate brain injuries in particular often go undiagnosed and untreated because emergency medical attention is directed toward more visible injuries such as penetrating flesh wounds (Belanger et al., 2005; Lew, 2005). However, even mild and moderate brain injuries can produce significant deficits, and when repeated can lead to sustained neuro-somatic damage and neurodegeneration (Lew, 2005; Lew et al., 2005).

Over the last several decades, a number of experimental animal models have been implemented to study the mechanisms of blast wave impact and include rodents and larger animals such as sheep (Savic et al., 1991; Stuhmiller et al., 1996). Shock tubes have been used as the fundamental research tool for the past several decades. However, because of the rather generic nature of the blast generators used in the

different studies, the data on injury mechanisms, including brain damage, have been difficult to analyze and compare (Jaffin et al., 1987; Elsayed 1997; Guy et al., 1998). Thus, there is still a lack of relevant reproducible models within the blast injury framework, including the development of generators that precisely control parameters of the blast wave, similarly to those present in classical mechanical controlled cortical impact (CCI) models. This makes it difficult to investigate the blast injury mechanisms and to develop relevant biomarkers for diagnostics and mitigation. However, preliminary analysis of the data on blast brain injury mechanisms suggests that it can be compared to the CCI injury model and potential biomarkers suggested by the latter can be examined.

The goals of this review are to examine potential biochemical/pathophysiological pathways and possible biomarkers of blast brain injury.

## Review of Existing Biomarker Candidates

According to a generally accepted definition, a biomarker has the characteristic that it can be objectively measured

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## Report Documentation Page

*Form Approved*  
*OMB No. 0704-0188*

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1. REPORT DATE <b>JUN 2009</b>		2. REPORT TYPE		3. DATES COVERED <b>00-00-2009 to 00-00-2009</b>	
4. TITLE AND SUBTITLE <b>Biomarkers of Blast-Induced Neurotrauma: Profiling Molecular and Cellular Mechanisms of Blast Brain Injury</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Center of Innovative Research, Banyan Biomarkers, Inc, Alachua, FL</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT <b>The nature of warfare in the 21st century has led to a significant increase in primary blast or over-pressurization injuries to the whole body and head, which manifest as a complex of neuro-somatic damage, including traumatic brain injury (TBI). Identifying relevant pathogenic pathways in reproducible experimental models of primary blast wave exposure is therefore vital to the development of biomarkers for diagnostics of blast brain injury. Comparative analysis of mechanisms and putative biomarkers of blast brain injury is complicated by a deficiency of experimental studies. In this article, we present an overview of current TBI biomarkers, as well as outline experimental strategies to investigate molecular signatures of blast neurotrauma and to develop a pathway network map for novel biomarker discovery. These biomarkers will be effective for triaging and managing both combat and civilian casualties</b>					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			

and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses for therapeutic intervention (Biomarkers Definitions Working Group, 2001). In contrast, a “surrogate biomarker” or “surrogate endpoint” is expected to predict clinical effects based on therapeutic, pathophysiological, or other scientific correlations. Despite the apparent consensus on definition, controversy still exists about how to clearly delineate these two groups that have been associated with different experimental approaches that employ biomarkers for discovery and validation (Lescuyer et al., 2007). Ideally, brain injury biomarkers should be biological substrates unique to the brain and should provide information on injury mechanisms, a criterion used to distinguish biochemical markers from surrogate markers of injury, since surrogate markers usually do not provide information on injury mechanisms. Although there are currently no biomarkers with proven clinical utility for the diagnosis of brain injury, whether caused by blast, mechanical trauma, stroke, or other acute brain injuries, research has uncovered several valuable candidates that have shown preclinical potential. The ones currently generating the most interest include S-100 $\beta$ , neuron specific enolase (NSE), glial fibrillary acid protein (GFAP), and myelin basic protein (MBP). Although these proteins are still being assessed, they appear to lack either the necessary sensitivity or brain specificity (except perhaps GFAP) to be used effectively alone (Bazarian et al., 2006; Piazza et al., 2007). However, the combination of these markers can effectively detect TBI and provide outcome predictions (Berger et al., 2005; Berger et al., 2007). Below is a brief analysis of these generally accepted putative biomarkers of TBI.

#### *S-100 $\beta$ protein*

S-100 $\beta$  is among the most well studied proteins for TBI and is considered a promising, non-proprietary brain injury biomarker. S-100 $\beta$ , a small dimeric calcium binding protein, is most abundant in glial cells of the central nervous system (astrocytes) and peripheral nervous system (Schwann cells). A weakness of this marker is that it is not exclusive to the brain; S-100 $\beta$  has been found to be expressed in melanocytes, adipocytes, and chondrocytes (Michetti and Gazzolo, 2002).

A number of studies have demonstrated S-100 $\beta$ 's relationship to injury magnitude and outcome in TBI (Pelinka et al., 2004; Berger et al., 2005; Berger et al., 2007; Korfiatis et al., 2007). This suggests that S-100 $\beta$  may be useful in assessing the efficacy of treatment after severe TBI (Korfiatis et al., 2007). Other studies, however, reported a poor value of S100 $\beta$  as a predictor of outcome after brain injury, particularly mild and pediatric TBI (Bazarian et al., 2006; Kleindienst and Bullock, 2006; Piazza et al., 2007). Also, several studies raised concern whether the serum level of S100 $\beta$  can be considered a true biochemical marker of brain damage given the definition of “biomarker” discussed above (see Kleindienst et al., 2007 for review). For example, in several studies a poor correlation was found between serum and brain S100 $\beta$  values, suggesting that the serum levels may depend primarily on the integrity of the blood–brain barrier and do not reflect the S100 $\beta$  levels in the brain (Kleindienst and Bullock, 2006; Piazza et al., 2007). Despite apparent controversy, S-100 $\beta$  still has potential as a brain injury biomarker, and its preclinical and clinical

utility should be further explored, especially after blast wave exposures.

#### *Neuron specific enolase*

NSE initially held promise as a brain injury biomarker, as it was originally believed to be strictly neuronal. The protein is located in the neuronal cytoplasm and is involved in regulating intracellular chloride levels (Vinores et al., 1984). However, follow-up research found that NSE is also present in red blood cells and platelets, decreasing its diagnostic utility as a marker due to possible cross-contamination that could occur in blood samples (Johnsson et al., 2000). In clinical studies, serum NSE levels have been frequently studied alongside S-100 $\beta$ . The sensitivity and specificity of serum NSE after pediatric TBI as determined by ROC curves was found to be 71% and 64%, respectively (Berger et al., 2005). After multiple trauma, elevated NSE levels have been observed, but systemic NSE increased by similar degrees with and without TBI, limiting its ability to discriminate brain injury magnitude (Pelinka et al., 2005). Additionally, NSE has a long half-life, more than 20 h in serum, and this may account for its limitation for use as a TBI biomarker (Ingebrigtsen and Romner, 2003). Reports on correlations of serum NSE levels alone with clinical and neurological measures of brain injury magnitude and overall outcome have been controversial (Berger et al., 2005; Berger et al., 2007; Ingebrigtsen and Romner, 2003; Pelinka et al., 2005), although assay of serum NSE together with S100 $\beta$  have been valuable in predicting TBI outcome (Berger et al., 2006; Berger et al., 2007).

#### *Glial fibrillary acidic protein*

Glial fibrillary acidic protein (GFAP), a filament protein found in the astroglial cytoskeleton, is not found outside the CNS (Galea et al., 1995). It has only recently gained attention as a TBI biomarker, when it was shown to be as good as if not more promising than S-100 $\beta$ . In a study of 92 severe TBI patients both GFAP and S-100 $\beta$  were shown to be equally good predictors of mortality, with sensitivity between 70% and 84% depending on the time they were measured after injury (Pelinka et al., 2004; Nylen et al., 2006). In addition to discriminating survival after TBI, GFAP, but not S-100 $\beta$ , could predictably discriminate between severe disability and vegetative state versus good and moderate outcomes as evaluated by the Glasgow Outcome Scale (GOS). Another study of 114 severe TBI patients confirmed its ability to predict mortality (Pelinka et al., 2004), and it was found to further discriminate outcome categories of the GOS. This study also indicated that GFAP had the ability to discriminate injury severity based on the Marshall scale classification of computed tomography (CT) scans. It could discriminate between patients that had intracranial pressure (ICP) greater or less than 25 mm Hg, patients that had cerebral perfusion pressure greater or less than 60 mm Hg, and patients with mean arterial pressure greater or less than 60 mm Hg. In a third study of 85 patients with severe TBI, levels of GFAP, NSE, and S-100 $\beta$  taken on hospital admission correlated with outcome measured by GOS at 6 months. Biomarker levels also correlated moderately with other indices of injury magnitude and were better correlated with Glasgow Coma Scale scores than was CT scan analysis. GFAP shows good diagnostic potential to predict outcome after injury, and may also be valuable for diagnosing

injury magnitude, although it has not been studied in mild and moderate injury. Since it appears to be specific for brain tissue and is not affected by hemorrhagic shock or extracranial trauma, it may be a better diagnostic tool than S-100 $\beta$ .

#### *Myelin basic protein*

Myelin basic protein (MBP), an abundant protein in white matter, has been examined as a marker of axonal damage in several insults germane to neurointensive care. In TBI, CSF and serum levels of MBP have demonstrated excellent specificity, but limited sensitivity (Berger et al., 2005). It was recently shown that serum MBP, as well as NSE and S100 $\beta$ , concentrations obtained at the time of TBI may be useful in predicting outcome after pediatric TBI (Berger et al., 2006; Berger et al., 2007).

More recently a number of new candidate biomarkers have been discovered. The emerging data suggest TAU (c-tau) proteins (Zemlan et al., 2002; Gabbita et al., 2005),  $\alpha$ II-spectrin protein breakdown products (SBDPs) (Pike et al., 1998a; Pike et al., 1998b; Pike et al., 2001; Pineda et al., 2004; Haskins et al., 2005; Wang et al., 2005; Kobeissy et al., 2006; Ottens et al., 2006; Pineda et al., 2007), and other proteins have strong potential. Currently, Banyan Biomarkers, Inc. is performing ELISA validation on some of these markers, and clinical validation of the biomarkers with human serum samples is in progress.

#### *Microtubule associated protein-tau (c-Tau)*

The same enzymes that cleave the cytoskeletal protein  $\alpha$ II-spectrin cleave the microtubule associated protein-tau, releasing breakdown products (BDPs) with molecular weights that profile cleavage activity as being induced by the pro-necrotic calpains or the pro-apoptotic caspases. Tau cleavage by calpains include a BDP at 17 kDa that has been noted in cells following  $\beta$ -amyloid peptide treatment (Park and Ferreira, 2005; Park et al., 2007). Caspases have been shown to produce a tau breakdown product (TBDP) around 45 kDa (Chung et al., 2001). TBDPs were found to be present after TBI in rats (Zemlan et al., 2002; Gabbita et al., 2005). In a study whose primary objective was to determine the relationship between serum S-100 $\beta$  and c-tau levels and long-term outcome after mild TBI, S-100 $\beta$  and c-tau levels in serum taken 6 h after injury for 35 mild TBI subjects were compared to 3-month post-TBI Rivermead Post Concussion Questionnaire (RPCQ) scores and post-concussive syndrome. The results showed a weak linear correlation between marker levels and RPCQ scores for both markers, and that there was no statistically significant correlation between marker levels and 3-month post-concussive syndrome. Thus, serum S-100 $\beta$  and c-tau levels are apparently poor predictors of 3-month outcome after mild TBI (Bazarian et al., 2006). Further studies are required to ascertain whether the MAP-tau protein is a valid biomarker for brain trauma.

#### *$\alpha$ II-Spectrin breakdown products*

$\alpha$ II-spectrin is a major structural component of the neuron axonal cytoskeleton and a major proteolytic substrate for cysteine proteases (calpain and caspase-3) involved in oncotic (necrotic) and apoptotic cell death, respectively (Wang et al.,

1998). Considerable evidence has been accumulated by our laboratory that  $\alpha$ II-spectrin possesses signature cleavage products with molecular weights of 150 (SBDP150) and 145 kDa (SBDP145) due to cleavage by calpain, and a transient fragment of 150 kDa (SBDP150i) and a major cleavage product of 120 kDa (SBDP120) by caspase-3 (Nath et al., 1998; Pike et al., 1998a; Pike et al., 1998b). Since calpains and caspases become hyperactivated after traumatic and ischemic brain injury, we have proposed that SBDPs would be useful biomarkers of brain injury. Indeed, we and others found that these signature cleavage products are apparent in brain tissue and CSF after brain injury in rats (Pike et al., 1998a; Beer et al., 2000; Pike et al., 2001), brain ischemia (Zhang et al., 2002), and in human CSF following TBI (Pineda et al., 2004; Ringger et al., 2004). While MBP and c-Tau are relatively specific to the brain, the cytoskeleton  $\alpha$ II-spectrin and its cleavage products SBDP150, 145, and 120 are not. Thus, plasma/serum levels of  $\alpha$ II-spectrin SBDPs reflect the magnitude of multi-organ damage (including brain) rather than TBI per se.

#### *NMDA-R fragments*

Potential biomarkers for brain injury may include the fragmented form of the glutamate-N-methyl-D-aspartate (NMDA) receptor (NR2A/2B subtype). Dambinova and colleagues investigated the diagnostic accuracy of serum autoantibodies to NR2A/2B, a subtype of NMDA receptor, in assessing transient ischemic attack (TIA) and ischemic stroke (IS), and its ability to distinguish cerebral ischemia from intracerebral hemorrhage (ICH). They showed that patients with TIA (n=56) and acute IS (n=31) had significantly higher NR2A/2B autoantibody concentrations than controls. In addition, levels of NR2A/2B autoantibodies within 72 h in the IS group differed significantly from those in ICH patients ( $p < 0.001$ ). This was confirmed by magnetic resonance imaging and CT (Dambinova et al., 2003). It was concluded that NR2A/2B autoantibodies are independent and sensitive serologic markers capable of detecting TIA with a high post-test probability, and this correlates well with neurologic observation and neuroimaging, thereby ruling out ICH. Also, this biomarker may help assess the risk of TIA in routine general practice, and may potentially be useful in assisting diagnosis of acute IS in the emergency setting.

#### *Neuro-inflammatory cytokine markers*

The role of inflammation in various CNS injuries has been shown in numerous studies (see Lucas et al., 2006 for review). Hayakata and associates investigated early changes in the concentrations of CSF S-100 $\beta$  and various cytokines after TBI, and evaluated the relations of both to ICP and prognosis. CSF concentrations of S-100 $\beta$  and CSF and serum concentrations of five cytokines (IL-1 $\beta$ , TNF- $\alpha$ , IL-6, IL-8, and IL-10) were measured and compared. The CSF concentrations of S-100 $\beta$  and the cytokines peaked within 24 h after severe TBI, and decreased gradually thereafter. The authors concluded that CSF S-100 $\beta$  and IL-1 $\beta$  may be useful as outcome predictors in cases of severe TBI (Hayakata et al., 2004). In a subsequent study, they compared the CSF and serum levels of pro- and anti-inflammatory cytokines in 35 patients with severe TBI with and without additional injury. CSF and serum concentrations of two pro-inflammatory mediators (IL-1 $\beta$  and TNF- $\alpha$ ), and three anti-inflammatory mediators (IL-1 receptor

antagonist [IL-1ra], soluble TNF receptor-I [sTNFr-I], and IL-10) were measured and compared at 6 h after injury. CSF concentrations of pro-inflammatory mediators were much higher than the corresponding serum concentrations in both patient groups. In contrast, serum concentrations of anti-inflammatory mediators were much higher than the paired CSF concentrations in patients with additional injury, but serum concentrations were lower than or equal to the corresponding CSF concentrations in patients without additional injury. CSF concentrations of IL-1 $\beta$ , IL-1ra, sTNFr-I, and IL-10 were significantly higher in patients with high ICP than in patients with low ICP, and were also significantly higher in patients with an unfavorable outcome than in patients with a favorable outcome. These studies suggest that increased serum concentrations of anti-inflammatory mediators after severe TBI were mainly due to the additional extracranial injury/polytrauma. In contrast, anti-inflammatory mediators in CSF may be useful indicators of the severity of brain damage in terms of ICP, as well as overall prognosis of patients with severe TBI (Shiozaki et al., 2005).

### General Approaches for Studies of Specific Mechanisms of Blast-Related Injuries

In the blast injury model, experimental animals are exposed to blasts that result from the detonation of known quantities of explosives at varying distances from the source, at different body orientations towards the blast wave, and in open or confined fields (Elsayed, 1997; Axelsson et al., 2000; Saljo et al., 2000). Also, a number of investigations have employed compressed air-driven shock tubes and nitrogen-driven blast wave generators to expose various animals to blast injury (e.g., rats, mice, and rabbits) to analyze the mechanisms of injury (Jaffin et al., 1987).

The majority of the blast injury-related work has been done using animals that have been exposed to total body blasts (Cernak et al., 1991a; Cernak et al., 1991b; Cernak et al., 1995; Stuhmiller et al., 1996; Elsayed, 1997; Elsayed et al., 1997; Gorbunov et al., 1997; Januszkiewicz et al., 1997; Mayorga, 1997; Cernak et al., 1999c; Cernak et al., 2001a; Elsayed and Gorbunov, 2003; Chavko et al., 2006; Elsayed and Gorbunov, 2007). Several groups reported that animals exhibited lung and abdominal injuries when they were exposed to a directed shock tube-generated blast. Guy and co-workers showed that rats exposed to moderate thoracic blast exhibited increased apnea, bradycardia, and hypotension before they returned to pre-blast values. No significant cardiovascular or respiratory changes were seen in animals subjected to abdominal blast (Guy et al., 1998). The most noticeable damage after blast exposure was found in the inner ear, lungs, and other gas- or fluid-containing interfaces such as the gastrointestinal tract (Cernak et al., 1991a; Cernak et al., 1991b; Elsayed, 1997; Elsayed et al., 1997; Januszkiewicz et al., 1997; Chavko et al., 2006; Elsayed and Gorbunov, 2007).

Several reports suggest that free radical-mediated injury in lung and blood of rats, rabbits, and sheep occurs in response to the blast (Elsayed et al., 1996, 1997). The biochemical changes included an increase in lipid peroxidation and hemoglobin associated with decreased Pao<sub>2</sub>, and antioxidant depletion and impairment of calcium transport across the cell membrane. These changes were found to correlate well with blast peak overpressure (Elsayed et al., 1996).

Hemoglobin and especially either of its oxidation products (metHb and oxoferrylHb) or its degradation products (heme and free iron) promote injury due to free radical-mediated consequences (Elsayed et al., 1996; Gorbunov et al., 1997). This may explain the continued pathological response after the blast wave exposure has ceased. Nitric oxide (NO) released from endothelial cells can form ligands that complex the active iron on the hemoglobin molecules rendering it unreactive (i.e., NO may function as an antioxidant by quenching the hemoglobin-mediated free-radical reactions) (Gorbunov et al., 1996). On the other hand, following blast exposure, endogenous NO was observed to increase in some rat tissues including lung and brain tissue.

### Identification of brain-specific blast injury mechanisms and potential biomarkers

Several decades of medical literature reports provide a number of cases in which brain injuries are likely to have resulted from primary blast forces (Cramer et al., 1949; Murthy et al., 1979). Reported neuropathological changes have included small hemorrhages within the white matter, chromatolytic changes in the neurons (due to degeneration of Nissl bodies, an indication of neuronal damage), diffuse brain injury, and subdural hemorrhage. It is still controversial whether primary blast forces directly damage the brain, and if they do, what the mechanisms are that mediate the injury (Taber et al., 2006).

Shear and stress waves from the primary blast could potentially cause TBI directly (e.g., concussion, hemorrhage, edema, and diffuse axonal injury). On the other hand, the primary blast can also cause formation of gas emboli, leading to infarction (Guy et al., 2000a; Guy et al., 2000b).

The most common types of TBI are diffuse axonal injury, contusion, and subdural hemorrhage (Vander Vorst et al., 2007). Diffuse axonal injuries are very common following closed head injuries. They result when shearing, stretching, and/or angular forces pull on axons and small vessels. Impaired axonal transport leads to focal axonal swelling and after several hours may result in axonal disconnection (Hurley et al., 2004). The most common locations are the corticomedullary (gray matter-white matter) junction (particularly in the frontal and temporal areas), the internal capsule, the deep gray matter, the upper brainstem, and the corpus callosum. However, it has been shown that severely injured axons do not necessarily swell. The presence of focal axonal swellings, the most commonly used neuropathological marker for TBI, may therefore seriously underestimate the magnitude of injury present. It has also been noted that the insensitivity of presently used neuropathological markers to the unmyelinated fine-caliber axons that make up 30% of the corpus callosum may also contribute to inaccurate injury evaluation. After rats had been exposed to a total body blast, a phosphorylated epitope of the heavy subunit of the neurofilament proteins (p-NFH) normally restricted to axons, accumulated in the neuronal perikarya in layers II–IV of the temporal cortex, and in the piriform cortices, the dentate gyrus, and the CA1 region of the hippocampus. At the same time, the p-NFH immunoreactivity disappeared from the axons and dendrites of cerebral cortex neurons. These findings suggested a dephosphorylation of NFHs in axons and dendrites, and an accumulation of p-NFHs in the perikarya due to disturbed

axonal transport (Saljo et al., 2000). The authors noted that these changes are likely the result of disturbed anterograde axonal transport.

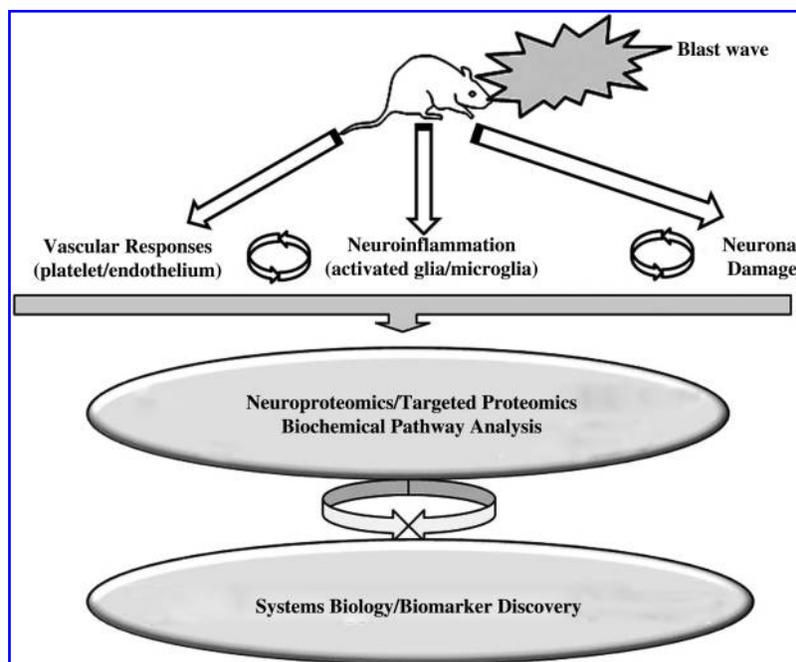
Cernak and co-workers studied the effect on the brain of exposure to primary blast (delivered by a shock tube) and noted that it was sufficient to cause a moderate level of lung injury in rats (Cernak et al., 1999b; Cernak et al., 2001a, 2001b). In some cases, the effect of exposing the whole body was compared to exposing only the thoracic region (with the head protected) (Cernak et al., 2001b). Both types of blast exposure resulted in ultrastructural evidence of neuronal injury (expanded perineuronal spaces, cytoplasmic vacuoles, myelin deformation, and axoplasmic shrinkage) in the areas examined (hippocampus and brainstem reticular formation). The authors noted that the pattern of neuronal abnormalities is similar to those seen in diffuse axonal injury. Biochemical changes indicative of oxidative stress were also present. The degree of neuronal damage correlated with impaired performance on an active avoidance task (Cernak et al., 1999b; Cernak et al., 2001a, 2001b).

In a recent controversial paper, Kato and co-workers exposed rats to a direct single shock-wave shot (produced by a silver azide explosion) after craniotomy. This high-overpressure (>10 MPa) shock-wave exposure resulted in hemorrhage and a significant increase in TUNEL-positive neurons exhibiting chromatin condensation, nuclear segmentation, and apoptotic bodies. The maximum increase was seen at 24 h after the shock-wave application. Low-overpressure (1 MPa) shock-wave exposure resulted in spindle-shaped changes in neurons and elongation of nuclei without marked neuronal injury. The administration of the caspase-3 antagonist Z-VAD-FMK significantly reduced the number of TUNEL-positive cells observed 24 h after high-overpressure shock-wave exposure. High-overpressure shock-wave exposure results in brain injury, in-

cluding neuronal apoptosis mediated by a caspase-dependent pathway. The authors speculated that the threshold for shock-wave-induced brain injury is under 1 MPa, a level that is lower than the threshold for other organs including lungs (Kato et al., 2007). However, the model of blast injury employed in this study is very similar to a controlled cortical impact (CCI) model, which our group has been using for a number of years in rat TBI studies (Pineda et al., 2004; Wang et al., 2005; Ottens et al., 2006). Specifically, Kato and colleagues imposed the pressure of blast wave locally on dura mater after craniotomy instead of using mechanical piston impact at various depths as is typical in our studies. It is not clear from the study whether the magnitude and duration of overpressure blast was measured at the surface of the dura mater. The morphological pattern of brain tissue injury in that model closely resembled the damage observed after CCI (Kato et al., 2007).

In a number of studies using a CCI model of TBI in rats, we have convincingly demonstrated the important role for caspase-3-mediated apoptotic pathways in neuronal injury, as well as the calpain-mediated oncosis/necrosis mechanisms (Pineda et al., 2004; Wang et al., 2005; Ottens et al., 2006). Several pathways and target proteins have been identified as potential biomarkers of TBI (Kobeissy et al., 2006). Moreover, a systems biology approach was implemented to construct a network map of proteins and pathways involved in the pathophysiology of TBI (Kobeissy et al., 2006). Whether these pathways are equally critical after blast-wave exposure of total body and/or direct head targeting without imposing mechanical/traumatic forces remains to be elucidated.

Importantly, the rats exposed to blast injury exhibited significant deficits in performance of an active avoidance task that persisted up to 5 days post-injury. Electron microscopy findings in several brain structures showed swellings of neurons, glial reaction, myelin debris, and increased pinocytotic



**FIG. 1.** Schematic presentation of a proposed research algorithm to elucidate molecular signatures and pathophysiological pathways of blast brain injury to develop a network map for blast brain injury.

activity on the fifth day following trauma. In blast-injured rats, there was also a significant elevation in total nitrite/nitrate levels 3 and 24h following injury, which were comparable with the changes in the expression of inducible nitric oxide synthase mRNA. The results indicate that blast injury-induced neurotrauma is able to cause cognitive deficits (Cernak et al., 2001a). In a later study, performance tests of coordination, balance, and strength were significantly impaired by exposure to a more intense explosion. An increase in the number of degenerating neurons was seen in cerebral cortex following these more intense explosions (Moochhala et al., 2004). An important finding was that administration of aminoguanidine, an antagonist of inducible NO synthase, before or after blast exposure ameliorated the blast-evoked behavioral changes (Moochhala et al., 2004).

On the other hand, as indicated above, NO may function as an antioxidant by quenching the hemoglobin-mediated free-radical reactions (Gorbunov et al., 1996). Thus the roles of NO and NO synthase, and particularly iNOS, in blast brain injury remains unclear. Moreover, recent studies using spin trap techniques and nitrosylation markers in iNOS knockout mice indicated that NO may play a dual role after TBI, that is both injurious and protective, depending on the time after traumatic impact (Bayir et al., 2005; Foley et al., 2008).

For rats subjected to an explosive-generated blast, Kaur and co-workers showed generalized activation of microglia and astrocytes 1 and 7 days after the blast, particularly in the superficial layers of the cerebral and cerebellar cortices, while the oligodendrocyte profile remained intact (Kaur et al., 1995, 1996, 1997a, 1997b, 1999). It was concluded from this study that the blast may have disrupted the integrity of the blood-brain barrier, resulting in possible abnormal entry of serum-derived substances, thereby leading to microglia/astrocytic activation.

#### *Approaches for the development of specific biomarkers of blast-induced brain injury*

Integrating the existing data obtained from different models of blast brain injury, as well as from classical CCI-like models, we propose that exposures to blast waves cause brain injury with distinct biochemical pathophysiological signatures, which when defined and integrated with systems biology tools, can be exploited to discover diagnostic biomarkers and blast brain injury mitigation strategies. For example, we recently employed Pathway Studio software for creation of a functional interaction map of proteins affected during controlled cortical impact TBI (Kobeissy et al., 2006). We propose the term BLAST-OMICS for the similar functional interaction map of proteins impacted by blast-wave exposure, which will be created during current and future studies.

We postulate that the following molecular pathways and/or targets are identifiable upon blast wave exposures of experimental animals (Fig. 1).

1. Vascular responses and dysregulation of cell adhesion molecules as bridges connecting vascular-endothelial-neural tissue disturbances, including but not limited to nitric oxide metabolism assessed in brain tissues, CSF, and blood by Western blot, sandwich ELISA, and immunohistochemical means.
2. Neuroinflammation as a further development of impaired vascular reaction in the brain, resulting in enhance-

ment of endothelial permeability/leakage, infiltration of macrophages, and activation of microglia that will involve cytokines and markers of activated microglia.

3. Neuron-glia interactions and cell death pathways, including apoptosis, oncosis/necrosis, and autophagy.
4. Additional pathways and biomarkers, such as dendrite/axonal disturbances including axonal transport dysfunction and damage to microtubules and neurofilaments.

The successful development of true biochemical markers of blast brain injury should help to distinguish between minor brain damage, particularly mild TBI, and post-traumatic stress disorder (Kennedy et al., 2007; King, 2008). The differentiation of PTSD and mild TBI is especially important for soldiers returning from Iraq, as mild TBI (e.g., concussion) that occurs among these soldiers has a strong association with PTSD and physical health problems 3 to 4 months after the soldiers return home (Hoge et al., 2008).

#### **Perspectives**

Despite a long history, critical need, and a vast array of recent data, the mechanisms of primary blast neurotrauma, particularly brain injury and potential biomarkers, remain elusive. A lack of unified models with defined blast-wave signatures has contributed significantly to existing controversies. Given data from both our laboratory and others, it has become increasingly clear that brain pathology, the underlying mechanisms and potential biomarkers associated with primary blast exposures, may be different from those imposed by focal mechanical head trauma (Bhattacharjee, 2008). This exciting new area of research clearly requires more adequate models and in-depth studies of biochemical/pathophysiological signatures of blast neurotrauma. Thus the design and implementation of a relevant experimental framework is of particular importance for elucidation of the mechanisms of injury, the identification of biomarkers, and eventually the development of strategies for mitigating blast-induced brain injury.

#### **Acknowledgments**

The authors wish to thank Ms. Qiushi Tang for her excellent technical assistance. This work was supported by grants N14-06-1-1029, W81XWH-8-1-0376 and X81XWH-07-01-0701 from Department of Defense.

#### **Author Disclosure Statement**

Dr. Svetlov, Dr. Larner, Dr. Hayes, and Dr. Wang have financial interest in Banyan Biomarkers Inc. Dr. Kirk and Dr. Atkinson have no conflict of interests.

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1. Patrick M. Kochanek , Richard A. Bauman , Joseph B. Long , C. Edward Dixon , Larry W. Jenkins . 2009. A Critical Problem Begging for New Insight and New TherapiesA Critical Problem Begging for New Insight and New Therapies. *Journal of Neurotrauma* **26**:6, 813-814. [[Citation](#)] [[PDF](#)] [[PDF Plus](#)]