Animal Models for the Study of Military-Related, Blast-Induced Traumatic Brain Injury

Joseph T. McCabe, Chantal Moratz, Yunbo Liu, Ryan Egan, HuaZhen Chen, Jiong Liu, Craig S. Budinich, Ellen E. Burton, Jonathan K. Danquah, and Matthew R. Myers

Abstract— In present war time conditions, traumatic brain injury (TBI) has moved to the forefront as a “signature injury.” In terms of prevalence and understanding the biological mechanisms that underlie the injury, blast-induced TBI — particularly in “mild” cases—has proven to be a significant challenge for military medicine. Basic research that employs animal models of TBI is a key element for furthering our understanding of the biological consequences of blast-related TBI and for the development of therapeutic approaches. This paper outlines the currently available prototypes for the study of TBI, particularly in rodent models, and how the most widely used approaches for modeling TBI can contribute to understanding the mechanisms of injury.

I. INTRODUCTION

TBI has always accounted for a significant proportion of the casualties of modern war. Close proximity to improvised explosive devices (IEDs) is still a tragic reality, with high mortality and loss of limb(s) and significant injury to the torso [1]. In the present conflicts in Afghanistan and Iraq, modern procedural tactics and protective equipment have significantly reduced the number of wounded and fatalities. Of great concern, however, is the fact that traumatic head and brain injuries continue to be a major source of casualties and long lasting disabilities. As many as 60% of the 25,000 war injuries to date are related to explosive blasts such as IED’s or roadside bombs, and many are cases involving the head region. As noted in Okie (cf. [2], citing the Joint Theater Trauma Registry), 22% of the casualties treated at Landstuhl Regional Medical Center sustained head, neck, or face injuries, and this report further indicates that this percentage rate is probably a modest estimate of the number of TBI cases (Deborah Warden, cited in [2]).

In this paper, several animal models of TBI are briefly described and discussed. The emphasis is on rodent models that have been employed widely or at least by more than one laboratory. Relevant biomechanical, in silico, in vitro, mathematical, and larger animal models are not considered. The focus, too, is upon developments in the last two years where there has been a more concerted effort to develop models that are relevant for the study of blast exposure.

II. ANIMAL MODELS OF TBI

A. The Fluid Percussion Model of TBI

The fluid percussion model induces brain injury by a brief, forceful displacement of the dura that causes a diffuse load pressure from a central site and through the surrounding cortical surface. It is considered a “mixed model” of TBI, since it produces focal and diffuse injury [4, 5]. It induces a range of injuries, including subarachnoid and intraparenchymal hemorrhage, blood-brain barrier dysfunction, neuronal cell death, axonal injury, altered brain electrical activity and behavioral abnormalities, increased intracranial pressure, cerebral ischemia and hypotension, mitochondrial dysfunction, and what has been described as a “gliding contusion” [3]. More recent studies have emphasized the wider extent of injury, including the fact that neuropathological changes continue over time, perhaps akin to human injuries, with changes observed even remote to the impact site [6], and that midline injury can be used to mimic diffuse axonal injury, demonstrating remote deafferentation [7]. A limitation for using the midline as a site of injury, however, is the fact that there can be increased morbidity based upon damage to hypothalamic and brain stem structures [8], requiring the employment of less force. A comparison of intracranial pressures for the fluid percussion model, the controlled cortical impact, and weight drop models (see below) found a greater rise in pressure in the contralateral cerebral hemisphere, and a state of apnea; attributed to the brain stem pressure changes after this injury model [9]. The fluid percussion...
model has been employed mostly in laboratory rats, although it can be used in mice [10]. As with other animal models, the fluid percussion model has limitations based upon the use of anesthesia and that a craniotomy is needed.

B. The Controlled Cortical Impact Model

The controlled cortical impact (CCI) device is an alternative method, with over 800 citations related to the use of this model. CCI injury is caused by the rapid depression of the dura by a flat-faced, cylindrical piston. Earlier models employed a pneumatically-driven impactor [11-13], while a more recently engineered device is electromagnetically-regulated, and reportedly results in better control of load velocity, impact depth, and reproducibility [14]. The Impact One™ Stereotaxic Impactor for CCI from myNeuroLab, for example, uses an electromagnetically-driven impactor. The CCI device induces primarily a localized contusion in the region of dural indentation [3, 5, 15], although the injury extends well beyond the impact site, including injury to underlying structures of the corpus callosum, hippocampus and thalamus [5, 16], internal capsule, cerebellum, midbrain, pons, and medulla [8], and its injuries include “gliding-like contusion,” superficial, subarachnoid, subdural and intraparenchymal hemorrhage, axonal injury and behavioral impairments [3], cerebral ischemia and hypotension [17]. The CCI model is used extensively in rat and mouse. Some reports directly compared CCI and fluid percussion and find differences in site of injury and biological effects [18, 19]. In the majority of work performed using a CCI device, a craniotomy is performed. The CCI device can be modified for use as a closed head injury. For example, Cernak has used a pneumatically-driven CCI device to strike a metal plate cemented to the rat skull [20]. This approach reportedly induced diffuse TBI, including disruption of the blood-brain barrier, cerebral edema, cell death in the cerebral hemispheres and brain stem, and behavioral impairments.

C. The Weight Drop Model

The weight drop model utilizes a free-falling weight or rod that impacts a metal plate or helmet fixed to the rodent’s cranium [21]; over 100 publications have employed this model for the study of rat and mouse TBI. A recent description of an apparatus by Flierl and colleagues [22] describes an approach that uses a falling metal rod and is applicable in mice. The dropped weight results in an impact acceleration injury that induces diffuse traumatic injury [21, 22], and thus well reflects the stimulus of human impact injuries such as automobile accidents, falls, and at least some components of impact injuries sustained in a blast. This model, then, differs from fluid percussion and controlled cortical impact models by producing less of a focal site of injury. This procedure can be used without performing a craniotomy, although the use of heavier weight can result in cranial fractures. A modification of the model was recently reported, The ‘Maryland Model’ Device [23], that utilized an intervening impactor to deliver the force to the malar processes of the rat; resulting in a frontally-induced impact.

D. Blast Overpressure Shock Tubes

Several designs to induce an atmospheric shock wave have been developed and were described in recent reports [24-26]. Perhaps the most widely employed version is the shock tube utilized by researchers at the Walter Reed Institute for Army Research/Naval Medical Research Center [27-30]. A 30 cm diameter, 5.33 m long galvanized steel tube is attached to a smaller pipe of the same diameter. The latter, expansion chamber, is bolted to the larger tube with a Mylar membrane placed between the tubes, allowing compressed air in the expansion chamber to rupture the membrane resulting in a shock wave. Intracranial measures in anesthetized rats demonstrated overpressure within the cerebral ventricles in the range of 30-40 kPa [31], and that blast effects upon intracranial pressure persisted for hours after exposure [24]. Long [28] reports that shock tube exposure at relatively higher levels (126-147 kPa) leads to cerebral hemorrhage, necrosis, cortical cell loss, gliosis, and widespread fiber degeneration, which is not reported in many studies with fluid percussion or control cortical impact injury; others also have reported little evidence of obvious focal cortical injury [25, 31]. Compared to the methods described in A-C, the shock tube permits whole-body exposure, which is relevant to body effects from blast on neurological function, and studies related to the impact of armor or protective clothing are feasible [25, 28]. Finally, an obvious feature of the shock tube is that rodents sustain a closed head injury, and one can relate neuropathological and behavioral changes to physical characteristics such as peak pressure amplitude and exposure duration.

E. High Intensity Focused Ultrasound

High intensity focused ultrasound (HIFU) may offer an alternative means for the study of blast effects upon neurological function [32]. HIFU-produced shock wave characteristics can be modified to reflect a blast wave and relate these characteristics to the nature and severity of tissue injury. The focusing feature of HIFU is advantageous by limiting the complexity of systemic response. By sonicating only the head region or a portion of the brain, there is a possibility that HIFU causes a reduced or less extensive endocrine/immune response and multifaceted trauma as is seen in a whole-body blast. For example, whole-body blasts can elicit inflammatory responses in organs such as the lung [30]. Signals from multiple organ injury from blast makes for an entangled, but perplexing variety of system responses. Comparable to the other models, one can limit the site of injury, reducing mortality, can have extended survival times to measure the progression of injury and impact of treatment, including behavioral effects, and can relate injury consequences directly to measures of blast intensity, including threshold values. HIFU is a non-surgical, closed-head injury model that lends itself to the study of repetitive TBI. The clinical relevance of HIFU-induced TBI, however, has not been tested, although it has been demonstrated that HIFU disrupts blood-brain barrier integrity [33-35]. Our group
After exposure, the protein extravasation with disruption of the blood-brain barrier. Evans blue binds to albumin, allowing visualization of plasma proteins each, ~1µs pulse, ~80 msec interpulse interval. Evans blue dye was seen on the cortical surface. At euthanasia, the brain was removed from the calvarium and animals regained consciousness and survived for 2 h before euthanasia. The brain was removed from the calvarium and visually inspected for evidence of Evans blue on the brain surface. Evans blue dye was seen on the cortical surface. At 540 nm excitation (TRITC emission range), Evans blue dye fluoresces cerebral vessels. 

The prototype has been used with mice that received an intravenous injection of a 1% Evans blue solution in saline 45-60 min before exposure to 610 HIFU pulses (~10 MPascal each, ~1µs pulse, ~80 msec interpulse interval). Evans blue dye binds to albumin, allowing visualization of plasma protein extravasation with disruption of the blood-brain barrier. After exposure, the

<table>
<thead>
<tr>
<th>Model</th>
<th>Injury Effects</th>
<th>Model Relevance to Human Blast TBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid Percussion</td>
<td>Disrupts the blood-brain barrier, cell death, altered brain metabolism and blood flow, increased intracranial pressure, diffuse axonal injury, behavioral abnormalities</td>
<td>Well-characterized model, replicates many aspects of injuries related to cranial acceleration relevant to secondary and tertiary injuries with focal and diffuse pathology.</td>
</tr>
<tr>
<td>Controlled Cortical Impact</td>
<td>Disrupts the blood-brain barrier, superficial, subarachnoid, subdural and intraparenchymal hemorrhage, axonal injury, behavioral impairments</td>
<td>Widely used model, producing focal injury, but pathology is more extensive than originally realized. Can be used as a closed head injury model.</td>
</tr>
<tr>
<td>Weight-Drop Models</td>
<td>Disrupts the blood-brain barrier, impact acceleration injury, can be employed as a closed-head injury</td>
<td>Replicates secondary and tertiary aspects of blast via forceful acceleration of brain and body.</td>
</tr>
<tr>
<td>Blast Overpressure Shock Tubes</td>
<td>Diffuse axonal injury, altered brain metabolism, closed-head injury</td>
<td>A unique model for blast research by inclusion of atmospheric overpressure wave(s) as primary stimulus.</td>
</tr>
<tr>
<td>HIFU</td>
<td>Disrupts the blood-brain barrier, closed head injury</td>
<td>Permits &quot;organ isolation&quot; studies by directing the blast to specific regions of the body.</td>
</tr>
</tbody>
</table>

Table 1 provides a summary of perhaps the most salient features of each experimental approach with respect to blast-induced TBI.

### III. CONCLUSION

At present, the pathology underlying blast-related brain injury is not well understood. It has been repeatedly stated by many experts in the TBI field that no single model replicates all of the mechanisms and consequences of human experienced TBI [3, 5, 22, 36], and this view is likely to be apropos for blast-related TBI model developments. Yet, models allow investigators to further our understanding of the biological effects of TBI—and this is an area of dire need with blast-related injuries where research has not determined the short and long term consequences of blast exposure. Movement forward in the development of animal models requires close consideration of clinical descriptions at many levels, including the nature of the exposure(s), initial neurological observations, imaging findings, and description of the short and long term cognitive and emotional picture. Basic research by neuroscientists, psychologists, clinicians, engineers, and physicists is needed for the identification of current research gaps to shape model development that replicates one or more aspect of central nervous system injury.

### REFERENCES
