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Research Paper

Neuropathology and neurobehavioral alterations in a rat model of traumatic brain injury to occupants of vehicles targeted by underbody blasts

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ABSTRACT

Many victims of blast-induced traumatic brain injury are occupants of military vehicles targeted by land mines. Recently improved vehicle designs protect these individuals against blast overpressure, leaving acceleration as the main force potentially responsible for brain injury. We recently developed a unique rat model of under-vehicle blast-induced hyperacceleration where exposure to acceleration as low as 50G force results in histopathological evidence of diffuse axonal injury and astrocyte activation, with no evidence of neuronal cell death. This study investigated the effects of much higher blast-induced accelerations (1200 to 2800G) on neuronal cell death, neuro-inflammation, behavioral deficits and mortality. Adult male rats were subjected to this range of accelerations, in the absence of exposure to blast overpressure, and evaluated over 28 days for working memory (Y maze) and anxiety (elevated plus maze). In addition, brains obtained from rats at one and seven days post-injury were used for neuropathology and neurochemical assays. Sixty seven percent of rats died soon after being subjected to blasts resulting in 2800G acceleration. All rats exposed to 2400G acceleration survived and exhibited transient deficits in working memory and long-term anxiety like behaviors, while those exposed to 1200 acceleration G force only demonstrated increased anxiety. Behavioral deficits were associated with acute microglia/macrophage activation, increased hippocampal neuronal death, and reduced levels of tight junction- and synapse- associated proteins. Taken together, these results suggest that exposure of rats to high underbody blast-induced G forces results in neurologic injury accompanied by neuronal apoptosis, neuroinflammation and evidence for neurosynaptic alterations.

1. Introduction

Brain injuries resulting from direct or indirect exposure to explosions represent up to 68% of more than 300,000 U.S. military neurotrauma victims who served in the recent wars in Iraq and Afghanistan (Abdul-Muneer et al. 2013; DePalma et al., 2005; Hoge et al., 2008; Okie, 2005; Owens et al., 2008; Rosenfeld et al., 2013). Blast-induced traumatic brain injuries (bTBI) are mainly classified into four sub-categories: 1. primary injury, which results from blast overpressure, 2. penetrating injuries caused by debris propelled by the explosion, 3. "coup-countercoup" head injury resulting from secondary head impact, and 4. exposure to intensive heat, toxic chemicals, and electromagnetic radiation (Cernak et al., 2011; Mellor, 1988). Missing from these classifications is TBI induced by sudden, intense acceleration force without significant exposure to blast overpressure. Such injuries occur to occupants of vehicles targeted by land mines, where the V-shaped armored hulls deflect the bulk of blast overpressure laterally, away from the occupants. Nevertheless, the occupants are subjected to uniquely rapid and intense acceleration.

Clinical research and epidemiological studies suggest that the majority of all bTBI victims suffer from mild TBI (Hoge et al., 2008; Lange et al., 2013). Although most victims of mild-bTBI pass the Acute Concussion Evaluation (Brenner et al., 2009; Elder et al., 2010), many return to the hospital later, exhibiting cognitive and memory impairments, increased anxiety, and depression (Elder et al., 2010; Jaffee and Meyer, 2009; Rosenfeld and Ford, 2010). Thus, a better knowledge of the

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pathophysiology of different forms of bTBI is needed to improve both short and long-term outcomes for bTBI casualties.

A number of different animal models of bTBI have been developed (Goldstein et al., 2014; Kovacs et al., 2014), with the goal of improving treatment for bTBI (Chen et al., 2013; Schultz et al., 2011). Such models have focused primarily on brain injuries caused by exposure to blast overpressure and shock waves, such as those experienced by "unmounted" warfighters on foot patrol. These models typically utilize rodents restrained within or just outside the end of a shock tube that exposes the animals to these forces. The results of these experiments demonstrate that exposure of laboratory animals to a single blast can cause significant pathological changes in the brain including the disruption of the blood brain barrier integrity (Abdul-Muneer et al., 2013; Elder et al., 2015), increased accumulation of inflammatory microglia/macrophages, neuronal cell death, and neurobehavioral deficits (Abdul-Muneer et al., 2013; Kwon et al., 2011; Vandevord et al., 2012).

To better understand the pathology of blast-induced brain injury sustained by occupants of military and other vehicles, we recently developed a unique rat model of underbody blast-induced vertical hyperacceleration where relatively low blast intensity (50G acceleration force) revealed histopathological evidence of diffuse axonal injury and astrocyte activation in rats, with no neuronal cell loss or obvious behavioral deficits (Proctor et al., 2014). The current study investigates the impact of much higher underbody blast-induced acceleration force of 1200–2800G on neuronal cell death, inflammation, levels of tight junction and synaptic proteins, behavioral impairments, and mortality at periods ranging from hours to 30 days post-injury.

2. Materials and methods

2.1. Animals and their environment

All animal research protocols were approved by the University of Maryland, Baltimore Animal Use and Care Committee and the US Army Animal Care and Use Review Office. Male Sprague-Dawley rats (Harlan Laboratories, CA), 300–350 g were maintained under a controlled environment with an ambient temperature of 23 ± 2 °C, a 12 h light/dark cycle, and continuous access to food and water ad libitum. Experimental groups consisted of 10 to 14 rats for behavioral studies, 4 to 8 rats for immunohistochemistry or biochemical analyses, and 8–14 rats for mortality incidence.

2.2. Exposure of rats to underbody blast-induced hyperacceleration force

Exposure of rats to underbody blast-induced hyperacceleration force was performed following scaling analysis to develop parameters to guide the design by a procedure modified from what we described previously (Proctor et al., 2014; Zhao et al., 2013). In brief, two rats were anesthetized concurrently with 4% isoflurane in room air for 5 min, allowing for placement within restraints without stress. Anesthesia was discontinued and the rats were secured, while still unconscious, within two fiberglass restrainers (Stoeling Inc., IL), with a custom addition of metallic cone to restrain the head and minimize secondary acceleration and head impact. The restrainers were bolted onto a 38 cm square and 2.5 cm thick aluminum platform. This platform was located immediately above a second 38 cm square aluminum platform that was either 5.0 cm thick (for 1200G acceleration force) or 2.5 cm thick (for 2800G acceleration). A 0.6 cm thick, hard rubber pad was present between the two platforms to dampen oscillatory acceleration forces. Both platforms had 2.5 cm wide holes located inside each of the four corners. Aluminum rods, 2.0 cm wide and 90 cm high, were inserted through each of the holes and secured to a steel base on which the platforms rested, thus allowing for only direct movement of the platforms vertically following an underbody blast. The steel base was bolted to the edges of a steel tank filled with water to different stand-off distances from the bottom platform, resulting in relatively low acceleration force at large distances and relatively high acceleration force at short distances.

An explosive charge of pentaerytritol tetranitrate (PETN) was secured in the water at a fixed 5 cm depth of burial from the water line. The explosives weighing 0.75 g, for 1200G blasts, and 2.00 g, for 2400G and 2800G blasts, were detonated electromagnetically at exactly 5 min after anesthesia was discontinued. Parallel exposure of rats to 4% isoflurane for 5 min indicated that they were fully conscious 5 min after discontinuing the anesthesia. Explosion within the water ensured a non-compressible transfer of the explosive energy onto the bottom of the lower platform and subsequently to the upper platform where the rats were located. Accelerometers were placed on the top of the higher platform near the front of the rat restraints and the velocity, acceleration force, and JERK (first derivative of acceleration force) measured using UERD-Tools software (U.S. NAVY). The peak acceleration force reached in these blast experiments was in the range of 0.30 to 0.50 m sec. Pressure sensors were also placed near the head-end of the restraints during several experiments, verifying that the pressure changes experienced by the rats were less than 1 lb/in^2. Sham animals were also anesthetized with 4% isoflurane for 5 min, secured on the platform and removed 5 min later.

All blast and sham animals were returned to their cages immediately after the blast or sham procedure and examined for any physical injuries every 30 min for 3 h. Necropsies were performed on all four animals that died immediately following the 2800G blasts.

2.3. Behavior

2.3.1. Y maze test

The Y maze is a Y-shaped platform that consists of three arms 50 cm long and 10 cm wide, each surrounded on three sides by 40 cm high walls. The open ends of the three arms are connected centrally and positioned radially at 120° angles (Stoelting Co., IL). The test was performed at approximately 1 h, and 6, 13 and 27 days post-injury, as previously described (Tchantchou and Zhang, 2013). Each rat was placed at the center of the maze and allowed to explore for 5 min. Movement was recorded using an overhead camera and analyzed using the Any-maze software (SD instruments, CA). Arm visit sequences and number of entries to each arm were recorded. Working memory was defined by the frequency of alternately exploring different arms and was determined using the formula: total number of alternations / (total number of arm entries − 2) × 100.

2.3.2. Elevated plus maze

This plus sign-shaped device consists of four perpendicular arms measuring 90 cm long and 10 cm wide, connected by a 10 cm × 10 cm central platform. Two of the arms are bordered on three sides by 20 cm high black plastic walls, while the other two arms together with the center of the maze are open. The arms are located 50 cm above the floor (Budde et al., 2013). The test was performed on days 1, 8, 14 and 28 post-blast, as previously described, with a minor modification (Budde et al., 2013). In brief, rats were individually placed on the central area and allowed to explore the maze for 10 min. Movement was recorded by an overhead camera and the data were analyzed by Any-maze software (SD instruments, CA), to provide information including the time spent in each arm and the central area and the total distance traveled. Anxiety like behavior is inversely proportional to the time the rats spend in the open arms.

2.4. Tissue collection and processing

At different times following blasts or sham procedure (Fig. 1), rats were deeply anesthetized by intraperitoneal injection of a mixture of ketamine (160 mg/kg) and xylazine (120 mg/kg), and euthanized by exsanguination via transcardial perfusion. Anesthetized rats were initially perfused for 5 min with oxygenated artificial CSF containing