Axonal alterations at acute stage of a non-impact, blast-induced rat brain injury model by *in vivo* diffusion tensor imaging

Shiyu Tang[1,2], Su Xu[1,2], William L Fourney[3,4], Ulrich H Leist[3,4], Julie L Proctor[5,6], Gary Fiskum[5,6], and Rao P Gullapalli[1,2]

[1]Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland, Baltimore, MD, United States, [2]Core for Translational Research in Imaging @ Maryland, University of Maryland, Baltimore, MD, United States, [3]Department of Mechanical Engineering, University of Maryland, Baltimore, Maryland, United States, [4]Center of Energetics Concepts Development, University of Maryland, Baltimore, MD, United States, [5]Department of Anesthesiology, University of Maryland, Baltimore, MD, United States, [6]Shock, Trauma, and Anesthesiology Research Center, University of Maryland, Baltimore, MD, United States

Introduction

Blast related TBI (bTBI) resulting from improvised explosive devices is the hallmark injury of recent wars affecting many returning veterans who have been exposed either to a direct or indirect exposure. Many of these exposed veterans suffer from long term neuro cognitive symtpoms. bTBI is considered clinically distinct from closed-head and/or penetrating TBI[1]. The underbody blasts exposes the occupants of a vehicle to vertical acceleration that can range from several to more than 1,000 G; however, it is unknown if blast-induced acceleration alone and in the absence of secondary impacts, can cause mild TBI. Our group has recently developed a novel rat model of brain injury caused by under-vehicle blast-induced hyperacceleration (BIH)[2]. This study investigated axonal alterations using *in vivo* DTI at 2-hours after under-vehicle BIH in rat brains.

Materials and methods

Adult male Sprague-Dawley rats weighing 250 to 300 g were randomly assigned to a sham group and bTBI group (n=6). Rats in bTBI group were subjected to BIH brain injury. BIH was performed with a device as described in previous publication[2] (Figure 1). Rats were initially anesthetized with ketamine (160 mg/kg) and xylazine (20 mg/kg), and then were placed in the two cylinders (one for each) in wrapped thick cotton blanket to minimize movement within the cylinders (see figure). An explosive charge was placed in the water precisely under the center of the plate at distances that generate a precise g-force (2000g) measured using accelerometers. When detonated, the explosion causes the plate to accelerate upwards extremely rapidly to heights of approximately 4-8 inches and the plate then drops back down to the original location. Following the blast exposure, all rats spontaneously recovered from the anesthesia within one hour and exhibited no signs of distress. Rats in sham group were anesthetized with the same dose ketamine but without undergoing the blast procedure.

Figure 1. Blast-induced hyperacceleration device

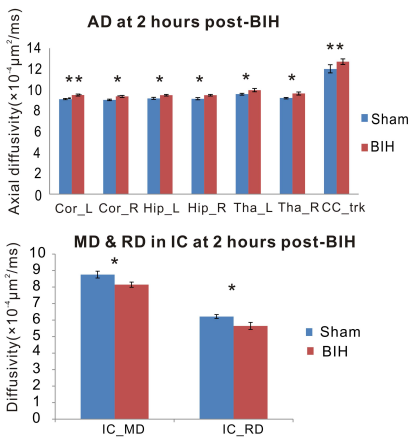

Figure 2. (A) Axial diffusivity of sham and BIH group (n=6) at 2 hours post-BIH; (B) Mean diffusivity and radial diffusivity in internal capsule of sham and BIH group (n=6). '*' $p<0.05$, '**' $p<0.01$

In vivo diffusion tensor imaging (DTI) experiment was performed prior to the BIH/sham procedure (baseline) and 2 hours post-BIH on a Bruker Biospec 7.0 Tesla 30 cm horizontal bore scanner (Bruker Biospin MRI GmbH, Germany) equipped with a BGA12S gradient system and interfaced to a Bruker Paravision 5.1 console. A Bruker ^{1}H 4-channel surface coil array was used as the receiver and a Bruker 72 mm linear-volume coil as the transmitter. The rat was under 2–3% isoflurane anesthesia and 1 L/min oxygen administration during experiment. An MR compatible small-animal monitoring and gating system (SA Instruments, Inc., New York, USA) was used to monitor the animal respiration rate and body temperature. The animal body temperature was maintained at 36–37 °C using a warm water bath circulation. A single shot, spin-echo echo-planar imaging (EPI) sequence in coronal view (TR/TE = 6000/49.90ms, FOV = 35mm ×35mm, slice thickness = 1 mm, number of averages = 1, slice numbers = 16) was used for DTI data acquisition. An encoding scheme of 30 gradient directions was used with the duration of each of the diffusion gradients being 4 ms with a temporal spacing of 20 ms between the two diffusion gradients. Three b-values (1000 s/mm^2, 1500 s/mm^2 and 2000 s/mm^2) was acquired for each direction following the acquisition of five images acquired at b= 0 s/mm^2. All animal procedures were approved by the University of Maryland, School of Medicine animal Care and Use Committee. Software written in Matlab (Mathworks, Natick, MA) was used to generate mean diffusivity (MD), fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD) maps. Regions of interest (ROI) for each rat were manually drawn on the FA map within the cortex (left, right), hippocampus (left, right), thalamus (left, right), internal capsule (left, right), and the corpus callosum (genu, trunk, splenium) respectively. One-way ANOVA was used to compare between the groups at each time point for each of the region and for each of the parameters. Statistical significance was defined as $p < 0.05$.

Results

The effects of BIH at 2 hours were evident in the cortex (L/R), hippocampus (L/R), thalamus (L/R) and the trunk of corpus callosum all of whom demonstrated significantly increased AD (Figure 2A). Decreased MD and RD were observed in the internal capsule of the animals at two hours folllowing BIH injury compared to the sham animals (Figure 2B). No significant differences were observed between baseline parameters of both groups.

Discussion

To the best of our knowledge, these experiments represent the first *in vivo* evidence to axonal alterations caused by pure BIH on the brains of laboratory animals in the absence of exposure to any significant blast overpressure. Significantly elevated axial diffusivity in multiple brain regions may indicate early diffuse axonal injury which is agreement with previously reported silver-stained histology[2] on a different set of animals. Decreased mean and radial diffusivities observed in the internal capsule reflected the presence of abnormal cell swellings which was also observed in our histology[2]. This novel model of bTBI can provide unique insights into the mechanisms of interaction between the brain tissue and blast induced hyperacceleration.

Acknowledgement

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References

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