Acute vitreoretinal trauma and inflammation after traumatic brain injury in mice

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Abstract

Objective: Limited attention has been given to ocular injuries associated with traumatic brain injury (TBI). The retina is an extension of the central nervous system and evaluation of ocular damage may offer a less-invasive approach to gauge TBI severity and response to treatment. We aim to characterize acute changes in the mouse eye after exposure to two different models of TBI to assess the utility of eye damage as a surrogate to brain injury. Methods: A model of blast TBI (bTBI) using a shock tube was compared to a lateral fluid percussion injury model (LFPI) using fluid pressure applied directly to the brain. Whole eyes were collected from mice 3 days post LFPI and 24 days post bTBI and were evaluated histologically using a hematoxylin and eosin stain. Results: bTBI mice showed evidence of vitreous detachment in the posterior chamber in addition to vitreous hemorrhage with inflammatory cells. Subretinal hemorrhage, photoreceptor degeneration, and decreased cellularity in the retinal ganglion cell layer was also seen in bTBI mice. In contrast, eyes of LFPI mice showed evidence of anterior uveitis and subcapsular cataracts. Interpretation: We demonstrated that variations in the type of TBI can result in drastically different phenotypic changes within the eye. As such, molecular and phenotypic changes in the eye following TBI may provide valuable information regarding the mechanism, severity, and ongoing pathophysiology of brain injury. Because vitreous samples are easily obtained, molecular changes within the eye could be utilized as biomarkers of TBI in human patients.
Introduction

Traumatic brain injury (TBI) is damage to the brain resulting from an external mechanical force including rapid acceleration/deceleration, pressure waves due to blast, crush, and impact or penetration by an object. TBI is the leading cause of death and disability in those under 45 years old and is a pervasive public health problem in both civilian life and on the battlefield, affecting persons of all ages, races, ethnicities, and socioeconomic status. Even with an annual incidence of 1.7 million in the United States, it has been estimated that as many as one fourth of persons who sustained a TBI did not seek medical attention. Survivors of TBI commonly suffer from disabling changes in personality, sensorimotor function, and cognition. The long-term sequelae of TBI can result in lower quality of life, often with the permanent loss of one or more physical or mental functions, which can prevent return to the workforce after the injury.

Recently, there has been increased recognition of the impact of TBI on ocular health and vision. Following even mild TBI, some form of visual disturbance occurs in up to 90% of patients. In cases of severe TBI, there are fewer systematic reviews of impact on vision and ocular injury, but significant ocular injury is known to occur. In cases of abusive head trauma in children, for example, an ophthalmologic exam routinely occurs given its role in determining etiology. Findings of retinal hemorrhages during a fundoscopic examination in a child with brain injury and unknown mechanism are highly suggestive of abusive head trauma. However, in accidental forms of severe TBI ocular injuries have also been noted including hyphema (blood within the anterior chamber), traumatic cataract, corneal injuries, choroidal ruptures, and intra-retinal hemorrhage. Because the eye exam is not routinely done regardless of TBI severity, the impact of TBI on vision and eye health is likely underestimated.

A significant increase in TBI in armed services has been seen largely due to an increase in blast exposures and increased survival due to the use of body armor. The increased prevalence of injuries due to improvised explosive devices (IEDs) in present day U.S. military conflicts necessitates a deeper understanding of the acute and long-term issues following blast-induced traumatic brain injury (bTBI). While penetrating eye injuries from fragmentation can cause lacerations and readily visible damage, closed-eye or nonpenetrating ocular injuries are much more difficult to assess. Additionally, closed-eye injuries are particularly difficult to identify within the context of trauma or altered mental status. A previous study evaluating 46 combat-veterans who had sustained documented TBI from blast exposures found that 43% of patients had evidence of closed-eye injuries upon further evaluation. Many had normal visual acuity, suggesting that damage occurred at areas away from the fovea and patients initially presented without symptoms. This study found that protective ballistic eyewear reduced the number of open-eye injuries from projectiles, but was not protective against closed-eye injury in their study sample.

As current standards for eye protection seem to be insufficient to prevent ocular injury and many veterans who experienced blast injuries have returned to civilian life without ophthalmic examinations, there is a large potential for undiagnosed closed-globe injuries with unknown long-term manifestations.

The retina and the optic nerve extend from the diencephalon during embryological development, are comprised of neuronal tissue, and are considered a continuation of the central nervous system (CNS). The axons of retinal ganglion cells within the retina come together to form the optic nerve, which respond to injury similarly to other CNS axons (e.g., retrograde and anterograde degeneration of axons, scar formation, myelin destruction). Additionally, the retina and the CNS are both immunologically privileged sites as they are protected by the blood–retina barrier (BRB) and blood–brain barrier (BBB), respectively, and further protected by anti-inflammatory and immunoregulatory mediators. The parallels between the retina and the CNS are further supported by similar eye manifestations in various neurodegenerative diseases, such as Parkinson’s disease, Alzheimer’s, multiple sclerosis, and stroke. Thus, the retina reflects the brain and spinal cord in terms of tissue structure, response to injury, and interactions with the immune system, allowing retinal manifestations to serve as an easily accessible surrogate in which to study CNS injury.

TBI can result from a multitude of forces applied in many distribution patterns in one of the body’s most complex organs. To address this heterogeneity, several preclinical models are used to study TBI that differ...
mechanism and severity and allow identification of pathophysiologic changes that may be unique to a single injury type or seen across various clinical TBI phenotypes. While visual disturbances secondary to TBI have been documented in patients, the phenotypic changes found in the eye in mouse models of traumatic brain injury have not been routinely evaluated. Similar to the primary brain injury, however, we would expect that ocular injuries may vary with different mechanisms of TBI. Characterization of eye manifestations in multiple preclinical TBI models will thus provide important information regarding mechanism of eye injuries in TBI. Furthermore, as the eye is an extension of the brain, an improved understanding of the molecular mechanisms underlying eye damage can provide information vital to the prevention, evaluation, treatment, and long-term management of patients suffering from TBI. This study was designed to evaluate acute histological and phenotypic changes observed in the eye from two different mouse models of TBI (Fig. 1). The blast-induced TBI (bTBI) model applies a shockwave and acceleration to the entire head including the eye, causing a direct mechanical deformation to the eye in addition to acceleration/deceleration forces causing traumatic axonal injury in the optic nerve. The lateral fluid percussion (LFPI) model causes direct damage to the brain, resulting in diffuse and traumatic axonal injury to the optic nerve from shearing forces from a fluid wave. Both models will also incur secondary injury following TBI due to the endogenous injury cascades triggered by trauma.

**Methods**

**Blast-induced traumatic brain injury model**

Three- to six-month-old wild-type C57BL/6 mice were assigned to either the blast-induced traumatic brain injury model (bTBI) (n = 6) or the sham (n = 4) group. The bTBI model consisted of a 76-mm diameter shock tube that was previously described in detail, with a 25-mm length driver section pressurized with helium gas and a 1240-mm long driven section. The mouse was anesthetized with isoflurane, the body secured within a rigid pipe 15 mm away from the shock tube exit to protect the...
torso and lungs. A metal nose bar and chin support were used to minimize motion of the head. Pressure transducers (Endevco 8530B-1000; Meggitt Sensing Systems, Irvine, CA) were flush-mounted at the shock tube exit, as well as inside the mouse holder in close proximity to the animal torso. Mice were exposed to a single shockwave exposure of 269 ± 9.8 kPa peak over pressure, 0.73 ± 0.021 msec duration, and 67 ± 2.3 kPa-msec impulse directed at the top of the head. Eyes were open during the blast exposure and were harvested 24 days post bTBI. Sham-exposed mice were handled similarly in all respects except that the shock tube was not triggered and as a result these animals received no shockwave exposure.

Lateral fluid percussion injury model

The lateral fluid percussion injury (LFPI) model was modified from a previously described model. Four-month-old wild-type C57BL/6 mice were assigned to either the LFPI (n = 4) or the sham (n = 3) group. On the day prior to TBI, a craniectomy was completed using a hand-held 3-mm outer diameter trephine microdrill (Research Instrumentation Shop, University of Pennsylvania), located lateral to the sagittal suture and centered between the bregma and lambda. A Luer-loc hub was secured to the skull around the craniectomy site and was used to connect mice to the FPI device (Custom Design Fabrication, Virginia Commonwealth University, Richmond, VA). Mice were allowed to recover overnight, and on the following day were anesthetized with 3% isoflurane and attached to the FPI device by the Luer-loc hub. The FPI device was triggered, generating a transient fluid pulse that impacted the exposed dura with a magnitude of 121.6–152.0 kPa. Control mice underwent sham injury, including craniectomy and identical treatment until connection to the FPI device, without the application of the pressure pulse. Eyes were harvested 3 days post TBI.

Acute neurological assessment after LFPI

The mice were disconnected from the device and underwent acute neurologic assessment by measuring the time to right after they regained consciousness; the righting reflex is a biological variable previously shown to correlate with injury severity. To ensure a consistent level of injury across each mouse, a Tektronix digital oscilloscope (TDS460A) was used to record the pressure produced by the fluid percussion apparatus. Injury severity data measured by the oscilloscope was validated using the righting reflex to confirm consistent level of injury. This reflex is commonly used in FPI to reflect the level of injury delivery across animals. The sham mice righted under 15 sec, while the LFPI-injured mice had righting times of 45 sec, 2.5 min, 4 min, and 6.5 min. The mice were then reanesthetized with isoflurane in order to remove the Luer-loc hub and to suture-close the craniectomy and injury site.

Eye histology

Mice eyes were enucleated by blunt dissection and fixed as previously described. Specimens were fixed with Excalibur’s Alcoholic Z-Fix (Excalibur Pathology), processed to paraffin, and 5-μm sections were placed on slides (Sakura VIP Processor, AO 812 microtome). Slides were air-dried and then placed in 60°C oven overnight. Slides were cooled, deparaffinized to water, and stained with Gill III Hematoxylin (StatLab Medical) and Eosin Y Alcoholic (BBC Biochemical). Pupil-optic nerve sections were processed with hematoxylin and eosin, and standard images were captured under light microscopy for review.

Retinal ganglion cell quantification

Whole globe sections were used to evaluate the number of cells within the retinal ganglion cell (RGC) layer. An observer was masked for the origin of the sections. To ensure consistency in regional sampling and quality from one sample to the next, any sections in which the RGC layer was not continuous with the exiting optic nerve, where the RGC layer was disrupted, or processing error led to poor quality images were excluded prior to quantification by a blinded evaluator. A total of 20 eyes were evaluated within the blast cohort, resulting in n = 5 and n = 8 that met inclusion criteria for the sham and blast-injured groups, respectively. Similarly, 14 eyes were evaluated within the LFPI cohort, resulting in n = 5 and n = 6 for the sham and LFPI-injured groups, respectively. Results are expressed as mean ± SEM and a Student’s t-test was performed. A value of P < 0.05 was considered significant.

Brain histology

Hematoxylin and eosin stains were used 24 days post injury on blast brain sections using standard methods.

Immunohistochemical staining of mouse IgG

Following LFPI, sections were stained for IgG for detection of blood-brain barrier leakage. Mice were deeply anesthetized with ketamine/xylazine and transcardially
perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 mol/L PBS. Brains were extracted, then immersed and stored in the same fixative. Brains were cryoprotected in 30% sucrose solution in PBS prior to cutting 40 µm sections on a freezing microtome. Sections were then stored in cryopreservative at 20°C until further processing. At time of staining, sections were washed with PBS, incubated in peroxidase blocking solution, washed, and then blocked in 1.5% serum solution. Staining for IgG was performed on free-floating sections using anti-IgG antibody (1:500) for 1 h at room temperature. Sections were then incubated in strep-HRP. Staining was developed using DAB.

Results

Blast-induced traumatic brain injury model

Our first model mimics blast-induced traumatic brain injury (bTBI) resulting from an air shock wave, such as that produced by an IED, being translated into a pressure wave within the skull-brain complex. A compressed gas-driven shock tube creates an air shock wave, which simulates blast effects (Fig. 1A). Twenty-four days post bTBI, there was no notable brain tissue destruction and no obvious inflammatory cells (Fig. 1B).

Histology of whole globe sections of blast-exposed animals were compared to control animals. Both the sham and blast-exposed groups had a normal corneal epithelium, stroma, and endothelium. The anterior chambers were of normal depth without cells, and the angles did not show recession (Fig. 2A and B, respectively). The irides showed normal pigmentation without ruberosis or pupillary membranes, and no cataracts were observed.

In the posterior segment, the ciliary bodies of all blast-exposed and sham eyes contained normal stroma, pigmented and nonpigmented layers, and cilia (Fig. 2A and B). In blast-exposed eyes there were posterior vitreous detachments (arrowheads in Fig. 2B and D), vitreous hemorrhage, and some inflammatory cells (Fig. 2C and D). The abnormal presence of macrophages were found within the vitreous hemorrhage (arrows in Fig. 2C and D). In these eyes, there were foci of photoreceptor degeneration and pigmentary changes (Fig. 3B, arrows in D). There was evidence of detached and degenerating photoreceptor outer segments (Fig. 3E). Activated macrophages were noted in blast-exposed eyes within the degenerating photoreceptor layer, as well (arrowheads in Fig. 3D). Subretinal hemorrhage also occurred (arrow in Fig. 3F). The retinal pigment epithelium and choroid showed normal pigmentation, Bruch’s membranes were intact, the optic nerve was not avulsed, and there were no neovascular membranes (Fig. 3F). We did observe a decrease in the retinal ganglion cell (RGC) count in the blast-exposed mice (Fig. 4A, *P* = 0.0002). While the sham mice had an RGC count of 390.8 ± 21.9 (Fig. 4B, *n* = 5), the bTBI mice had an RGC count of 260.4 ± 12.8 (Fig. 4C, *n* = 8).

Lateral fluid percussion injury model

We utilized a lateral fluid percussion injury (LFPI) model to examine the effects of another mechanism of TBI on the eye. The LFPI model, which applied a brief, high-pressure fluid wave directly to the exposed dura and brain tissue, mimics the effects of blunt trauma or inertial mechanisms such as a fall. A pendulum was released, hitting a saline-filled reservoir, which produced a fluid pressure wave that impacted the dural tissue and brain parenchyma through a craniectomy (Fig. 1C). As a result of impact by the fluid wave, there was significant destruction of cortical tissue and disruption of the blood–brain barrier (BBB) at the injury site as detected by immunohistochemistry staining for mouse IgG 24 h post LFPI (arrows in Fig. 1D).

Histology of whole globe sections of LFPI-exposed animals were compared to control animals. Both the sham and LFPI-exposed groups had a normal corneal epithelium, stroma, and endothelium. The irides showed normal pigmentation without ruberosis or pupillary membranes (Fig. 5A and B, respectively). Within the LFPI mice there was anterior chamber exudate (Fig. 5B–F) and the presence of inflammatory cells (arrowheads in Fig. 5E and F) when compared to the sham mice (Fig. 5A and C). This was seen within both the ipsilateral and contralateral eye relative to the injury site. In the posterior segment, the ciliary bodies of all sham and LFPI-exposed eyes contained normal stroma, pigmented and nonpigmented layers, and cilia. Additionally, we found a subcapsular cataract in one of the LFPI mice (arrows in Fig. 5E). Unlike the eyes of the bTBI mice, both the LFPI and sham eyes demonstrated a normal posterior retina with no cellular infiltrates, no subretinal hemorrhage, and no photoreceptor degeneration. The retinal pigment epithelium and choroid showed normal pigmentation, Bruch’s membrane was intact, the optic nerve was not avulsed, and there were no neovascular membranes. We did not observe a difference in the RGC count between sham and LFPI-exposed mice (Fig. 6A, *P* = 0.3554), with RGC counts of 382.0 ± 18.31 (Fig. 6B, *n* = 5) and 409.8 ± 21.08 (Fig. 6C, *n* = 6), respectively.

Discussion

The mammalian eye is similar between mice and humans, with most differences due to the fact that the mouse eye
is smaller, has a larger lens, and the retina does not have a fovea. While humans do have higher visual acuity, the mouse eye is the main animal model for human eye diseases. A histological evaluation of mouse eyes post TBI allowed us to identify several associated ocular injuries capable of causing long-lasting visual dysfunction. After sustaining a blast injury, posterior vitreous detachment and vitreous hemorrhage with macrophages was noted. bTBI mice also showed foci of photoreceptor degeneration and subretinal hemorrhage. Again, we found abnormal cells along the edge of the inner nuclear layer that are believed to be activated macrophages. A reduction in the cellularity of the RGC layer was noted in the bTBI mice as well. A different phenotype was found following our second model of TBI, the lateral fluid percussion injury. These mice showed a large amount of anterior

Figure 2. Posterior vitreous detachment and hemorrhage due to bTBI. Both the sham (A) and blast-exposed (B) mice had anterior chambers of normal depths without cells, and the angles did not show recession. Vitreous detachment in the posterior chamber was seen in bTBI mice (arrowheads in B, D) in addition to vitreous hemorrhage with some inflammatory cells (C, D). The abnormal presence of macrophages was noted as well (arrows in C, D). Hematoxylin and eosin stain. bTBI, blast traumatic brain injury.
chamber exudate and debris with some inflammatory cells, representing an anterior uveitis. Although bTBI mice showed evidence of retinal ganglion cell loss, the LFPI mice did not show a difference in retinal ganglion cell counts. Of note, RGC loss was evaluated just 3 days following LFPI compared to 24 days post blast. In
unpublished data, however, we also saw no difference in RGC counts 24 days post FPI which is consistent with what others have reported post FPI. The early retinal cell death in the blast model is striking, since reduction in retinal ganglion cell layer cellularity and damage to the optic nerve has been observed at later time points, such as after 10 months post bTBI. A longitudinal evaluation of our models could help us determine the long-term effects of LFPI and bTBI on the retinal ganglion cell layer.

Veterans involved in blast-related injuries have seen a chronic progression of visual symptoms following closed-globe injuries causing optic nerve and retinal damage, suggesting an ongoing neurodegenerative response over time post blast. Within the eye, blast trauma can cause injuries such as hyphema, retinal detachment, retinal edema, traumatic optic neuropathy, and loss of visual field. Another mouse model of closed-globe blast trauma directly applied to the eye saw a similarly delayed decrease in visual acuity, potentially due to the gradual increase in oxidative stress, neuroinflammation, and focal cell death. This delay in the progression of cell injury and death warrants further investigation, as early interventions could greatly improve visual outcomes for patients experiencing bTBI. While we did not assess visual acuity in the bTBI mice, the loss of the photoreceptor layer observed in the LFPI model where instead delayed axonal swelling and damage to the optic nerve has been documented, as well as disruption of the blood–brain barrier (BBB). We did see BBB damage 24 h post LFPI, demonstrated by the immunohistochemical staining of mouse IgG at the injury site; additionally, a loss of BBB integrity using the bTBI model occurring within the 24 h post injury has been previously well established. There was no visible disruption of the brain architecture at 24 days post blast injury. It has been well documented that the integrity of BBB is reestablished 24 h post blast injury, but we wanted to verify that overt tissue disruption did not occur. Damage to the BBB in both of these models leads to the loss of the brain’s normal immunologic privilege and isolation from the systemic immune system. This can lead to secondary injury due to increased inflammation, and the brain itself can produce immunologic mediators, contributing to immune dysregulation and the production of local tissue damage.

Numerous systemic inflammatory conditions are associated with anterior uveitis, and uveitis frequently coincides with CNS diseases stemming from inflammatory, infections, and neoplastic processes. In the absence of direct trauma to the eye, uveitis has not been causally linked to TBI, however, it is possible the aberrant immune over activation after injury could contribute to the development of the inflammatory exudate we found in the anterior chamber of our LFPI mice. While the LFPI-injured mice demonstrated various righting times, suggesting different levels of injury severity, we did not see a correlation between LFPI injury severity and ocular pathology. However, this study was not powered to determine if a dose response is present within the ocular injury we observed.

Figure 4. RGC count decreased in bTBI. A significant decrease was observed in the bTBI mice (A, \(P = 0.0002\)). While the uninjured sham mice had an RGC count of 390.8 ± 21.9 (B, \(n = 5\)), the bTBI mice had an RGC count of 260.4 ± 12.8 (C, \(n = 8\)). Eyes were assessed by a masked observer and were excluded if they did not meet inclusion criteria to ensure consistent regional sampling and quality. RGC, Retinal ganglion cell; bTBI, blast traumatic brain injury.
Figure 5. Anterior chamber exudate due to LFPI. Anterior chamber exudate and the presence of inflammatory cells was noted in the LFPI-exposed mice (B, D, E) when compared to the sham (A, C). A subcapsular cataract was identified (arrows in E) and abnormal inflammatory cells were found along the outer surface of the lens and beneath the cornea (c) in LFPI-exposed mouse eyes (arrowheads in E & F). Hematoxylin and eosin stain. CB, ciliary body. LFPI, lateral fluid percussion injury.
As brain trauma may occur through several different mechanisms, identification of biomarkers that correlate with injury type, severity, and outcome would facilitate the ease of diagnosis as well as the translational aspect of developing and evaluating the efficacy of novel therapies. Using the eye as a continuation of the CNS, potential biomarkers identified within the eye could be utilized to reflect what is happening within the brain, as fluid samples from the vitreous are easily obtained.43–46 Additionally, advances in ocular imaging techniques could lead to noninvasive diagnosis of CNS injury. Many neurodegenerative diseases often have ophthalmic findings and imaging using optical coherence tomography (OCT) has been proposed as a way to aid in diagnosis as well as monitoring progression of diseases that affect both the CNS and the eye.15

Immediately following TBI, eye health is not the primary concern for physicians and vitreoretinal trauma may be overlooked. However, the varied phenotypes we found in this study suggest that TBI may be accompanied by several types of eye injury. This data in combination with the high rate of visual symptoms reported after even mild TBI6,7 suggest that a comprehensive ocular evaluation should be done for all patients following TBI.

Future studies should include histological evaluation of mice subjected to multiple TBI models across a rigorous time course, considering the presence of a dose response to TBI, as well as analyzing vitreous samples to screen for markers that can be utilized clinically, allowing the eye to serve as an early indicator of brain trauma. Additionally, to fully develop this work as a model for TBI-induced eye injury, we would need to correlate histological changes with brain function and behavioral studies. Our work suggests that studies of the eye could have significant implications on developing and translating new targeted therapies for brain injury, a clinical problem that currently lacks effective treatments and results in significant impairment and decreased quality of life.

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**Conflict of Interest**

The authors have no commercial or financial interests associated with this article.

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