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Repetitive mild traumatic brain injury with impact acceleration in the mouse: Multifocal axonopathy, neuroinflammation, and neurodegeneration in the visual system

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ABSTRACT

Repetitive mild traumatic brain injury (mTBI) is implicated in chronic neurological illness. The development of animal models of repetitive mTBI in mice is essential for exploring mechanisms of these chronic diseases, including genetic vulnerability by using transgenic backgrounds. In this study, the rat model of impact acceleration (IA) was redesigned for the mouse cranium and used in two clinically relevant repetitive mTBI paradigms. We first determined, by using increments of weight dropped from 1 m that the 40 g weight was most representative of mTBI and was not associated with fractures, brain contusions, anoxic–ischemic injury, mortality, or significant neurological impairments. Quantitative evaluation of traumatic axonal injury (TAI) in the optic nerve/tract, cerebellum and corpus callosum confirmed that weight increase produced a graded injury. We next evaluated two novel repetitive mTBI paradigms (1 time per day or 3 times per day at days 0, 1, 3, and 7) and compared the resulting TAI, neuronal cell death, and neuroinflammation to single hit mTBI at sub-acute (7 days) and chronic time points (10 weeks) post-injury. Both single and repetitive mTBI caused TAI in the optic nerve/tract, cerebellum, corticospinal tract, lateral lemniscus and corpus callosum. Reactive microglia with phagocytic phenotypes were present at injury sites. Severity of axonal injury corresponded to impact load and frequency in the optic nerve/tract and cerebellum. Both single and repeat injury protocols were associated with retinal ganglion cell loss and optic nerve degeneration; these outcomes correlated with impact load and number/frequency. No phosphorylated tau immunoreactivity was detected in the brains of animals subjected to repetitive mTBI. Our findings establish a new model of repetitive mTBI model featured by TAI in discrete CNS tracts, especially the visual system and cerebellum. Injury in retina and optic nerve provides a sensitive measure of severity of mTBI, thus enabling further studies on mechanisms and experimental therapeutics. Our model can also be useful in exploring mechanisms of chronic neurological disease caused by repetitive mTBI in wild-type and transgenic mice.

1. Introduction

There are at least 2–3 million new cases of civilian TBI in the US every year, most of them from motor vehicle accidents (MVA) or falls. A recent trend that has attracted much attention is an increasing number of cases of progressive tauopathy in professional and amateur athletes with careers in collision sports such as football (Omalu et al., 2005, 2006; McKee et al., 2009; Goldstein et al., 2012). These cases

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; BBB, blood–brain barrier; CCI, controlled cortical impact injury; CST, corticospinal tract; CTE, chronic traumatic encephalopathy; CV, Cresyl violet; DAB, 3,3′-diaminobenzidine; DAPI, 4′,6-diamidino-2-phenylindole; FPI, fluid percussion injury; GOS, Glasgow Outcome Scale; H&E, hematoxylin and eosin; IA, impact acceleration; IHC, immunohistochemistry; mTBI, mild traumatic brain injury; MVA, motor vehicle accidents; NFL, national football league; NSS, Neurological Severity Score; ON, optic nerve; ONc, optic nerve segment proximal to chiasm; RGCs, retinal ganglion cells; ROIs, regions of interest; TAI, traumatic axonal injury.

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have been linked to repetitive concussions and appear to be identical to cases of dementia pugilistica (Martland, 1928; Millspaugh, 1937; Corsellis et al., 1973), with which they are classified under the rubric of chronic traumatic encephalopathy (CTE) (Goldstein et al., 2012). Repetitive mild blast TBI from exposure to explosive munitions is the signature injury in the Iraq and Afghanistan war theaters (Warden, 2006) and the Defense and Veterans Brain Injury Center has recently developed return-to-duty guidelines to prevent further TBI incidents in soldiers with concussive histories (Barth, 2011). The public concern over repeat concussions is growing not only because of the risk among NFL professionals and active-duty soldiers or veterans, but also because of the exposure of millions of non-professional athletes playing football, soccer and other contact sports (Langlois et al., 2006).

The development of animal models that approximate human concussion scenarios can provide a much-needed proof of concept by linking repetitive injury to adverse long-term effects, including neurodegeneration. Furthermore, animal models of repetitive TBI can be applied to transgenic rodents to work out molecular mechanisms that may help identify subjects with genetic predispositions to traumatic tauopathy. Such genetic backgrounds may include pathogenic tau mutations (Ballatore et al., 2007) or H1 tau haplotypes (Ferrari et al., 2011; Vandrovcova et al., 2010). In addition, animal models can serve as vehicles for therapeutic targeting and experimental therapeutics.

In this paper, we modified a well-characterized rat model of closed, non-contusive head injury, i.e. the impact acceleration (IA) model (Marmarou et al., 1994; Beaumont et al., 1999), for repetitive use in mice at a mild impact level. Although the IA model can produce a graded, widespread injury involving neurons, astrocytes, axons, and the microvasculature, it does not cause focal damage regardless of injury severity. We believe that the mild, repetitive, non-contusive IA injury by this IA mouse model that approximates conditions encountered in repetitive concussion in contact sports, is instructive for repetitive blast TBI occurring in combat, and may provide general insights for situations featured by repetitive TBI such as epilepsy, self-injurious behaviors, and child and domestic abuse. Our findings are consistent with the view that repetitive TBI causes cumulative axonopathy that can lead to degeneration of axotomized CNS neurons.

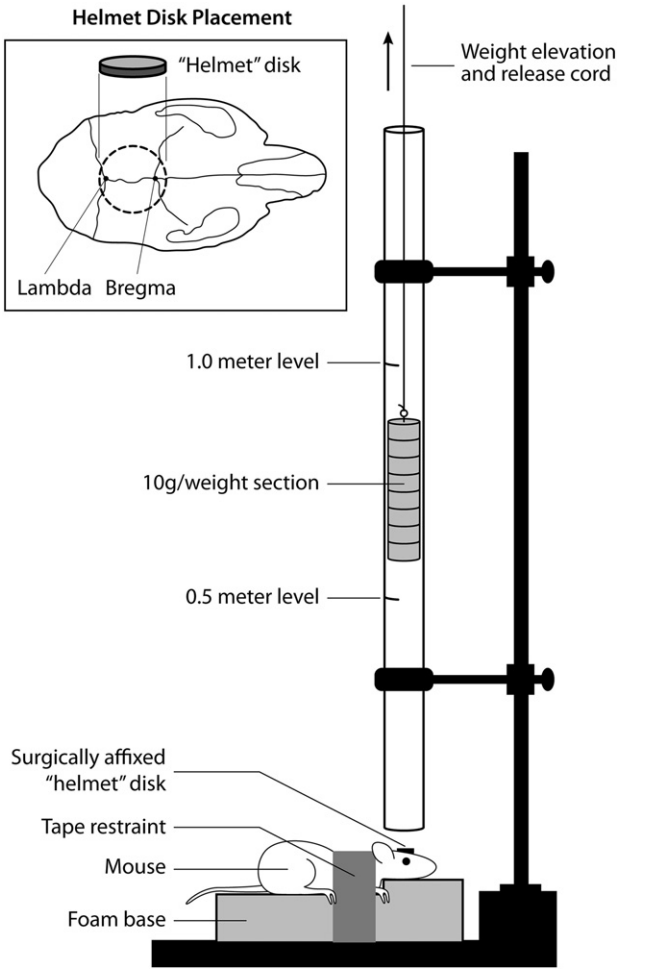

Fig. 1. A simplified sketch of our mouse IA model that was adapted from the original rat Marmarou model.

2. Materials and methods

2.1. Experimental animals and surgical procedures

The subjects of these experiments were 5–6 weeks old C57BL6/J mice (male, n = 117; Charles River Laboratories, Wilmington, MA). The animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals, 8th Ed. (National Academy Press, Washington, DC, 2011) and all animal care, surgical and post-operative procedures were approved by the Animal Care and Use Committee of The Johns Hopkins Medical Institutions. The animals were housed in the vivarium with 12 h light/12 h dark cycles and given access to pellet food and water ad libitum.

The animals were exposed to a modified version of the IA method of TBI initially described for rat by Marmarou et al. (1994) (Fig. 1). The Plexiglass tube was 1 m high with an inner diameter of 10 mm, designed to clear a column of 10 g, 3.6 mm-diameter brass cylinder weights. The diameter of the steel disk fixed on the mouse skull was set at 3.2 mm, designed to ride over bregma and lambda sutures when affixed with cyanoacrylate to the skull, and thickness was set at 1 mm. As described in original rat IA model (Foda and Marmarou, 1994; Marmarou et al., 1994), the main purpose of the stainless disk was to prevent fracture. In this position, acceleration was confined to the sagittal plane and caused both translational and rotational displacement. The foam bed (4–0 spring constant foam, Foam to Size Inc., Ashland, VA) was used as initially described (Foda and Marmarou, 1994; Marmarou et al., 1994), but cut at smaller dimensions of 20 × 8 × 6 cm to accommodate the mouse body placed in the prone position.

All surgical procedures were carried out with gas anesthesia (isoflurane:oxygen:nitrous oxide = 1:33:66) and under aseptic conditions. Briefly, the steel disk was glued on the saline washed, air dried cranium between bregma and lambda under microscopic guidance (Fig. 1). The mouse was placed prone on the foam bed under the hollow Plexiglass tube, and secured with strapping tape. The injury was induced by dropping the column of brass weights through the Plexiglass tube from a distance of 1 m onto the disk (Fig. 1). The foam bed was moved quickly right after the impact to avoid secondary rebound injury. The mouse was then placed on warm pad for recovery. At the same time, self-righting time was recorded. Unlike the rat IA model, mechanical ventilation was not applied, in order to imitate real-life concussion conditions. After righting itself, the mouse was re-anesthetized, the steel disk removed, and the skull checked under the surgical microscope for skull fractures. The scalp incision was then closed with surgical staples, and the animals were returned to cage for full recovery. Animals with skull fractures were excluded from further study. Sham animals were subjected to the same procedures minus the weight drop maneuver. A list of experimental groups and numbers of animals is provided in Table 1.

2.2. Grading severity of single TBI regimens

To generate a range of injury severities based on lethality (n = 61), the impactor height was set at 1 m. Weight was initially set at 10 g and increased by 10 g increments. Weight increase stopped when mortality