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Traumatic brain injury impairs sensorimotor function in mice

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ARTICLE INFO

Article history:
Received 9 August 2016
Received in revised form
15 December 2016
Accepted 16 February 2017
Available online 23 February 2017

Keywords:
Traumatic brain injury
Neurologic assessment
Injury severity score
Sensorimotor function
Acoustic startle response

ABSTRACT

Introduction: Understanding the extent to which murine models of traumatic brain injury (TBI) replicate clinically relevant neurologic outcomes is critical for mechanistic and therapeutic studies. We determined sensorimotor outcomes in a mouse model of TBI and validated the use of a standardized neurologic examination scoring system to quantify the extent of injury.

Materials and methods: We used a lateral fluid percussion injury model of TBI and compared TBI animals to those that underwent sham surgery. We measured neurobehavioral deficits using a standardized 12-point neurologic examination, magnetic resonance imaging, a rotating rod test, and longitudinal acoustic startle testing.

Results: TBI animals had a significantly decreased ability to balance on a rotating rod and a marked reduction in the amplitude of acoustic startle response. The neurologic examination had a high inter-rater reliability (87% agreement) and correlated with latency to fall on a rotating rod ($R_s = -0.809$).

Conclusions: TBI impairs sensorimotor function in mice, and the extent of impairment can be predicted by a standardized neurologic examination.

Introduction

Each year, over 1.7 million Americans sustain a traumatic brain injury (TBI), which is known to be a contributing factor to over 30% of all injury-related deaths in the United States.[1,2] Although improvements in emergency medicine and neurosurgical care have increased the survivability of TBI, survivors frequently endure a range of long-term cognitive and sensorimotor sequelae, or secondary injuries, including headaches, loss of concentration, difficulty in recalling information, impaired coordination, decreased reaction time, aggressive behavior, frustration, anxiety, and even seizures.[3-6] These aforementioned symptoms of clinical outcomes after TBI can vary greatly among individuals and persist for long periods of time, due to variables which are poorly understood, and few currently available therapeutic options.[7]

Abnormalities in sensorimotor function that occur after TBI are of special interest to certain populations including professional athletes and military troops for whom even subtle changes in such function can be devastating. The acoustic startle response (ASR) paradigm has been used in both human and rodent models of TBI to screen for

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0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2017.02.016

abnormalities in sensorimotor pathways. In several studies, a decreased ASR was found after TBI in humans and rodents.[8-12] Several models of TBI have been established in rodents such as controlled cortical impact,[13] controlled concussion,[14] the weight-drop method,[15] blast, and fluid percussion injury (FPI). In the FPI method, injury severity can be titrated to deliver a consistent focal force that produces long-term secondary injuries with significant neurologic deficits.[16-18] It is important to understand the extent to which murine models of TBI replicate clinical outcomes, so that investigators can conduct studies into pathophysiological mechanisms and perform preclinical trials of potential therapeutic interventions.

Our study aimed to determine the natural history of sensorimotor outcomes in a mouse model of TBI. We used magnetic resonance imaging (MRI) techniques to visualize the brain after the insult and a standardized neurologic and behavioral assessment scoring system to quantify neurological deficits after surgery. We measured sensorimotor function both by determining latency to fall on a rotating rod and by measuring ASR amplitude. We also validate the utility of a rapid neurologic scoring system to predict sensorimotor dysfunction following a TBI in a mouse model.

Methods

Animals

Adult male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME), aged 8-12 wk, were allowed to acclimate to their cages and the facility for at least 1 wk before experimentation. Mice were kept on a 12 h light/dark cycle, with ad libitum access to food and water and were housed in groups of five. All studies were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals (National Institute of Health), Guidelines for Survival Rodent Surgery (National Institute of Health), and approved by the Institutional Animal Care and Use Committee of the University of Vermont.

Fluid percussion injury and survival studies

An FPI method was used to induce a TBI, similar to that previously described by Larson *et al*..[19] Briefly, mice were anesthetized with 2%-5% isoflurane, and the animal's head was placed into a restraint. A craniotomy was performed at a point midway between lambda and bregma, approximately 2 mm lateral to the sagittal suture. A 3-mm diameter hole is drilled through the skull into the underlying extradural space using a variable speed power drill (Dremel, Racine, WI). A custom engineered stainless steel, hollow intracranial screw with a 2-mm internal diameter was fitted tightly into the burr hole. Once secured, the intracranial screw was filled with 0.9% normal saline and attached with tubing to the fluid percussion device. A pendulum was released to impact the fluid percussion device and induce TBI. Sham operated animals received anesthesia and a scalp incision only. During surgery, the intracranial pressure and its instantaneous elevation by the fluid percussion wave were recorded using an in-line pressure transducer connected to a high-speed data acquisition unit (Instrumentation and Modeling Facility, University of Vermont, Burlington, VT). The peak level of FPI, equivalent to the measured intracranial pressure during percussion, was measured in pounds per square inch (PSI). Changing the height of the pendulum allowed titration of the level of injury in a stepwise fashion across groups of mice. We sought to determine a level of injury which was survivable in the majority of animals, but with demonstrable neurologic deficits. For each set of three mice, the level of injury was incrementally increased. FPI was started at 20 PSI for the first set, and survivability was measured 48 h after the injury. The FPI was then increased by 10 PSI for each set of mice, survivability was measured 48 h after injury, and the process was repeated to a PSI of 50, which yielded 0% survival. Mortality increased along with the incremental increases in PSI, and the level of injury used for all subsequent trials was 37 PSI, which yielded 66% survival. This intensity was chosen as it was between 40 and 35 PSI, which yielded 100% and 33% survival, respectively. All animals received 0.02-mg/kg buprenorphine for analgesia while under anesthesia, and 5-mg/kg of meloxicam was used to treat postoperative pain, to reduce interference with behavioral studies. Animals were recovered from surgery and allowed access to food and water ad libitum.

Neurologic examinations and assessment

Once the survivable level of injury was established, additional animals were used to develop and validate a neurologic scoring system sensitive to TBI. To develop the scoring system, we performed serial neurologic examinations on cohorts of TBI and sham animals in a pilot period. We chose to study the majority of animals which received an FPI at 37 PSI, as this level of injury was survivable (66%) and generated demonstrable neurologic deficits. In addition, a second subset of animals in the TBI group received a lower level of injury (20 PSI) to establish a correlation between the level of injury and the neurologic assessment score. Using these two levels of injury allowed us to further validate the neurologic scoring system at different degrees of FPI. To develop our scoring system, we selected those neurologic examination features based on previously published literature for rodent brain injury, which could be performed "at the bedside", without conditioning the animals or requiring special equipment.[20,21] During the pilot phase, neurologic examinations were performed in TBI and control animals before surgery and at longitudinal time points after surgery. The examination findings that were observed by multiple examiners in multiple TBI animals remained in the standardized examination (Table 1). Based on pilot survival studies and preliminary neurologic scoring results, we chose 37 PSI as the level of injury for all further TBI experiments. This level of injury resulted in a consistent insult, with the majority of animals surviving and clearly observable changes in neurologic functions as quantified by the neuroscore.

The first part of the examination assessed sensorimotor deficits. The first three evaluations were completed as passive observations of the mouse outside of the cage. Contortions of the body with or without a tilt of the head qualified as an abnormal position, which was marked as a positive finding. A gait deficit was considered present if the mouse was unable to maintain coordination while ambulating. If the mouse was