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Novel pharmaceutical treatments for minimal traumatic brain injury and evaluation of animal models and methodologies supporting their development

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ABSTRACT

Background: The need for effective pharmaceuticals within animal models of traumatic brain injury (TBI) continues to be paramount, as TBI remains the major cause of brain damage for children and young adults. While preventative measures may act to reduce the incidence of initial blunt trauma, well-tolerated drugs are needed to target the neurologically damaging internal cascade of molecular mechanisms that follow. Such processes, known collectively as the secondary injury phase, include inflammation, excitotoxicity, and apoptosis among other changes still subject to research. In this article positive treatment findings to mitigate this secondary injury in rodent TBI models will be overviewed, and include recent studies on Exendin-4, *N*-Acetyl-L-cycteine, Salubrinal and Thrombin.
Conclusions: These studies provide representative examples of methodologies that can be combined with widely available in vivo rodent models to evaluate therapeutic approaches of translational relevance, as well as drug targets and biochemical cascades that may slow or accelerate the degenerative processes induced by TBI. They employ well-characterized tests such as the novel object recognition task for assessing cognitive deficits. The application of such methodologies provides both decision points and a gateway for implementation of further translational studies to establish the feasibility of clinical efficacy of potential therapeutic interventions.

1. Traumatic brain injury

Concerns about traumatic brain injury (TBI) are rising amidst contemporary military deployments, reports on contact sport athletes, motor vehicle accidents and an aging population. To date, an estimated 1.7 million people sustain a TBI in the USA annually, not including those who seek care outside of the hospital Emergency Room setting or no care at all (CDC 2011). Of these cases, 80–90% fall under the classification of mild TBI (mTBI). Although the least severe classification, the term "mild" refers to the severity of the initial injury and not to the severity of the injury's consequences, which can include cognitive and behavioral deficits lasting from months to many years (Brain Injury Association of America; Schreiber et al., 2008). Additionally, mTBI has been linked to an increased risk of developing a number of neurodegenerative conditions, such as Alzheimer's disease and Parkinson's disease, later in life (Daneshvar et al., 2011), and repetitive mTBI is a conduit to the development of chronic traumatic encephalopathy (Kondo et al., 2015).

TBI can be appreciated as having two separate, but connected injury components. First, an externally derived mechanical injury to the head and second, an internal cascade of molecular mechanisms understood to cause further neurological damage. Mechanisms of the secondary injury are still subject to research, but are known to include inflammatory, excitotoxic, and apoptotic processes (Werner and Engelhard, 2007). In the case of mTBI where

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the primary injury is substantial but far less severe than other forms of TBI, it is the secondary injury that likely is responsible for most of the ensuing damage. Fortunately, it is also the secondary injury that could prove treatable, should an experimental treatment option be both effective against components that drive the secondary phase and administered early enough to inhibit it. As secondary injury spans minutes to days after the immediate insult, this creates a valuable window of opportunity for mTBI treatment once the time-dependent molecular mechanisms are understood. If effective treatments are identified to minimize the dysregulation resulting from mTBI, then cell death can be minimized to positively affect patient outcomes.

Currently there are no pharmaceutical treatments for mTBI; however, a continually improving understanding of the mechanisms of secondary injury is allowing ever better drug development and repositioning of existing drugs. Activation of antioxidant production and inhibition of pro-apoptotic proteins and pro-inflammatory cytokines are among drug features of recent interest. Using rodent models of mTBI and relevant cognitive and biological tests, numerous treatments with mechanism-based drug features have been reported to ameliorate mTBI induced cognitive deficits. As a recent example among many, we have lately shown that an increased thrombin concentration induced by mTBI may cause amnesia through the activation of its receptor, the proteases activated receptor 1 (PAR1) (Itzekson et al., 2014, Maggio et al., 2014); thereby exposing a novel therapeutic target potentially underpinning cognitive detriments of trauma. In the following sections, several widely used rodent models of mTBI are highlighted that can be combined with well characterized behavioral assays, epitomized by the novel object recognition (NOR) task for assessing cognitive deficits, to provide a methodological toolbox for evaluating molecular mechanisms involved in mTBI. To this, other techniques can be added, as required, to evaluate cell death or survival (e.g., by the use of immunohistochemistry, Western blotting, etc.), functional assays (e.g., electrophysiology, neuroimaging, etc.) and biomarkers of disease progression or remission (plasma, CSF), to both test hypotheses as well as potential treatment strategies.

2. Rodent modeling of mTBI

At present, at least a dozen distinct experimental methods exist for modeling TBI in rodents aimed at reproducing the multiplicity of human injury types. These protocols vary widely in their likeness to real-life human injury scenarios. Some are highly comparable and possess strong face validity, as exemplified by open field blast injury mTBI models involving mice that are elevated to the height of a standing human and are placed at a combat relevant distance from an actual TNT explosion (Rubovitch et al., 2011). Others are highly controlled and calculated, like in-laboratory trials of vacuum pulses applied directly to surgically exposed brain tissue (Shreiber et al., 1999). Whereas no animal model can replicate all aspects of the human injury, specific ones have benefits as well as disadvantages in relation to their utility and translational relevance. Nevertheless, across all models of injury, method requirements are to reproduce clinical TBI sequelae reliably, quantifiably, and with adjustable severity (Morales et al., 2005).

Even with all model requirements met, for those using rodents limitations may come from dissimilarities between animal and human brains including brain size, brain complexity, craniospinal angle, and white-to-gray matter ratio (Greig et al., 2014). Still, current methods have successfully replicated many specific human biopathological mTBI features, both focal and diffuse in nature. Three such widely used models are described below that can be valuably integrated with numerous neuroscience techniques.

2.1. Fluid percussion injury (FPI)

The fluid percussion device is one of the most extensively used in animal models of TBI. Initially described by Lindgren and Rinder (1965) in the rabbit, this model has been widely applied to larger and small animal species, including rodents (Kabadi et al., 2010). To induce injury, initial surgery is undertaken that involves a small, defined craniotomy to expose the dura, and the placement of a female Luer lock that is cemented into place. This procedure often is undertaken the day preceding TBI to minimize associated damage. The method of injury is by transient compression/deformation of the underlying brain by a fluid-mediated pressure pulse that is generated by a classical piece of equipment. This comprises of a cylindrical reservoir that is filled with sterile isotonic saline, and on one end has a transducer connected via a tube and male Luer lock to the female Luer lock attached to the skull. Injury is initiated by the development of an acute fluid pulse against the intact dural surface, which is induced by the release of a pendulum (from a precalibrated height or angle associated with a desired injury severity) that strikes a piston at the other end of the reservoir (Thompson et al., 2005). A number of micro-FPI devices are widely available that possess microprocessor-controlled pneumatically driven devices to accurately generate pressure waves (Kabadi et al., 2010). A sample protocol for a mild FPI injury is the delivery of a 0.9 ± 0.1 atm fluid pressure pulse. Generation of a larger pulse can be induced to create a more moderate to severe form of TBI, as necessary to meet focused research needs. Depending on the type of injury being modeled, the craniotomy may be placed at the midline for focal damage or laterally to generate a coup-contrecoup injury, which will result in both focal and diffuse damage (Morales et al., 2005). An experiment testing axonal damage at five time points within six weeks after a mild FPI found the localization of the damage to move across different brain regions over time, just as is found in human mTBI (Spain et al., 2010).

No TBI model recapitulates the full diverse spectrum of the human condition, with each model mimicking certain clinically translatable aspects. Whereas FPI models have the advantage of inducing graded levels of brain injury and creating axonal injuries and contusions, accompanied by impaired neurologic motor function and cognitive impairments, the fluid pulse that underpins the injury does not directly relate to the type of mechanical impact to the brain associated with TBI in the human scenario. Hence a comparison between tissue injuries generated by a fluid percussion model and that evident in the clinical setting is difficult. Furthermore, fluid flow characteristics within brain likely are dependent on the brain geometry of the animal species under study, making biomechanical analyses across species complex (O'Connor et al., 2011).

2.2. Controlled cortical impact (CCI)

Controlled cortical impact, often referred to as rigid percussion, imparts a solid mechanical injury force directly to the surface of the brain, and thus, like FPI, CCI requires craniectomy before injury induction. Nevertheless, CCI-induced injury force has the most biomechanical control of any model, as it is delivered by a rigid impactor that is precisely adjustable over the parameters of time, velocity and depth (Morales et al., 2005). This method also has no risk of rebound injury associated with other models. An example protocol for a mild injury used by Gao and Chen (2011) in mice is a 0.2 mm deformation delivered at a piston velocity of 2.8 m/s to an intact dura. The injury produced is mostly focal in nature, and is of strong relevance to those that occur in sports collisions or car accidents. Of value, this model – originally developed in the ferret (Lighthall, 1988), is adaptable across animal species – from small (rodents) to large (pigs and nonhuman primates, by varying