PERSPECTIVE

Novel rodent models of penetrating traumatic brain injury

A penetrating traumatic brain injury (pTBI) occurs when an object impacts the head with sufficient energy to penetrate skin, cranial bone and meninges to inflict injury directly to the brain parenchyma. This type of injury is particularly common in areas plagued by armed conflicts or gun-related violence. However, other causes of pTBI exist such as violent stabbings, various types of accidents and even animal attacks. pTBI is often associated with intracranial hemorrhage, edema, ischemia and a high risk of infection. Therefore, pTBI is classified as a severe form of TBI and special guidelines for the care of these patients have been developed (Esposito and Walker, 2009). In addition to the secondary injuries mentioned above, fragments from bullets, grenades or bone may be present within the wound and can further complicate the situation. Finally, pTBI patients are at higher risk to develop post-traumatic seizures and coagulopathy (Talving et al., 2009) compared to closed TBI patients.

Previous experimental research in pTBI has been performed by firing a projectile (steel sphere or bullet from 0.22 caliber rifle) into the head of an animal such as cat, dog, monkey, or sheep (see Cernak et al., 2014 and references therein). Given the obvious practical and/or ethical issues associated with these models, none of them are currently in routine use. Even though larger animals might be more similar in size and physiology to humans, rodents have the advantage of being easier and cheaper to handle making measurements of morphological, biochemical, and cellular parameters easier. Moreover, standardized behavior analyses are mainly available for rodents. Although several rodent models of TBI such as weight drop, fluid percussion and controlled cortical impact (CCI) (reviewed by Xiong et al., 2013) has been in use since the late 1980s and early 1990s, a rodent model of pTBI was not available until 2005, when Williams and co-workers published their first paper on a device called the penetrating ballistic-like brain injury (PBBI) model (Williams et al., 2005). In this model, a small inflatable probe is inserted into the brain of anesthetized rats and rapidly inflated. By controlling the pressure by which the balloon is inflated, the injury can be graded with good precision and reproducibility. The PBBI model was designed to reproduce the temporary cavity formed when a high-speed missile penetrates tissue. This means that the force in this model is directed perpendicularly to the injury tract and this model is therefore less well suited to model the actual impact or the entry wound and its effect on meninges, blood vessels and skull. Moreover, in this model, it is also difficult to assess and grade the amount energy that causes the injury.

Therefore, we set out to develop a novel non-fatal rodent model on pTBI, where the injury is caused by an actual high-speed penetration of the brain. In our model, an anesthetized animal is placed in a stereotactic frame (**Figure 1A**) and the head of the animal is advanced to the tip of a small steel (penetrator) probe placed in a custom-made holder (**Figure 1A**). A pellet is then shot from a modified air-rifle (**Figure 1A**), connected to an air-filled pressure tank. The pellet then impacts the back of the probe and as the probe advances forward (**Figure 1B** and **C**), it penetrates the brain of the animal, creating the injury. By varying the pressure in the loading chamber of the rife, probe speed and penetration depth can be regulated. One of the greatest challenges in designing this device was to achieve a reasonable magnitude of velocity of the probe, combined with a limited penetration depth. Our solution to this problem was the use of a brass ferrule fitted around the penetration probe (**Figure 1D**). After a few millimeters of free-flight, the ferrule is engaged and the tip part of it is compressed (**Figure 1D**) (note the increase in deformation of the ferrule as the loading pressure is increased). This causes the probe to come to an abrupt stop. Although we can measure speed and weight of the probe, a current drawback is that we cannot with certainty assess the amount of kinetic energy that is transferred to the brain tissue upon impact. However, high-speed video recordings of probe impact into a brain-simulant material reveals that impact creates a temporary cavity, indicating transfer of kinetic energy into the surrounding material/tissue (**Figure 1E**). In our rat model, we used a probe velocity in the rat version of around 90 m/s and a penetration depth of 4.5 mm (Plantman et al., 2012). This was chosen as our standard injury severity, as initial technical tests with higher loading pressure (and thus higher penetration velocity) resulted in unacceptable wear of the pTBI rig, and lower survival rates of animals. The mice version was created by decreasing penetration depth and size of the penetration probe (Cernak et al., 2014).

The injury as such causes extensive tissue destruction and hemorrhage in the lesion center, with cavity formation developing over time (**Figure 1F**). In addition, massive astro-, and microgliosis, neuronal death and blood-brain barrier disruption is noted in both rats and mice. Interestingly, both our model and the PBBI model causes an early peak in neuronal death (as assessed by fluororjade staining) in contrast to the CCI model, where the period of neuronal death is more prolonged (Hall et al., 2008), suggesting a possible hallmark of penetrating, rather than closed TBI. Both species displayed a loss of about 6% body weight at 24 hours post-injury, but weight was gradually recovered. Motor disturbances was present in both rats (Plantman et al., 2012) and mice (Cernak et al., 2014) after injury and in the rat model we have also detected disturbance in reference memory (but not working memory) and attention, using radial arm maze and the five-choice serial reaction time task (5CSRTT), respectively (Plantman et al., 2012). Spontaneous recovery in motorfunction after experimental TBI is common

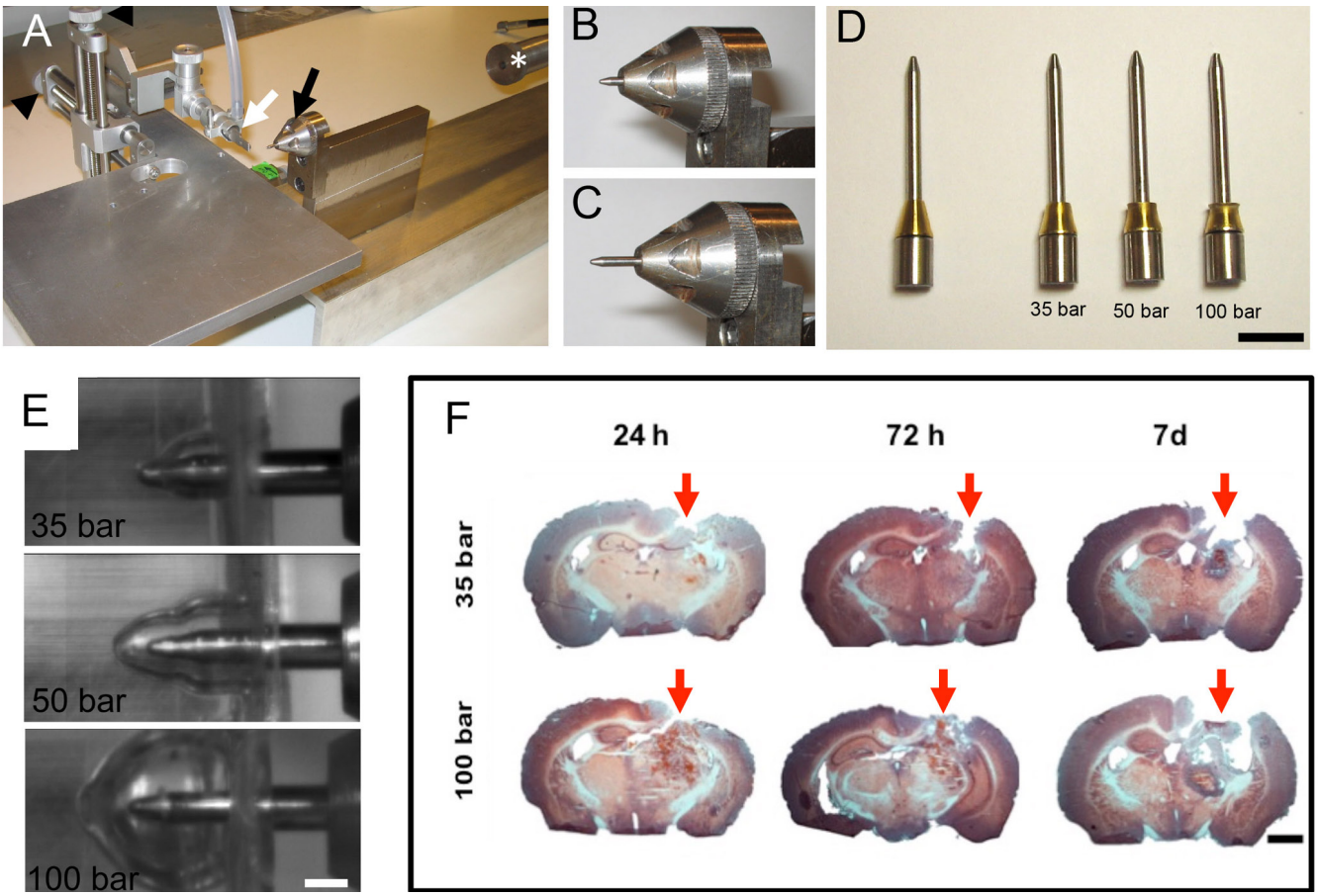

Figure1 Technical description of the penetrating traumatic brain injury (pTBI) device and morphological characterization of injury.
(A) Photo of the penetration device showing the mouthpiece (white arrow), probe holder (black arrow), stereotactic manipulators (black arrowheads), and barrel of air-rifle (white asterisk). (B, C) Close-up of the probe holder and probe before (B) and after (C) impact of pellet. Note the probe displacement (penetration) after impact (C). (D) Photo of probes after impact at loading pressures of 35, 50, and 100 bar. Note deformation of the brass ferrule (scale bar: 10 mm). (E) Images from high-speed videos of ballistics gel impact, showing the temporary cavity at different loading pressures (scale bar: 2 mm). (F) Hematoxylin and eosin stained coronal sections of mice brains subjected to pTBI with 35- and 100-bar loading pressure at 24 hours, 72 hours and 7 days after injury. Red arrows indicate site of lesion (scale bar: 2 mm).

(Hamm et al., 1994), and this was also the case in our experiments with the exception of mice subjected to the most severe injury level (100 bar loading pressure), where a motor deficiency was still prominent at 7 days post injury. However, studies with longer observation periods are needed to verify whether this motor deficit is truly permanent. So far, we have only examined the outcome of one specific injury location (3 mm posterior and 3 mm lateral to bregma for rats and 1.5 mm for mice), but future studies will be conducted in which the effects of changes in injury location will be studied, as discussed by Plantman et al. (2012). On a similar note: in its current design, the pTBI rig can only be used to create injury trajectories in a straight angle in the coronal plane; oblique injuries, for example, cannot be performed. We are currently designing a new version of the rig with an increased range of possible lesions. In addition, we are currently examining the effects of varying shapes of the head of the penetrator probe. One shortcoming of animal TBI models is that they do not accurately mimic the clinical situation in that most models produce either focal or diffuse injuries, whereas human TBI in many cases display a combination of the two. In our model, we have in addition to neuronal degeneration at the injury side, also seen injuries of a more diffuse character (β-APP) accumulation, and silver staining in remote white matter areas, suggesting that our models capture a somewhat broader scope of injury, compared to current rodent TBI models, although the full extent of injury post-pTBI still remains to be determined.

Potential future uses of our pTBI model fall into three general categories. First of all, the model could be used alone to investigate the efficacy of treatments that would affect one or several of the parameters specific for pTBI, such as intracranial hemorrhage or the massive tissue destruction (leading to the formation of the large expanding cavity). Also, the model could serve as a biological testbed to verify findings from computer simulations of pTBI. Finite element analysis suggest that the geometry of the penetrating object is of importance for the subsequent brain deformation (Pintar et al., 2001), but little is known about if and how this affects the biological

and/or behavioral outcome. Finally, the mouse version of pTBI could be used to examine mice with deletions in genes known to alter outcome after TBI. In addition, mice carrying human genevariants that are known to affect outcome of TBI (such as the Val66met allele of BDNF, that has been shown to affect cognitive recovery in Vietnam veterans suffering from pTBI (Barbey et al., 2014)), could be an interesting avenue of future research.

The second category of potential usefulness is based on comparison with other TBI models. A full understanding of which pathological processes, triggered by TBI that are common to all forms, and which are type-specific is currently lacking. A better understanding of this issue could potentially help tailor therapies for each specific type of TBI. We therefore used gene-array to look for distinctive molecular signatures that differ between penetrating-, blast- and rotation-induced TBI (Risling et al., 2011). By gene ontology grouping and enrichment for biological themes, we found a number of injury type-specific patterns of gene regulation. For example, genes related to neurogenesis were downregulated in the hippocampus after blast TBI but not after rotation or penetrating TBI. Interestingly, pTBI (but not blast or rotation-TBI) induced increase in activity of genes related to metabolic function, such as the cytochrome p450 system. Also, by use of electron microscopy we also found that pTBI and rotation induced TBI gives rise to two different forms of edema: in pTBI the edema is perivascular (Plantman et al., 2012) and in rotation TBI we observed intracellular edema (Risling et al., 2011). Further, the search for blood- or CSF borne biomarkers that can differentiate between different forms of TBI holds great promise for improved TBI diagnostics and as a research tool. A thorough experimental validation of current biomarkers and the search for new, more specific, ones requires a broad methodological base, including several different TBI models. We hope that our system could serve as a reliable and relevant model of pTBI in future efforts to move forward on these issues.

Finally, our model could be used in combination with a blast- or shocktube to investigate the combination of TBI caused by the primary blast wave and pTBI caused by flying debris (sometimes referred to as tertiary blast TBI). This could be of importance since around 70% of severe blast-TBI cases also include elements of penetrating TBI (Bell et al., 2009).

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