Title: Development of a mouse model of pediatric closed head injury

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Introduction: Traumatic brain injury (TBI) is a leading cause of pediatric morbidity and mortality. It is associated with life-long neurologic and psychiatric disease including deficits in executive functioning, memory, cognition, and motor function, as well as seizures, psychiatric disorders, and emotional and behavioral disability. However, the pathophysiology and long-term progression of TBI in children are much less thoroughly studied than in the adult population. As the central nervous system matures throughout childhood, a thorough understanding of injury evolution is important for development of therapeutic targets. Herein we describe our process of developing a mouse model of pediatric closed head injury to study disease pathobiology with a view to the development of age-appropriate therapies.

Methods: All surgical procedures were conducted in accordance with the rules and regulations of the Institutional Animal Care and Use Committee at Drexel University College of Medicine (DuCOM). On postnatal day 11, CD1 mouse pups (Charles River Laboratories) were randomly assigned to either a closed head injury (n=53) or sham-injured controls (n=28). Animals were anesthetized, a midline incision was made to expose the skull, a piston tip was zeroed over the left parietal cortex midway between the lambda and bregma sutures, and the impactor was driven into the intact skull at a velocity of 5 m/s with a 100 ms dwell time. Animals were placed on their backs, the times to normal breathing (apnea) and right themselves (righting reflex) were recorded. Injury severity was varied by altering the distance (2.0 mm or 3.0 mm) the head traveled when impacted by the impactor; additionally, we used either a metal or a silicone-tipped indenter. One set of animals was euthanized for histology (n=10) at 3 days post-injury while a second set underwent behavioral testing (Morris Water Maze, Elevated Plus Maze; n=27) at 3-4 weeks post-injury.

Results: Eighty-one total mice were included; 32 animals were injured at 2 mm depth, 19 served as 2 mm control, 21 animals were injured at 3 mm depth, and 9 served as 3 mm control. There were 27 injured females, 17 female controls, 26 injured males, and 11 male controls. The mean apnea time increased with increasing injury depth (2 mm = 7.4 s (SD 3.6) vs 3 mm = 315.5 s (SD 209.6), $p<0.01$) and was 0 s in all control mice. The righting reflex also increased with increasing injury depth (2 mm = 278.1 s (SD 141.5) vs 3 mm = 2026.9 s (SD 1795.5), $p<0.01$); sham controls had a mean righting reflex of 191.6 s (SD 166.45). Qualitative evaluation of the injuries revealed that 8 mice (25%) injured at 2 mm had mild hematoma formation, 17 (53%) had moderate, and 7 (22%) had severe hematoma formation with 18 (56%) having a skull fracture while 7 mice (33%) injured at 3 mm had moderate hematoma formation ($p=0.26$), 14 (66%) had severe hematoma formation ($p=0.003$) and 14 (66%) had a skull fracture ($p=0.64$). Three pups (14%) injured at 3 mm died acutely while an addition 2 (10%) died before post-injury day 3 (24% mortality), 1 sham in the 3 mm cohort died (11%) while none died in any other cohort ($p=0.002$). Three days after closed head injury (n=10; 1 sham, 3 injured at 2 mm with metal tip, 3 at 3 mm with metal tip, and 3 at 3 mm with silicone tip), animals demonstrated activation of microglia, axonal injury, neuronal degeneration, cortical tissue loss, and development of ventriculomegaly with more abnormality with increased depth. Brain-injured animals (n=27, all injured at 2 mm depth) demonstrated a deficit in spatial learning based on the observation of increased time to find a submerged platform in the Morris Water Maze compared to sham-injured controls ($p=0.02$). Brain-injured animals also demonstrated less time in the transition zone (center) of the Elevated Plus Maze suggestive of impaired inhibition and/or decision-making ($p=0.02$), and brain-injured female mice demonstrated impaired stress response/increased risk-taking behavior as evident by increased time spent in the open arm of the elevated plus maze ($p=0.03$).

Conclusion: Development of a mouse model of pediatric closed head injury will help to better understand the evolution of pediatric traumatic brain injury over time. Injury depth is associated with severity, as evident by increased apnea time, righting reflex, and histologic changes. Moderate induced injury (2 mm) was associated with small but statistically significant behavioral deficits while severe injury (3 mm) was associated with high acute and sub-acute mortality (24%). Current efforts are directed at determining the appropriate injury parameters that will lead to chronic behavioral deficits and allow us to evaluate the underlying mechanisms.