AGED MICE DEMONSTRATE BETTER HISTOPATHOLOGIC OUTCOMES COMPARED TO YOUNG MICE AFTER TRAUMATIC BRAIN INJURY

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Introduction

Traumatic brain injury (TBI) is defined as an external mechanical injury leading to brain insult. Damage suffered from a TBI is the leading cause of death for individuals under the age of 44 and increases the number of long-term or lifelong disabilities by 80,000-90,000 annually (Peterson, Sarniento et al. 2019). TBI follows a bimodal distribution with peaks in those aged less than 24 and above 60 years of age. However, few preclinical and no clinical trials have assessed age as an independent variable in outcomes post-TBI.

TBI is a heterogeneous injury process that can result in direct mechanical tissue disruption, neuronal excitotoxicity, free-radical generation, disruption in energy metabolism, and a spectrum of both beneficial and detrimental neuroinflammation (Makinde, Cuda et al. 2017; Kaur and Sharma 2018). The phenotypic effect of unconstrained response is the acute or chronic decline in normal cognitive, behavioral, and motor functions (McCullister 2011). TBI has also been linked to systemic maladies in children and young adults and to neurodegenerative diseases like Alzheimer’s in aged populations. Additionally, there are markedly disparate recovery patterns in young and aged patients following a TBI (de la Plata, Hart et al. 2008). Taken together, the differences in age-based outcomes suggest separate pathophysiology of injury in young vs. aged subjects despite similar injury patterns. We hypothesized that aged mice would demonstrate a more severe histopathological phenotype as compared to young mice after TBI. To test this hypothesis, we performed an open-head CCI to induce a severe TBI. Following a 30-day recovery period, we harvested, fixed, and stained, brains of young and aged cohorts of mice to assess for cerebral edema, neuronal loss, and gliosis.

Contrary to our hypothesis, young mice demonstrated significantly worse pathophysiological outcomes as compared to aged mice. These data suggest a different pathophysiology of injury in young vs. aged subjects.

Methods

Fig 1: Open head controlled cortical impact (CCI) injury and 30-day brains. A1(Ap) Activator and stereotactic impactor and brains with damage and recovery.

80-week-old (N=5) and 14-week-old (N=5) male C57Bl/6 mice were subjected to TBI via an open-head controlled cortical impact vs. sham-injury. Brains were harvested 30 days post-TBI and embedded in paraffin blocks. Sections were stained with H&E, NeuN, and GFAP and to assess for edema, neuronal degeneration, and gliosis, respectively. Sections were scored using the standardized NACC system by a neuropathologist blinded to the experimental group. Group analyses were performed using Two-way ANOVA followed by Tukey’s post hoc test.

Figure 2. Controlled Cortical Impact TBI Induces Hippocampal Edema, Neuron loss and Gliosis

Controlled cortical injury results in severe traumatic brain injury in young adult and aged mouse brains. Cortex sections were stained using hematoxylin and eosin (H&E), neuronal nuclei antibodies (NeuN), as well as Glial fibrillary acidic protein (GFAP), and evaluated for damage by neuropathologist.

Figure 3. Neuron loss and Gliosis Attenuated in aged TBI animals

(A) The average number of NeuN-positive cells in the dentate gyrus and CA3 regions per hemisphere were not different within the groups (p=0.95; however TBI caused a significant decrease in the average number of NeuN-positive cells in young animals compared to young and aged sham (p= 0.05; 0.001). (B) GFAP was evaluated over whole coronal slice. Young mice demonstrated severe and extensive edema, neuron loss, and gliosis (p<0.05) within the cortex, hippocampus, and subcortical grey matter where aged mice which demonstrated moderate and variable edema and neuronal loss. Scoring data in mice were analyzed by ANOVA (3-way, or 1-way) followed by multiple comparison tests to assess differences. P-values of 0.05 or less were considered statistically significant.

Results

Aged mice demonstrated less cerebral edema and attenuated neuronal loss within the cortex and subcortical grey matter as compared to young mice. Histopathological injury was scored from 0-3, with “1” indicating mild and “3” representing severe injury. Astrocytic reactivity was elevated in young TBI animals. Hippocampal gliosis was severe in both TBI groups. Young mice demonstrated severe and extensive edema, neuron loss, a lower number of mature neurons and more gliosis within the cortex, hippocampus, and subcortical grey matter compared to aged mice which demonstrated moderate and variable edema and neuronal loss (mean score of 2; p< 0.0001). Taken together we believe the data indicate a higher level of damage and repair response in young TBI mice as compared to aged TBI mice.

Limitations

Limitations to CCI include the need for craniotomy and the expense of acquiring the impactor and actuating device.

Conclusions

Contrary to our hypothesis, aged mice demonstrated markedly less cerebral edema and neuronal loss compared to young mice. Data demonstrate unique or divergent routes of pathophysiology in the aged vs. young brain after TBI. These data suggest that different treatment and rehabilitation strategies are needed for aged and young TBI patients.

Acknowledgments

NIH Grant # 3R01GM130662-01A1S1