Examining the Blood-Brain Barrier Following Mild Traumatic Brain Injury

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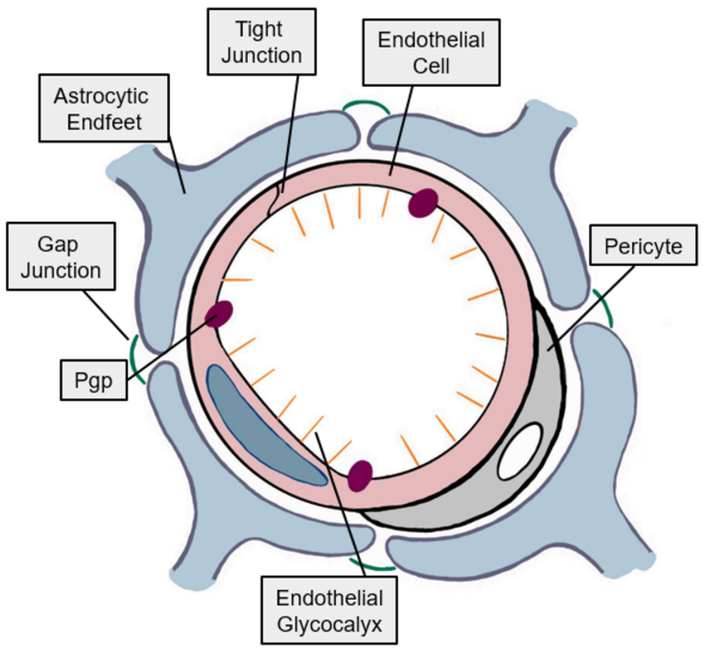
Introduction

Traumatic brain injury (TBI) is the most common cause of disability and death in Americans under 40. TBIs are classified by severity and/or mechanism with between 75-90% estimated to be closed head, mild traumatic brain injuries (mTBIs). TBIs may lead to impairments that are temporary or even permanent in nature. The mechanisms through which brain functions and pathology are altered are currently not well understood. Many incidents of mTBI are unreported due to the lack of availability of effective treatments. In our study, we define TBI as a non-degenerative, non-congenital insult to the brain from an external mechanical force. This may result from physical impact, blast waves, penetration by a projectile, or a combination of the three. The blood-brain barrier (BBB), an intricately linked cellular and molecular structure that maintains homeostasis of the brain, is a protective feature often disrupted by mTBI.

With an aim toward the further validation of our Weight-Drop model of mTBI, we are interested in examining the effects of a single mTBI the BBB. Using immunohistochemistry, we looked for the presence of albumin, a large, endogenous protein typically excluded by the BBB, and we examined the endothelial glycocalyx, a range of negatively-charged macromolecules that actively repel blood-borne substances from the luminal endothelium, acting as the first defense of the BBB.

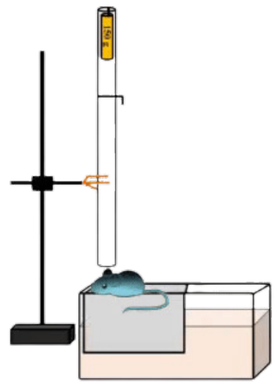
We hypothesize that our Weight-Drop model of mTBI will result in an increase of albumin in the brain as well as adverse effect on the integrity of the endothelial glycocalyx.

Blood-Brain Barrier



Methods: TBI

- Adolescent male Wistar rats were anesthetized with isoflurane and received an injection of bupivacaine, a local anesthetic, into the scalp.
- Rats were placed upon a perforated foil sheet taped over top of a chamber containing a collection sponge.
- A 300g weight was dropped from a height of 1m, impacting the dorsal surface of the head.
- The rat then fell through the foil sheet, landing on the sponge, and was immediately transferred to a recovery chamber and monitored for the loss of righting reflex (LRR), a measure associated with loss of consciousness in humans.
- At 24h post injury, animals were euthanized, and their brains harvested.
- Brains were cryosectioned at 40µm.



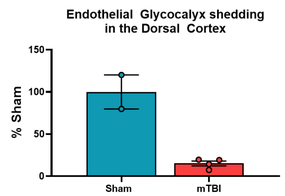
Endothelial Glycocalyx

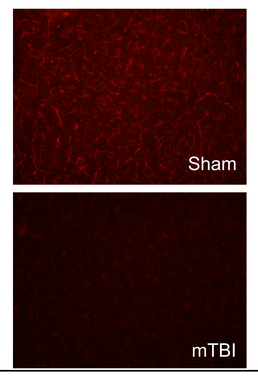
Methods

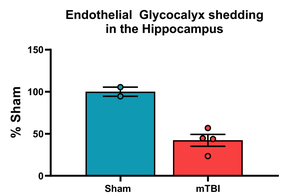
- Brain sections of animals receiving either a single mTBI (n=4) or sham procedure (n=2) were incubated overnight with a biotinylated tomato lectin and visualized using a streptavidin 647
- We imaged the dorsal cortex and the hippocampus at 20x
- Results are qualitative, not quantitative

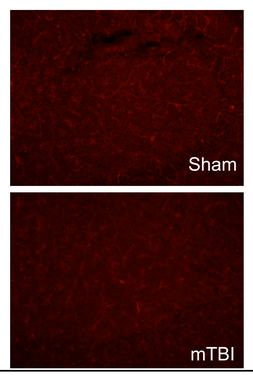
Results

- Reduction of tomato lectin labeling at 24h following a single mTBI compared to sham









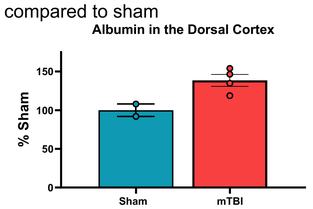
Extravasation of Albumin

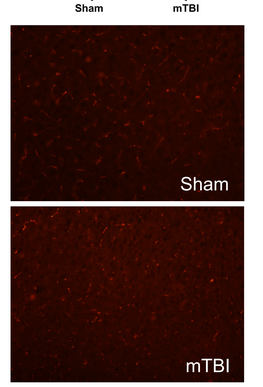
Methods

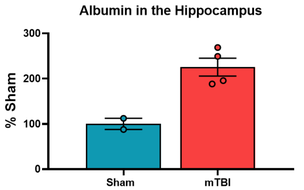
- Brain sections of animals receiving either a single mTBI (n=4) or sham procedure (n=2) were incubated overnight with an albumin antibody and visualized using an alexafluor 555
- We imaged the dorsal cortex and the hippocampus at 20x
- Results are qualitative, not quantitative

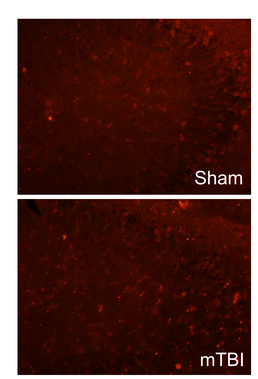
Results

- Increase in the amount of albumin allowed into the brain at 24h following a single mTBI compared to sham









Conclusions

- We observed a decrease in tomato lectin labeling corresponding to the shedding of the endothelial glycocalyx.
- We observed an increase in albumin in the brain tissues, suggesting a loss of BBB integrity.
- These results combine to suggest that our model of mTBI does indeed cause a disruption of the BBB.
- Future directions will be to examine other markers of BBB integrity, such as the tight junctions, astrocytes, and endothelial cells.

Acknowledgements

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