Carbon Dioxide Shows Promising Preclinical Ability to Prevent Traumatic Brain Injury in Mice

Owen D. Traubert,[1] Evan L. Reeder,[2] Christopher J. O'Connell,[2] Roman A. Caceres,[3] Savannah H. Savage,[4] and Matthew J. Robson[2]

[1]University of Cincinnati, College of Arts and Sciences, Department of Biology, Cincinnati, OH, USA
[2]University of Cincinnati, James L. Winkle College of Pharmacy, Division of Pharmaceutical Sciences, Cincinnati, OH, USA
[3]University of Cincinnati, College of Medicine, Department of Cancer Biology, Cincinnati, OH, USA
[4]University of Cincinnati, James L. Winkle College of Pharmacy, Division of Pharmacy Practice and Administrative Sciences, Cincinnati, OH, USA

Background

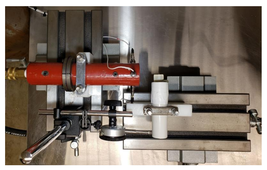
- Traumatic brain injury (TBI) is one of the leading causes of death and long-term disability worldwide
- In 2014, there were nearly 3 million TBI-related hospitalizations and deaths in the United States alone
- TBI often leads to secondary complications like Parkinson's disease, chronic traumatic encephalopathy (CTE), and Alzheimer's disease
- The "signature injury" of the military intervention in the Middle East
- Common among contact sports players
- Common due to accidents and falls
- $4 to $15 billion are spent on costs related to TBI each year
- Mild TBI, or mTBI is also known as concussion, and it is the most common type of TBI
- Advances in helmet technology have failed to improve incidence or outcomes for TBI due to design constraints
- There is an urgent need to develop new preventative measures against TBI to address this public health crisis

Mouse Model for Blast-Induced TBI

Blast TBI Model

Generates a short duration blast wave reminiscent of improvised explosive devices

Subject is PVC holder which acts to shield internal organs

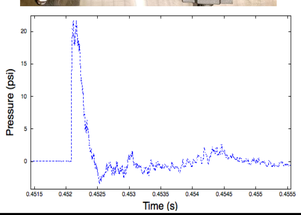


TBI Procedure

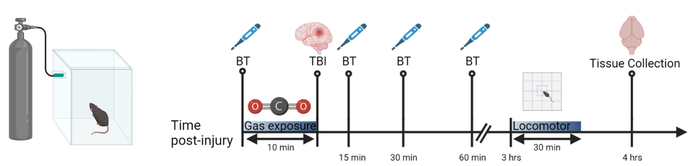
Adult, WT, male, mice (9 weeks of age) are subjected to blast-induced TBI from the shockwave of a pressure-burst mylar membrane

Creates blast wave with a peak overpressure of approximately 20 psi, which is scaled to mice

Sham subjects undergo same experimental protocol without blast exposure, including exposure to isoflurane anesthesia



Experimental Methods and Timeline



We anesthetized mice using isoflurane with a gas composition of either medical grade atmospheric air or 5% carbon dioxide, with atmospheric concentrations of oxygen

Mice were then subjected to either blast TBI or a sham treatment, consisting of being placed in the PVC tube

We recorded righting reflex time immediately after injury, as a validated measure of loss of consciousness

Body temperature was taken at four time points as a measure of physiological response to treatment

Locomotor activity was recorded three hours after treatment to gauge locomotor activity and arousal

Tissue was collected for RNA sequencing analysis of gene expression four hours after treatment

Concussion and the Compensatory Reserve Volume

Acceleration-deceleration event imparts force to skull

Brain moves relative to skull

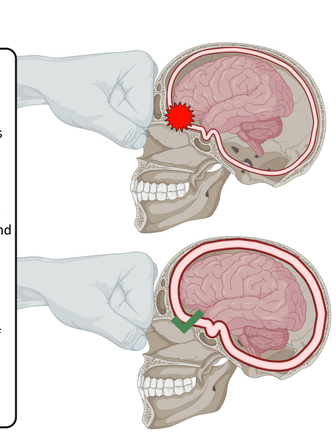
Helmets cannot prevent this type of injury effectively (they are located outside of the skull)

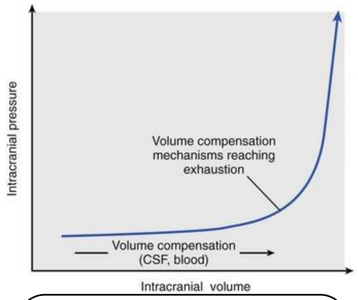
Brain impacts skull or twists relative to the spinal cord and brainstem

Injury occurs through numerous modalities

Brain can move relative to skull due to the tolerance of the compensatory reserve volume

If only we could exhaust ("fill") this volume…





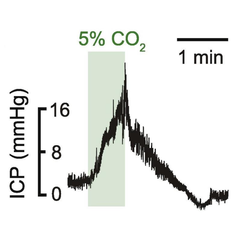
The skull is normally full of the brain, blood, and cerebrospinal fluid

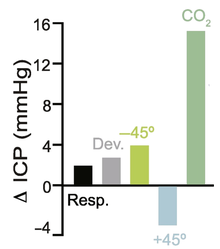
There is a small amount of compliancy due to the compensatory (cranial) reserve volume (CRV)

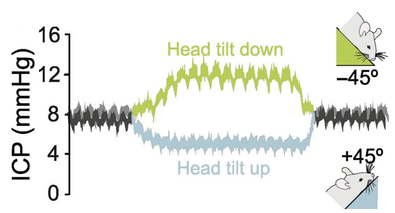
Adding additional fluid starts to exhaust the CRV and eventually raises intracranial pressure

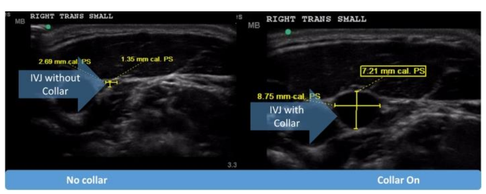
The most amenable component of intracranial pressure for intervention is the blood

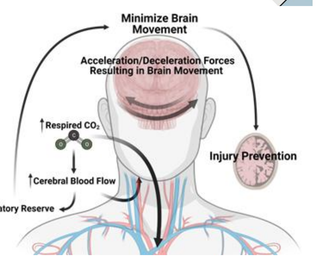
Filling the Compensatory Reserve Volume











There are numerous ways to increase intracranial pressure, and therefore exhaust the compensatory reserve volume, creating a tighter fit of the brain within the skull

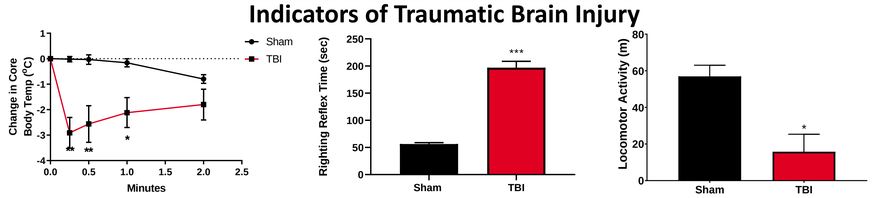
The first FDA-approved brain movement mitigation TBI preventative, the Q-Collar, accomplishes this using mild internal jugular vein compression, causing backfill of blood into cerebral veins, which has been shown to prevent indicators of concussion in studies of athletes

Respiration and simply changing the angle of the body are enough to elicit significant changes in intracranial pressure in mice

Carbon dioxide has an even more powerful on intracranial pressure and thus the compensatory reserve volume than other factors due to its robust ability to dilate cerebral arteries

We hypothesize that by using carbon dioxide's ability to exhaust the compensatory reserve volume via arterial dilation, we can reduce the effects of traumatic brain injury caused by brain movement

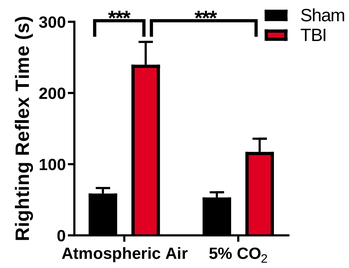
Indicators of Traumatic Brain Injury

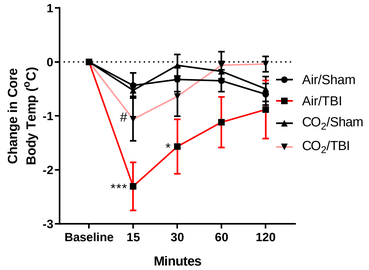


Mice exposed to blast TBI display reductions in body temperature serious loss of consciousness immediately after injury, and serious deficits in locomotor activity and arousal three hours after injury

* - less than 5% probability results arose by chance, ** - less than 1% probability results arose by chance, *** - less than 0.1% probability results arose by chance

Carbon Dioxide Normalizes Indicators of TBI

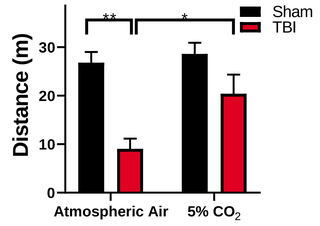




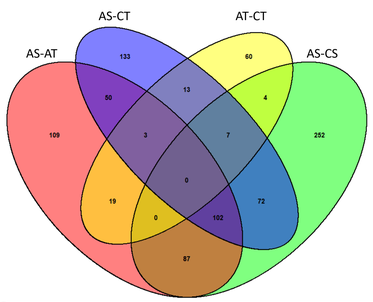
Mice exposed to carbon dioxide before TBI display near-baseline righting reflex time immediately after injury, indicating their loss of consciousness is no worse than non-injured mice

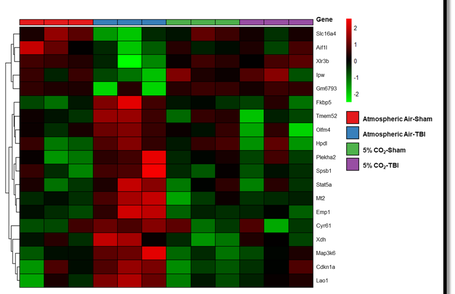
Carbon dioxide-treated mice have normalized body temperatures after TBI, indicating their physiological state after injury is similar to non-injured mice

Carbon dioxide exposure also normalizes the distance traveled in the open field test three hours after injury, indicating the treated mice have no deficits in locomotor activity or arousal deficits compared to the control group



Carbon Dioxide Normalizes Gene Expression





We examined gene expression to investigate which specific aspects of TBI carbon dioxide can prevent, given TBI induces a miasma of gene transcription alterations (370 genes in our data)

We identified 19 genes that are differently expressed by mice exposed to air TBI but not expressed by mice exposed to carbon dioxide TBI or by mice exposed to carbon dioxide alone

These, therefore, are the genes relevant to the post-injury processes carbon dioxide prevents and protects against via reducing brain movement during injury, and using these genes, we can deduce additional and unmet therapeutic targets

Conclusions and Future Work

- By exhausting the compliancy of the compensatory reserve volume via robust dilation of cerebral arteries, carbon dioxide shows a promising ability to reduce the incidence and severity of traumatic brain injury
- Mice treated with carbon dioxide before injury appear normal behaviorally and physiologically, and many genes with altered expression in TBI are normalized in mice exposed to carbon dioxide
- Inspired similar therapeutics, this premise could be turned into a medical device to protect humans at high risk of TBI

Preconditioning?

Carbon dioxide has a large role in the body, and is relevant to countless biological pathways

Many of the genes carbon dioxide changes on its own overlap with the genes TBI changes on its own, and furthermore, genes change in the same direction

Ischemic preconditioning uses exposure to brief interruptions of blood flow before organ transplant or stroke to improve outcomes, and works via changes in gene expression also caused by inflammatory compounds, hypoxia, and hyperthermia

Many of the genes involved in ischemic preconditioning are in the same neighborhood as those involved in carbon dioxide exposure and TBI, and TBI itself elicits ischemia, hypoxia, and inflammation