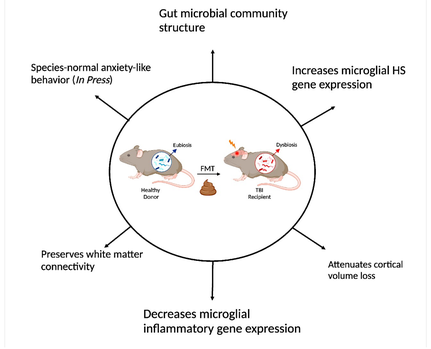
Department of Surgery, Northwestern University

Post-TBI Administration of Short Chain Fatty Acids Supports Gut Microbial Structure and Attenuates Cognitive Deficits

Booker T Davis IV, PhD, Kacie P. Ford, BS, Zhenhua Shang, PhD, Zhangying Chen, MS, Mecca B.A.R. Islam, MS, Daniele Procissi PhD, and Steven J. Schwulst, MD

[1]Division of Trauma and Critical Care [2]Department of Surgery , Feinberg School of Medicine

Background



Traumatic brain injury (TBI) is an underrecognized public health threat. There are limited therapeutic options for TBI, and supportive care remains the mainstay of treatment. Our previously published data demonstrate that post severe TBI fecal microbiome transplantation (FMT) can reverse TBI-induced depletion of commensal bacteria, preserve white matter connectivity, protects cognition, and decrease brain lesion size in mice after TBI.

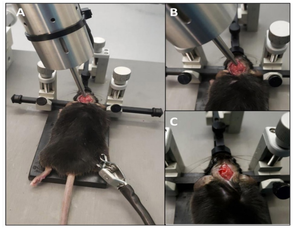
Research Objectives

To develop a basic understanding of the mechanisms within the Brain Gut Axis that impact TBI outcomes.

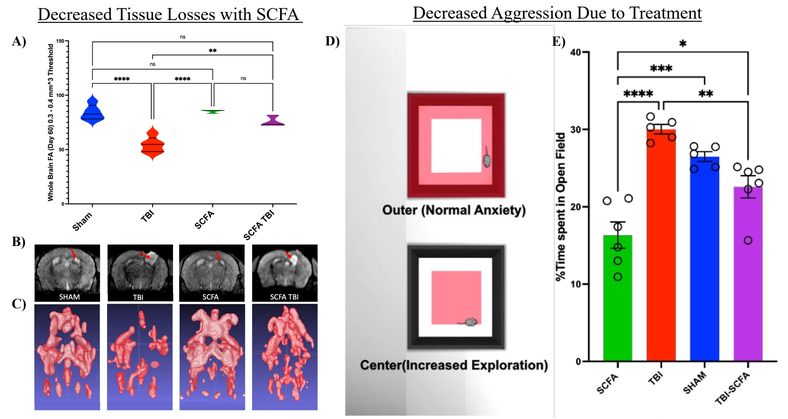
We hypothesized that post-injury treatment with Short Chain Fatty Acids (SCFA), metabolites of commensal gut bacteria, would attenuate neurocognitive deficits after TBI in mice.

Methods

14-week-old (n=52) male C57BL/6 underwent TBI via controlled cortical impact vs. sham injury. Post-TBI, each group was treated with acetate, butyrate, and propionate vs. salt vehicle via free access to drinking water for four weeks post-TBI. We measured white matter connectivity using magnetic resonance at 60 days post injury(DPI). and measured for species-normal anxiety levels with open field testing at 50DPI. We extracted at brain Microglia to assess signs of neuroinflammation at the 30DPI. Animal stool was collected 59DPI to assess the gut microbial community structure via 16s RNA gene amplicon sequencing.

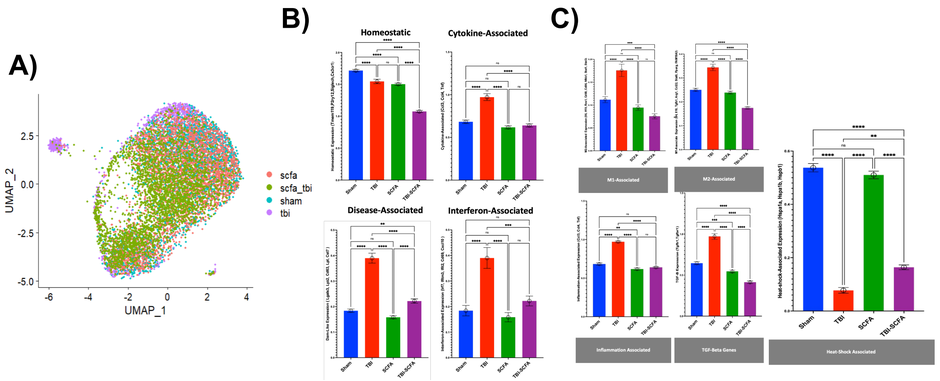


SCFA Treatment Decreases White Matter Loss and Behavioral Deficits



A) TBI is known to impede Fractional Anisotropy (white matter connectivity) in the brain. We used MRI scans of animals to measure FA through water diffusivity (60DPI). SCFA treated TBI mice were comparable sham (NS) and showed significant difference from untreated injured animals (p-value <.001)(N=3). B) Representative 3D contrast T2 weighted MRI images. C). Representative renderings of white matter connectivity. D) Diagram of Open Field test used to measure Levels of Anxiety/Seeking behavior. E) SCFA White matter connectivity protections correlated well with amelioration of behavioral deficits in TBI. While untreated animals showed an increase in aggressive/seeking behaviors compared to Sham (p<.0001), SCFA treatment decreased TBI induced aggression by nearly 1/3rd compared to the TBI groups (p<0.001) (Two-way ANOVA)

Microglial Gene Expression Altered by Injury and Treatment



(A) Extracted & sorted Cd45+/dim cells (microglia) were grouped by gene expression according to function. Plot demonstrates clear clustering of different microglia phenotypes due to treatment/injury (10X genomics) B) Functional gene expression was normalized to cell number. Boxplots demonstrate Homeostatic gene expression altered by TBI (p<.0001)and SCFA (p<.0001) compared to sham. SCFA also attenuated TBI-mediated increases in cytokine, disease, & interferon associated genes. C) TBI results in a significant upregulation of classically inflammatory (M2) and anti-inflammatory (M1) gene expression in TBI microglia compared to sham (p<.0001). This effect was blocked with FMT treatment (p<.0001). Neuroprotective heat shock genes were increased in SCFA_TBI animals compared to untreated (p<.0001).

Short Chain Fatty Acids Support Gut Microbes

- In TBI we saw the unexpected protection of specific microbial species with SCFA treatment
- DESEQ analysis found 11 differentially abundant bacteria, some associated with:
  - Inflammation – (Clostridia[1], Bacteroides[3])
  - Cognition & Neurodevelopment – (Oscillobacter[1], Lachnospiracae[2])
  - SCFA production – (Blautia[1], Lachnospiraceae[3] & Bacteroides[3])

Differentially Abundant Bacterial Taxa

Genus	Sham vs.TBI	Sham vs. SCFA	SCFA vs. TBI	SCFA vs. SCFA_TBI	TBI vs. SCFA_TBI
Clostridia_UCG_014	0.010569198	0.662382307	0.115076613	0.664650932	0.01226447
Lachnospiraceae_UCG_001	0.152724142	0.940933281	0.10950574	0.400838898	0.095726112
Colidextribacter	0.133814648	0.306968718	0.386283733	0.877050609	0.331683588
Bacteroides	0.012584015	0.564244839	0.000205032	0.081449707	0.022538713
Blautia	0.006789119	0.322162956	0.028373873	0.571796455	0.077117149
Lachnospiraceae_NK4A136_group	0.007000569	0.068212133	0.25003614	0.702998836	0.156890715
Lachnospiraceae_FCS020_group	0.045063515	0.009247725	0.781398773	0.20989872	0.214623636
Oscillibacter	0.052941586	0.034418339	0.512248726	0.075678649	0.130422485
Butyricicoccus	0.043222293	0.102594566	0.983830343	0.277639528	0.176022803
Parasutterella	0.089747094	0.127113232	0.052994821	0.013815154	0.009623174
Turicibacter	0.179936959	0.421835796	0.013798177	0.965080533	0.06352819

P-value 1.0 0.0

Limitations

- Limitations to CCI include the need for craniotomy and the expense of acquiring the impactor and actuating device.
- There is a dearth of consistent foundational literature highlighting TBI in females. As sex is a confounding factor in TBI, female mice are being assessed in a separate study.

Conclusions

- In the context of a TBI, short chain fatty acid treatment improved white matter connectivity and protected species normal behavior patterns. SCFA administration also protected a limited number of healthy microbial species within the gut and attenuated microglial inflammatory gene expression, increasing the expression of protective heat shock protein gene within microglia.
- These data highlight the potential of SCFA to mimic some of the benefits of FMT as a novel therapeutic intervention for TBI. FMT is already an established, albeit non-traditional, therapy for other clinical disease entities; repurposing FMT and the addition of SCFA administration for TBI treatment may represent a novel treatment paradigm for an injury process that is notorious for having few treatment options other than supportive care.

Northwestern Medicine®
Feinberg School of Medicine