Disease Modifying Effects of 4-Aminopyridine (4-AP) on Axonal Injury and Neurodegeneration after Traumatic Brain Injury

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MTBI²

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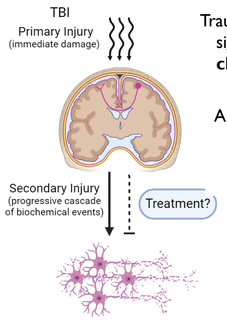
Background



Traumatic brain injury (TBI) poses a significant health threat by initiating a **chronic degenerative process.**

A primary driver of persistent disability is damage to white matter (WM) tracts, particularly the corpus callosum (CC), which connects the brain hemispheres.

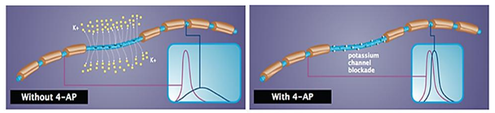
Initial impact triggers secondary injury cascades that **evolve over weeks**, leading to irreversible axonal fragmentation and WM atrophy.

Currently, no therapies exist to prevent acute axonal injury from progressing to irreversible chronic neurodegeneration.

To address this need, we evaluated 4-aminopyridine (4-AP) as a disease-modifying treatment to support damaged axons and prevent long-term WM loss in a mouse model of TBI.

4-AP: Repurposing Opportunity for Acute TBI

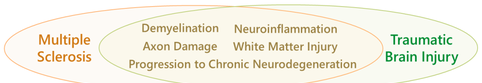
4-AP is a CNS-penetrant inhibitor of voltage-gated potassium (Kv1) channels used clinically to improve function at nodes of Ranvier that become disrupted by demyelination.



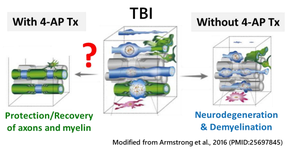
4-AP prevents dysregulated K^+ efflux and delays repolarization, mitigating conduction failure in damaged axons.

Low dose 4-AP (dalfampridine; Ampyra®) is FDA-approved to improve walking speed in patients with multiple sclerosis (MS).

➔ MS and TBI exhibit common pathological characteristics which warrants evaluation of **repurposing 4-AP for TBI**.



Hypothesis



Modified from Armstrong et al., 2016 (PMID:25697845)

Early 4-AP intervention sustains neuroaxonal activity in vulnerable axons, reducing early-stage damage and mitigating the progression to chronic neurodegeneration.

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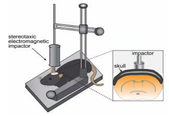
Study Design and Injury Model

Randomized, blinded, vehicle-controlled preclinical trial.

Animals: Adult (8-10 weeks old) C57BL/6J mice randomized to injury and treatment groups.

Study plan pre-registered in OSF.org. Pre-defined exclusion criteria.

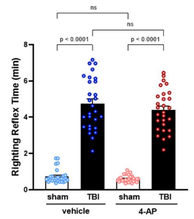


Single Impact Concussive TBI in Adult Mice	
Impactor	Impact One™ Stereotaxic Impactor Device
Parameters	3 mm tip – 4 m/s velocity – 100 ms dwell time
Impact Location	Exposed skull directly at bregma – 1.5 mm depth
Sham Controls	Received anesthesia and skin incisions only

Clinical relevance to human concussive TBI

- Brief loss of consciousness (righting reflex in mice)
- No overt structural damage or bleeding
- Dispersed damaged axons in corpus callosum (CC)
- MRI-DTI changes (decreased fractional anisotropy)
- Impaired processing speed
- Chronic neuroinflammation
- Progression of axonal injury to late stage CC atrophy

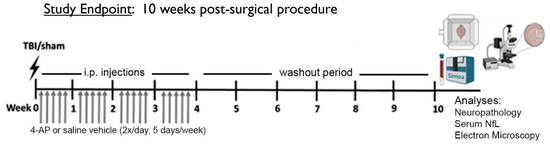
The mean righting reflex time across groups confirms that the TBI procedure significantly prolonged the latency to right from a supine position with no pre-treatment differences between injured cohorts.



Therapeutic Intervention

Drug: 4-AP (Millipore-Sigma Cat# 275875) Vehicle: 0.9% sterile saline

Dosing: Intraperitoneal injections (**0.5 mg/kg**) twice daily (clinically relevant dose)
Tx Onset: 24 hours post-surgical procedure (clinically reasonable window)
Tx Duration: Administered for 4 weeks (5 days/week)
Tx Washout: Treatment discontinued for 6 weeks
Study Endpoint: 10 weeks post-surgical procedure

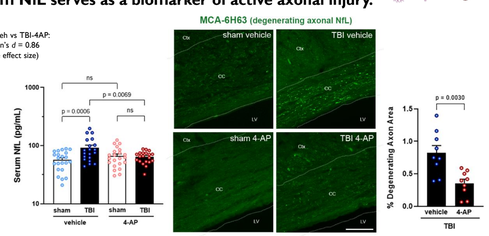


① 4-AP reduces biomarker of axonal injury

Neurofilament light (NfL) is a structural protein exclusive to the axonal cytoskeleton. When axons are damaged, higher levels of NfL enter the bloodstream.

Serum NfL serves as a biomarker of active axonal injury.

TBI-veh vs TBI-4AP: Cohen's d = 0.86 (large effect size)



(Left) Serum NfL. Ultrasensitive Simoa® immunoassay technology detected significantly higher serum NfL levels in TBI+vehicle mice compared to sham controls. Early 4-AP treatment post-TBI normalized serum NfL to sham levels.

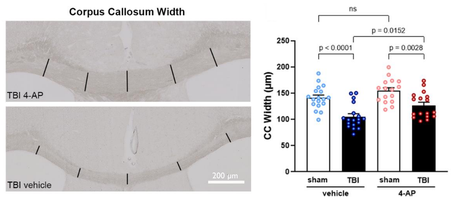
(Right) DegenoTag™ (MCA-6H63). Immunostaining with an antibody specific to *degraded* NfL epitopes confirmed that the serum signal originated from degenerating axons in the corpus callosum (CC). Abbr.: Ctx, cortex; Lv, lateral ventricle.

Serum NfL remained at healthy levels 6 weeks after discontinuing drug treatment, indicating that **4-AP reduces the extent of secondary axonal injury**.

② 4-AP attenuates white matter degeneration to late atrophy

CC atrophy is a clinically relevant measure of irreversible white matter degeneration.

To determine if 4-AP altered the trajectory of neurodegeneration, we quantified the width of the CC at 10 weeks post-injury (6 weeks after discontinuing drug treatment).



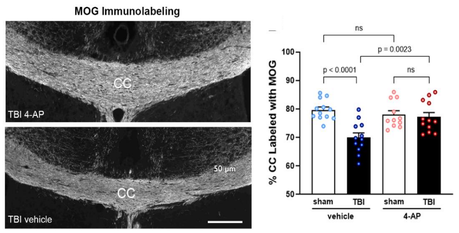
TBI-veh vs TBI-4AP: Cohen's d = 0.90 (large effect size)

TBI caused significant thinning of the CC compared to sham controls, modeling the progressive white matter loss seen in human patients. Early 4-AP treatment significantly reduced the extent of permanent white matter loss after TBI.

Early 4-AP intervention alters the progression of pathology after TBI, significantly mitigating the severity of chronic white matter degeneration.

③ 4-AP preserves myelin integrity

To evaluate the extent of myelination within the CC, we quantified the percent area of MOG (myelin oligodendrocyte glycoprotein) immunoreactivity.



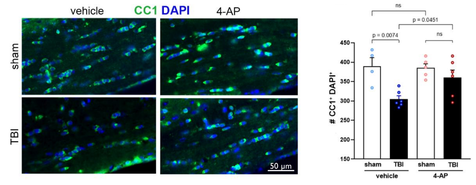
TBI-veh vs TBI-4AP: Cohen's d = 1.32 (large effect size)

TBI resulted in chronic myelin loss. In contrast, early 4-AP treatment maintained myelin coverage close to sham levels.

Early 4-AP treatment mitigates the extent of chronic myelin loss.

④ 4-AP mitigates oligodendrocyte loss

To determine if myelination levels corresponded with changes in oligodendrocyte population, we quantified the number of mature oligodendrocytes ($CC1^+$) within the CC.



TBI-veh vs TBI-4AP: Cohen's d = 1.52 (large effect size)

TBI caused a significant loss of $CC1^+$ cells compared to sham controls.

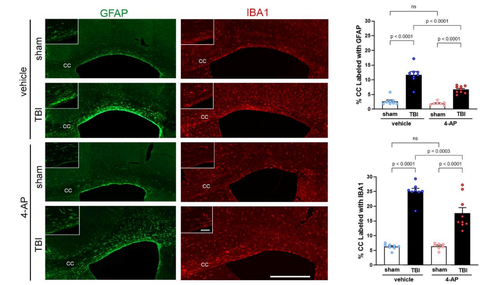
4-AP treatment effectively prevented this mature oligodendrocyte cell loss.

Early 4-AP treatment preserves the critical myelinating cell pool.

⑤ 4-AP dampens neuroinflammation

Persistent gliosis models chronic neuroinflammation in human TBI, a key driver of progressive neurodegeneration.

To assess if 4-AP modified the chronic neuroinflammatory response, we quantified the percent area of GFAP and IBA1 immunoreactivity in the CC.



TBI-veh vs TBI-4AP: Cohen's d = 2.12 (large effect size)

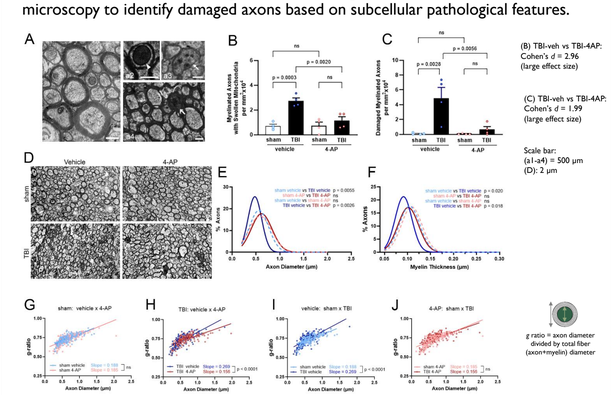
TBI-veh vs TBI-4AP: Cohen's d = 1.63 (large effect size)

TBI induced robust chronic hypertrophy of astrocytes (GFAP) and microglia (IBA1) which was significantly mitigated by early 4-AP intervention.

Early 4-AP treatment attenuates the chronic inflammatory response, reducing the long-term glial reaction in the CC after TBI.

⑥ 4-AP reduces axon-myelin pathology

To assess chronic axonal damage in the CC at high resolution, we utilized electron microscopy to identify damaged axons based on subcellular pathological features.



(B) TBI-veh vs TBI-4AP: Cohen's d = 2.96 (large effect size)

(C) TBI-veh vs TBI-4AP: Cohen's d = 1.99 (large effect size)

Scale bar: (a1-a4) = 500 µm (D): 2 µm

g ratio = axon diameter divided by total fiber (axon+myelin) diameter

4-AP reduced intra-axonal damage (impaired transport; cytoskeleton breakdown) and mitochondrial swelling, normalized axon diameters and myelin thickness to sham levels, and restored the myelin thickness relative of axon diameter (g-ratio).

Early 4-AP treatment reduces the chronic stage pathology of myelinated axons post-TBI.

Conclusions

4-AP has disease-modifying effects on the progression of evolving TBI pathology that endure long after treatment is discontinued.

This preclinical data supports repurposing of 4-AP (dalfampridine-ER) to acute TBI and, more broadly, provides a proof-of-concept for advancing interventions to treat axon damage and white matter degeneration.