Cellular mechanism involved in hemorrhagic progression of contusion following traumatic brain injury

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Background & Purpose

A severe sequelae of head trauma is a cerebral contusion, which bruises, toxifies, and irreversibly defunctionalizes tissues. Previous studies have shown that the resulting lesion will often expand into new or non-contiguous hemorrhages shortly after impact, a process known as hemorrhagic progression of a contusion (HPC)[1] (Figure 1). Glibenclamide is a sulfonylurea drug that reduces HPC after a traumatic brain injury (TBI) by acting as a SUR1 antagonist. It was previously discovered that after a TBI, brain microvessels upregulate SUR1-TRPM4 channels (Figure 2) prior to undergoing catastrophic failure and forming petechial hemorrhages, the underlying pathophysiology of the HPC[2]. However, the cellular and molecular mechanism of HPC has not been fully elucidated. Moreover, it is unclear which microvessel layer - the inner endothelial, gliovascular basement membrane containing pericytes, or outer layer of perivascular astrocyte endfeet - is the cellular target of glibenclamide. Identifying the cell-specific targets of glibenclamide is essential as it may aid in future drug discovery and provide a therapeutic benefit for TBI patients. In addition, we can better recognize existing and future drugs that are working through binding to SUR1-TRPM4 channels.

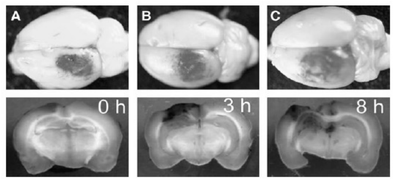


Figure 1. Hemorrhagic progression of contusion secondary to brain injury. Panels A–C show surface and coronal views of the same brains from rats euthanized immediately after, 3 h after, or 8 h after contusion injury, as indicated.

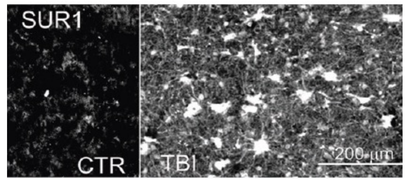


Figure 2. Immunolabeling for SUR1 displaying sparse immunoreactivity in the control specimen (CTR) vs. widespread expression in a GFAP-positive specimen from contusion-TBI.

Project Aims

Project Goal: Study the role of glibenclamide as a potential therapeutic agent for TBI and better understand the mechanism of action of glibenclamide on a cellular level as it relates to preventing HPC

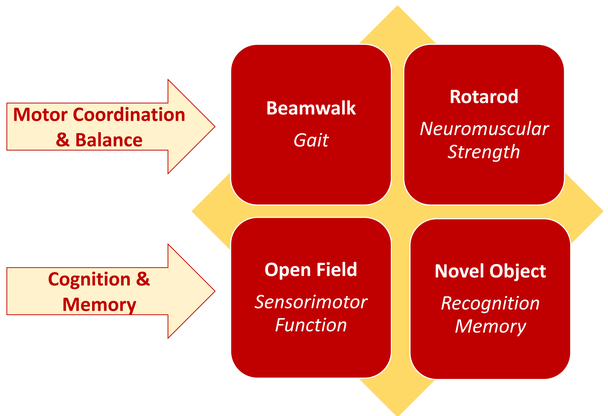
Compare neurofunctional outcomes pre- and post- brain injury amongst groups of mice given either a SUR1 antagonist or agonist at varying dosages. Neurofunctional status was assessed by scoring mice for forelimb and hindlimb function, resistance to force, cognition, memory, motor coordination, and balance.

Determine the volume of hemorrhage secondary to contusion for mice belonging to the previously described groups. Relative quantification of the size of the hemorrhage was done via imaging the epicenter of the lesion with a flatbed scanner.

Methods

Neurofunctional Testing[5,6]

Four neurofunctional tests were carried out in order to interpret motor coordination and balance, cognition, and memory. Each assessment was done pre-TBI and 24 hours post-TBI.



Controlled cortical impact (CCI) contusion-TBI model

Mice were anaesthetized (ketamine/xylazine) and fitted in a stereotactic apparatus (Stoelting Wood Dale, IL). Core temperature was maintained at 37ºC. Hair was clipped from the dorsum of the head, the skin was prepared with Betadine solution, and the skull was exposed via a midline incision. For CCI, a 3-mm craniotomy was centered at x, 2.5 mm; y, 3 or 2.5 mm. CCI was induced using an ImpactOneTM stereotaxic CCI instrument (Leica Biosystems, Buffalo Grove, IL), as described,[3,4] with precise parameters of velocity, displacement, duration, and angle towards the midline. The skull defect was closed by replacing the bone removed during the cranioplasty and fixing it in place with dental cement. The skin was closed, and the animal recovered from anesthesia.

Drug Induction

Group #	Drug Treatment	Frequency
1	Glibenclamide	1 injection: @ t=0 hours, immediately after contusion
2	Glibenclamide	2 injections: @ t=0 hours and t=10 hours
3	NN414*	1 injection: @ t=0 hours, immediately after contusion

* NN414 is a SUR1 agonist

Tissue blood at 24 hours[1,2]

After euthanasia, mice were perfused with heparinized saline to remove intravascular blood. The brain was harvested, and a coronal cut was made at the lesion epicenter. A flatbed scanner was used to image the lesion, to document lesion area and the involvement of various brain structures.

Results

Rotarod Scores

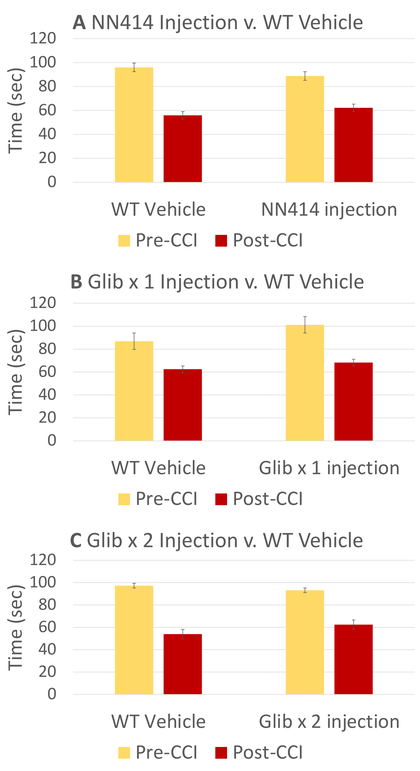


Figure 3. Rotarod scores taken pre-CCI and 24 hours post-CCI for each drug treatment group. (A) rotarod scores display a minimal difference between NN414 and vehicle groups. (B) 1 injection of glibenclamide improves rotarod scores, with (C) 2 injections demonstrating maximal therapeutic benefit.

Beamwalk Scores

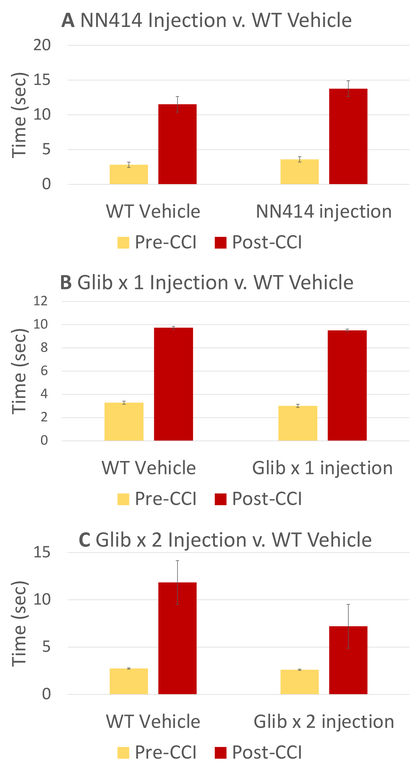


Figure 4. Beamwalk scores taken pre-CCI and 24 hours post-CCI for each drug treatment group. (A) beamwalk scores display a minimal difference between NN414 and vehicle groups. (B) 1 injection of glibenclamide improves beamwalk scores, with (C) 2 injections demonstrating maximal therapeutic benefit.

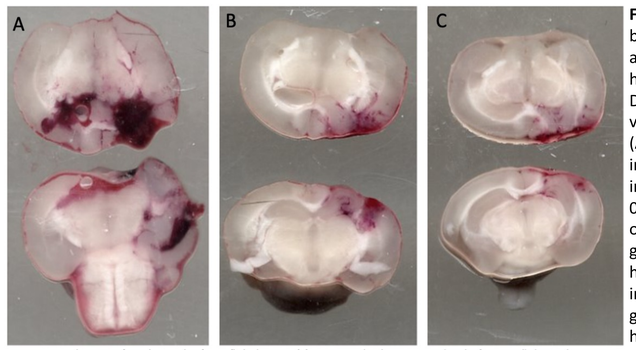


Figure 5. Mice coronal brain sections taken from a flatbed scanner 24 hours post-CCI. Decreasing hemorrhagic volume is seen from A-C: (A) Mice given one injection of NN414 immediately after CCI (t = 0 hours) (B) Mice given one injection of glibenclamide at t = 0 hours (C) Mice given two injections of glibenclamide at t = 0 hours and at t = 10 hours.

Conclusions

1. Group 1 improved in cognition and memory, but not in motor coordination and balance
2. Group 2 improved in cognition and memory as well as gait function; however, it had no significant effect on neuromuscular strength
3. Group 3 displayed similar neurobehavioral outcomes compared to controls, indicating that the CCI procedure produces maximal SUR1 activation and injury
4. Image analysis of coronal brain sections post-contusion aligned with neurofunctional outcomes
5. Glibenclamide has a therapeutic effect in a TBI model, especially within mice with a 2.5 mm displacement impact given two doses of glibenclamide

Future Directions

1. Compute hemorrhagic volume 2° to brain contusion for each treatment group and their respective vehicles via:

Spectrophotometric hemoglobin assay → **Digital quantification[7] using Photoshop and Image J**

The hemisphere encompassing the lesion will be homogenized and processed using Drabkin's reagent

Photoshop will identify pixels containing hemorrhage and Image J will measure the optical intensity

2. Evaluate histological and neurobehavioral outcomes of mice with cell-specific deletion of *Abcc8*/SUR1 in endothelial and astrocytic cells
3. Based on post-HPC SUR1 expression studies[1,2] and microvessel structural characteristics, the cell type that's mostly likely critical to HPC is the inner endothelium cell layer. Thus, mice with endothelium-specific deletions of SUR1 are expected to produce better neuroscores (beam walk and rotarod) and will have the lowest hemorrhagic contusion volume compared those with the astrocyte-specific deletion of SUR1

References

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