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Abstract Details:

Breakout Session: Acute Interventions for Battlefield TBI

Category: Oral Presentation

Title: Secondary Brain Injury Following Focal Ischemia in Mice is Reduced by a Synthetic Isoflavone Treatment that Inhibits G α q – Mediated Ca²⁺ Flux

Abstract:

Background: Traumatic brain injury (TBI) is a severe, debilitating health issue faced by soldiers on and off the battlefield. Greater than 300,000 US service members have sustained a TBI in training or combat, with 8% being moderate to severe types of TBI and the remainder are mild TBI. A key factor in the morbidity and mortality arising from TBI is the prodigious expansion of the injury volume in the hours and days after the primary head injury. This stems from a decline in local blood flow and edema leading to metabolic failure in neurons and glia triggering ionic pump failure, alongside excessive unregulated discharge of excitatory neurotransmitters, especially glutamate. Together this causes sustained buildup of the second messenger Ca²⁺ in the cytosol, activating caspase-coupled, pro-apoptotic signaling. Currently, treatment for TBI is limited to decompressive surgery or physical therapy, but pharmacological treatments that blocks the progressive expansion of the injury size in the days following the trauma do not exist.

Nyrada Inc. is developing a small molecule compound that blocks sustained Ca^{2+} build up in cells, tied to activation of $\text{G}\alpha\text{q}$ -type G protein-coupled receptors. Expressed across a wide range of cell types including neurons, astrocytes, microglia, platelets and endothelial cells, the activation of $\text{G}\alpha\text{q}$ receptors switches on the enzyme phospholipase $\text{C}\beta$ leading to production of secondary messengers - inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 activates the IP3 receptor on the endoplasmic reticulum, releasing Ca^{2+} internal stores while DAG activates Ca^{2+} re-entry via canonical transient receptor potential ion channels. This sustained activation of $\text{G}\alpha\text{q}$ -coupled receptors, especially the glutamate dependent class I metabotropic glutamate receptors (mGluR1 and mGluR5), on neurons and astrocytes, is central to the pathophysiology of secondary brain injury expansion.

Methods and Results: Nyrada's synthetic isoflavone compounds were initially screened for their activity in CHO cells stably expressing the $\text{G}\alpha\text{q}$ -type human M3 muscarinic receptor (hM3R), with activation via the M3R agonist carbachol. The Ca^{2+} flux was measured with a FLIPR® Calcium 5 Assay Kit and the IC50 of the lead compound (NYX-104) was determined as 9.8 μM .

NYX-104 was evaluated for its potential to block Ca^{2+} entry into the cytosol of Purkinje neurons following activation of mGluR1 in mouse brain slices. To assess this, the cerebellae of postnatal day 3 mice (C57BL/6J) were injected with 3×10^9 vg of a recombinant adeno-associated virus vector (AAV1) encoding the genetically encoded Ca^{2+} indicator, GCaMP5g. Ca^{2+} imaging was performed on parasagittal acute brain slices (400 μm) 8 – 12 weeks later using the Zeiss confocal system (710NLO LSM; excitation 488 nm). Slices were continuously superfused with artificial cerebrospinal fluid and the activation of mGluR1 was achieved by adding the agonist (s)-3,5-Dihydroxyphenylglycine (DHPG, 100 μM) to the bath for 10 min. During washout, NYX-104 (40 μM), or DMSO vehicle (0.1 %), was delivered to the brain slices for 30 mins and then DHPG was re-applied in the presence of NYX-104, or DMSO vehicle. Ca^{2+} dynamics in the Purkinje neuron soma were analysed for 40 seconds from DHPG-response onset. In relation to the initial baseline stimulation, DHPG - induced Ca^{2+} entry in Purkinje neuron soma incubated with NYX-104 was significantly reduced compared to the vehicle control ($p = 0.0175$, paired t-test, two-tailed).

NYX-104 was assessed for in vivo neuroprotection in a photothrombotic model of focal ischemia in mice (C57BL/6J strain; aged 8 - 12 weeks, males and females). Focal ischemia was achieved via tail vein injection of the photosensitive dye rose bengal (50 mg/kg), followed by irradiation of the somatosensory cortex via transcranial illumination with green light (532 nm, 1mW), which triggered localised thrombus formation, blood vessel occlusion and oxygen-glucose deprivation. Mice were rectally administered with NYX-104 (100 mg/kg/d), or a carrier control, commencing 45-minutes after injury induction, and then once daily for four days. In a reference group of mice, the primary injury volume, determined at 2-hours post injury, was 7.87 mm^3 . The secondary expansion of the brain injury doubled the infarct volume in the control mice by five days (15.21 mm^3 ; $p < 0.001$, one-way ANOVA). In comparison, NYX-104 demonstrated a 38% decrease in the penumbra expansion during this time (12.42 mm^3 infarct volume; $p = 0.025$; t-test comparing control and NYX-104 infarct volumes at 5 days post injury).

Conclusion: NYX-104 shows block of $\text{G}\alpha\text{q}$ -mediated Ca^{2+} flux in vitro, including reduction in mGluR-mediated Ca^{2+} loading in cerebellar Purkinje neuron soma. In an in vivo focal ischemia brain injury model, NYX-104 conferred significant protection from secondary brain injury expansion. Nyrada aims to further optimise this promising lead candidate to enhance potency against the block of Ca^{2+} and formulation to enable treatment of soldiers on the battlefield following TBI to improve their survivability.

Disclaimer: All animal experiments were undertaken in accordance with the UNSW Animal Care and Ethics Committee Code. The information presented in this abstract is the proprietary information of Nyrada Inc.

Learning Objectives

1. Identify that TBI is a major health problem in the DoD, with no current treatment that limits the ongoing brain damage from secondary injury mechanisms.
2. Recognize the significance of excessive loading of Ca^{2+} in brain cells as a key driver of secondary brain injury.
3. Evaluate the significance of NYX-104 in reducing pathophysiological Ca^{2+} loading in brain cells for a neuro-rescue therapy for TBI.

Submit for Young Investigators Competition? No

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