Neurons Selectively Impairs Complex Cognition
Eliminating System xc- Signaling Between Astrocytes and Potential Drug Therapy for Fragile X Tremor/Ataxia Syndrome

OBJECTIVES/GOALS: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a devastating rare neurological disorder that negatively impacts movement and cognition. To date, there are no effective pharmacological treatments for FXTAS. Our goal was to develop a cell culture model of FXTAS to investigate promising therapeutics. METHODS/STUDY POPULATION: To establish mitochondrial dysfunction, normal human cell lines and human-induced pluripotent cells were treated with multiple concentrations of glucose/glucose oxidase (GluOx) at 2, 12, and 24 hour time points to induce varying intensities of oxidative stress. The degrees of oxidative stress were measured by apoptosis and mitochondrial reactive oxygen species (ROS) production. Curcumin and MSKE compounds effective against oxidative damage in mitochondria were used to rescue glucose oxidase-induced oxidative damage in both cell lines. To test the ability of these drugs to restore mitochondrial health, cell viability and cellular superoxide production were assessed by propidium iodide and the MitoSox fluorescence assay, respectively.

RESULTS/ANTICIPATED RESULTS: We anticipated that GluOx at varying concentrations and time points would proportionally increase levels of apoptosis and mitochondrial ROS, reflective of mitochondrial damage, with the most severe dysfunction occurring at a dose of 25 nM and the longest duration of 24-hr exposure. Administration of MSKE in concentrations ranging from 10-8 to 10-5 M in half-log increments, did not reverse the oxidative defects induced in the cell lines. However, curcumin concentrations increased cell viability at the 2, 12, and 24 hour time period. Results indicate that the research design should be modified by increasing the concentration of both glucose and MSKE to provide a reliable test of the hypothesis.

DISCUSSION/SIGNIFICANCE: These studies illustrate the usefulness of this in vitro model to test novel therapeutics in neuronal FXTAS models and expand the discovery of mitochondrial markers for the syndrome.

Potential Drug Therapy for Fragile X Tremor/Ataxia Syndrome

OBJECTIVES/GOALS: Our hypothesis is that micronneedle array (MA) extraction of interstitial fluid (ISF) will enable minimally invasive quantitation of heavy metal (HM) exposure. We aim to establish analytical parameters for ICP-MS analysis of HMs, quantify baseline HM content in ISF vs other fluids, and characterize a mixed HM exposure model. METHODS/STUDY POPULATION: Ten healthy human volunteers were recruited into the study, approved by the UNM Human Research and Resource Committee. Each subject had blood and urine collected. ISF was also collected using 3D-printed MAs inserted into the forearm. Additionally, twelve Sprague Dawley rats were unexposed (n=6) or exposed (n=6) to ad libitum water containing a mixture of uranium (U), cadmium (Cd), vanadium (V), and arsenic (As), each at 5X the maximum contaminant level (MCL) for drinking water under a protocol approved by the UNM animal care and use program. Human and animal fluids were analyzed, using ICP-MS, to quantify the levels of U, Cd, V, and As.

RESULTS/ANTICIPATED RESULTS: Recent advances in ISF extraction and analysis suggest a minimally invasive method that can be adapted to monitor HM exposure and biological loads to produce highly specific, narrow changes in brain function that would benefit CNS disorders. To do this, we investigated cognitive changes produced through manipulating the activity of the astrocytic glutamate release mechanism system xc-.

METHODS/STUDY POPULATION: System xc- (Sxc) activity was eliminated by mutating the gene Slc7a11 through pronuclear injection of zinc-finger nucleases into Sprague Dawley rat embryos to create a line of rats lacking Sxc (MSxc rats). To confirm a lack of Sxc activity, we verified that tissue from MSxc rats had a complete lack of xCT, which is the regulatory subunit of Sxc that is encoded by Slc7a11. We also verified that astrocyte cultures generated from MSxc tissue lacked cystine-evoked glutamate release. Next, we measured development (body weight), CNS regulation of metabolism, and other indicators of generalized, non-specific brain function as well as behaviors that are reliant on executive function, such as cognitive flexibility, impulse control, decision-making, and response inhibition.

RESULTS/ANTICIPATED RESULTS: Eliminating Sxc was not lethal and did not impair development or produce widespread changes in brain function. Increasing the concentration of both glucose and MSKE to provide oxidative stress were assessed by propidium iodide and the MitoSox fluorescence assay, respectively.

DISCUSSION/SIGNIFICANCE: Eliminating Sxc activity in rats produced deficits in behaviors reliant on executive function without impacting development or simple brain function. These results highlight the potential of targeting Sxc to enhance cognition without generating therapeutically limiting adverse effects resulting from non-specific changes in brain function.