CAT-4001 improves mitochondrial function in a Friedreich’s ataxia model

John F. Reilly1, Giuseppe Yañez2, Pradeep Bista1, Dominic Picarella1, Diana Lee1, Chi Vu1, Andrew Nichols1, and Jordi Magrané2
1Catabasis Pharmaceuticals, Cambridge, MA, US; 2Brain and Mind Research Institute, Weill Cornell Medical College, New York, NY, US

Abstract

The frataxin deficiency that underlies Friedreich’s ataxia (FA) leads to oxidative stress and decreased mitochondrial function, which may play significant roles in disease pathology. Impaired nuclear translational of frataxin may be the causative factor for oxidative neuronal damage in FA. CAT-4001 is a novel, CNS-penetrant small molecule conjugate of monomethyl fumarate (MMF), which activates Nrf2, and the omega-3 fatty acid docosahexaenoic acid (DHA), which inhibits NF-κB, coupled via a linker designed to be enzymatically cleaved, enabling simultaneous intracellular release of the active components with concomitant synergistic pharmacology.

Degeneration of large sensory neurons is a hallmark of FA, and associated defects can be observed in dorsal root ganglion (DRG) derived neurons from frataxin-deficient mice (KIKO). KIKO DRG neurons show mitochondrial abnormalities in terms of mitochondrial fragmentation and altered bioenergetics. Treatment of DRG neurons from normal (WTWT) or KIKO mice with CAT-4001 increased expression of the Nrf2 target gene, Hmox1, indicating pharmacological modulation of the target pathway. To assess CAT-4001 effects on mitochondrial abnormalities, cultures were treated with CAT-4001 and mitochondrial lengths were measured using fluorescence microscopy. Compared to WTWT neurons, KIKO neurons showed a decreased mitochondria length within their axons, consistent with mitochondrial fragmentation. However, the axonal mitochondria in CAT-4001 treated KIKO neurons were longer, and of comparable lengths to the mitochondria in the WTWT neurons, suggesting that CAT-4001 could prevent mitochondrial fragmentation.

We evaluated mitochondrial respiration in a mouse muscle cell line (C2C12). Treatment with a pro-oxidant stressor leads to markedly decreased oxygen consumption in these cells. CAT-4001 reversed the H2O2-mediated decrease in oxygen consumption in a concentration-dependent manner. Combined with the effects in reversing mitochondrial fragmentation seen in the KIKO neurons, these results suggest that CAT-4001 is likely to improve mitochondrial bioenergetics.

In conclusion, CAT-4001 may represent an effective approach to improve mitochondrial function for the treatment of FA.

The Catabasis SMART Linker™ Platform

Conjugates engineered from a variety of proprietary, enzyme-degradable small chemical linkers (“SMART Linkers”):
- Inactivated bioactives
- Unavailable in gut & circulation
- Cellular uptake by endocytosis
- Driven by linker and/or bioactive components
- Intracellular hydrolysis of linker
- Bioactives “reactivated” upon cleavage
- Freed to interact with intended targets

CAT-4001 Potential in Neuroinflammatory Diseases

Friedreich’s ataxia (FA) – Deglutting life-shortening degenerative neuro-muscular disorder resulting in ataxia, fatigability, vision and other sensory impairment and potential scoliosis, heart disease and diabetes
- Caused by a defect in the frataxin gene; neuroinflammation believed to be involved

Amyotrophic lateral sclerosis (ALS) – Progressive neurodegenerative disease that affects nerve cells in the brain and spinal cord leading to muscle weakness, gradual loss of motor function and eventually, death
- Exact etiology unknown; NF-κB and neuroinflammation believed to be involved

CAT-4001 Activates Nrf2 in FA Patient Cells

Lymphoblast cell lines from FA patients (GM16197 and GM16215, repeat lengths as indicated) or unaffected/heterozygous parent (GM16200 and GM16215) were treated with CAT-4001 for 6 hr, and mRNAs and expression of the Nrf2-regulated gene, Hmox1, was assessed by quantitative RT-PCR, normalized to the expression of the housekeeping gene, 18S.

Data are mean ± SD of duplicate determinations

Summary and Conclusions

- CAT-4001 reduces mitochondrial fragmentation and normalizes mitochondrial length in KIKO DRG neurons
- CAT-4001 decreases ROS production in cerebellar mitochondria isolated from KIKO mice
- CAT-4001 may represent an effective approach to improve mitochondrial function for the treatment of FA

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CAT-4001 Activates Nrf2 in KIKO DRG Neurons

KIKO (knock-in knock-out) mice are engineered to completely remove one allele of the mouse frataxin gene (knock-out) and the second allele replaced with a version containing 230 expansions of GAA as an intron 1 (knock-in)

CAT-4001 reduces mitochondrial fragmentation and normalizes mitochondrial abnormalities when cultured ex vivo

CAT-4001 Normalizes Mitochondrial Length in Cultured KIKO DRG Neurons

Dorsal root ganglion (DRG) neurons were isolated from a mouse model of Friedreich’s Ataxia (KIKO mouse, E13.5) and cultured in vitro for 6 days (DIV8), followed by a single addition of CAT-4001 (at the indicated concentrations) and analyzed 3 days later (DIV11) for mitochondrial lengths in axons

Box plots show median and 25th – 75th percentiles

P-values are calculated using ordinary one-way ANOVA, adjusted for multiple comparisons against KIKO vehicle (top) or WTWT vehicle (bottom) using Dunnett’s test

CAT-4001 Reduces ROS Production in KIKO Mouse Cerebellar Mitochondria

Cerebellar mitochondria from KIKO mice had elevated ROS production, and CAT-4001 treatment reduced the levels of ROS produced during mitochondrial respiration

Mice were fed CAT-4001 in the diet for 8 weeks, and mitochondria from the cerebellum were isolated (at 3 months of age) State 2 respiration was induced (by adding substrates to drive respiratory chain) ROS (H2O2) was measured using Amplex UltraRed fluorescent

CAT-4001 Enhances Mitochondrial Respiration in C2C12 Cells

In C2C12 myoblasts, CAT-4001 treatment enhanced both basal mitochondrial respiration and reserve capacity and counteracted the loss of mitochondrial function during stressed conditions

Responses of C2C12 myoblasts in basal and hypoxic conditions were assessed with the Seahorse XF Cell Mito Stress Test assay

Compounds were added to the cells as indicated at the onset of the experiment, followed by oligomycin to block mitochondrial ATP production, then the unspecific FCFP to stimulate maximal respiration

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