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

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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 translocation of Nrf2 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 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 H_2O_2-mediated decreases 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.

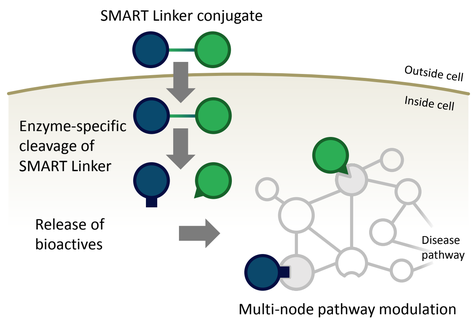
The Catabasis SMART Linker℠ Platform

Conjugates engineered from a variety of proprietary, enzyme-cleavable small chemical linkers ("SMART Linkers")
- Inactivated bioactives
- Uncleavable in gut & circulation

Cellular uptake by endocytosis
- Driven by linker and/or bioactive components

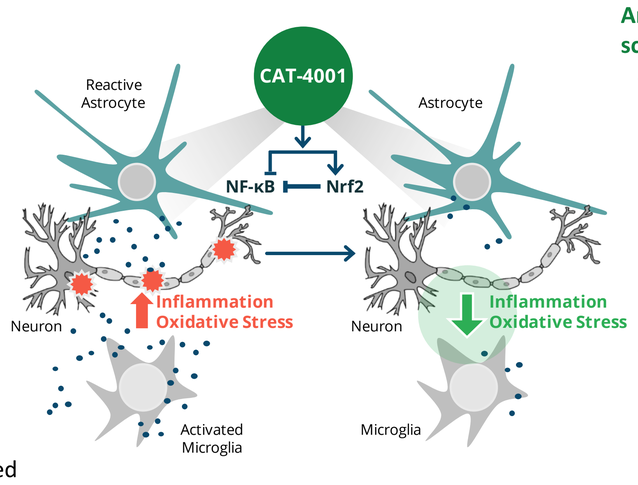
Intracellular hydrolysis of linker

Bioactives "reactivated" upon cleavage
- Freed to interact with intended targets



CAT-4001 Potential in Neuroinflammatory Diseases

Friedreich's ataxia (FA)
- Debilitating life-shortening degenerative neuro-muscular disorder resulting in ataxia, fatigue, 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



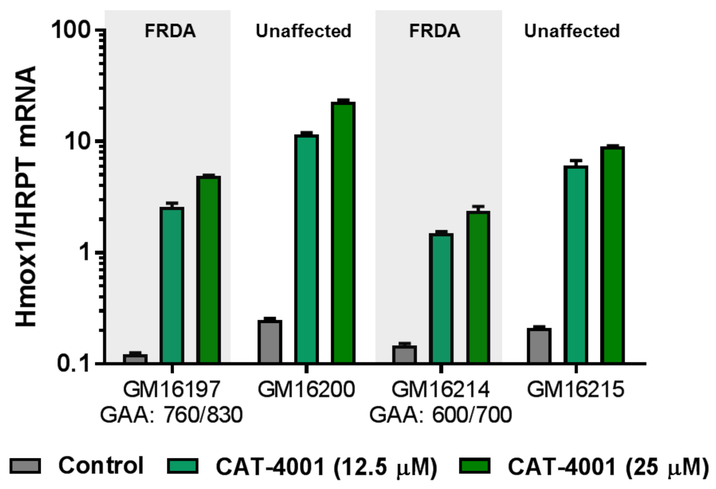
Amyotrophic lateral sclerosis (ALS)
- Progressive neurodegenerative disease that affects nerve cells in the brain and spinal cord leading to muscle weakness, gradual loss of motor function and, eventually, death
- Exact etiology unknown; NF-κB and neuroinflammation believed to be involved

CAT-4001 Activates Nrf2 in FA Patient Cells

Nrf2 activation by CAT-4001 across different GAA-repeat lengths found in FRDA

Lymphoblastoid cell lines from FA patients (GM16197 and GM16214, repeat lengths as indicated) or unaffected/heterozygote parent (GM16200 and GM16215) were treated with CAT-4001 for 6 hr, and mRNA expression of the Nrf2-regulated gene, *Hmox1*, was assessed by quantitative RT-PCR, normalized to expression of the housekeeping gene, *HPRT*

Data are mean ± SD of duplicate determinations

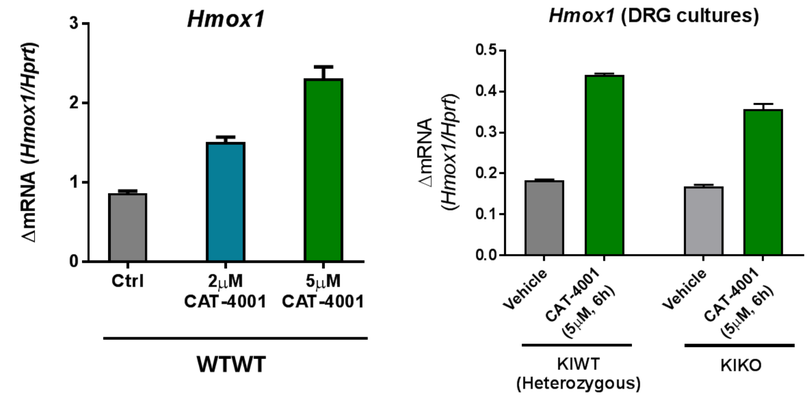


CAT-4001 Activates Nrf2 in KIKO DRG Neurons

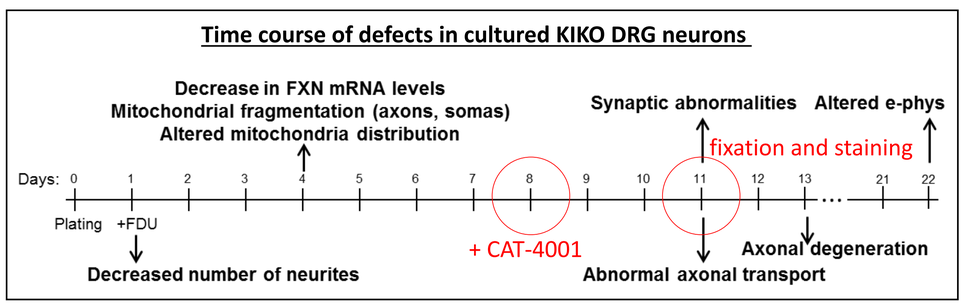
CAT-4001 treatment induces the Nrf2 target gene, *Hmox1*, after 6h of treatment in cultures of DRG neurons from WTWT, KIWT, and KIKO mice

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 on intron 1 (knock-in)

DRG neurons isolated from these mice show mitochondrial abnormalities when cultured *ex vivo*



CAT-4001 Normalizes Mitochondrial Length in Cultured KIKO DRG Neurons

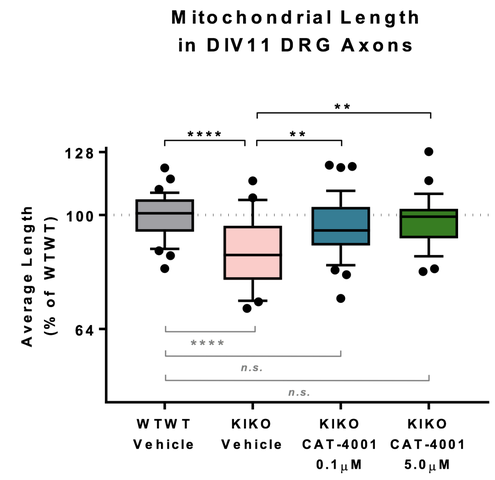


Dorsal root ganglion (DRG) neurons were isolated from a mouse model of Friedreich's Ataxia (KIKO mouse, E13.5) and cultured *in vitro* for 8 days (DIV8), followed by a single addition of CAT-4001 (at the indicated concentrations) and analyzed 3 days later (DIV11) for mitochondrial lengths in axons

Box plots show median and 25th – 75th percentiles

Whiskers are 10th – 90th percentiles with points outside this range represented as dots

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



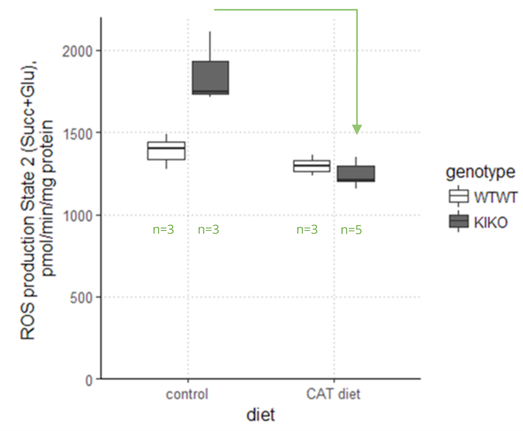
CAT-4001 Reduces ROS Production in KIKO Mouse Cerebellar Mitochondria

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 (H_2O_2) was measured using Amplex UltraRed fluorescence

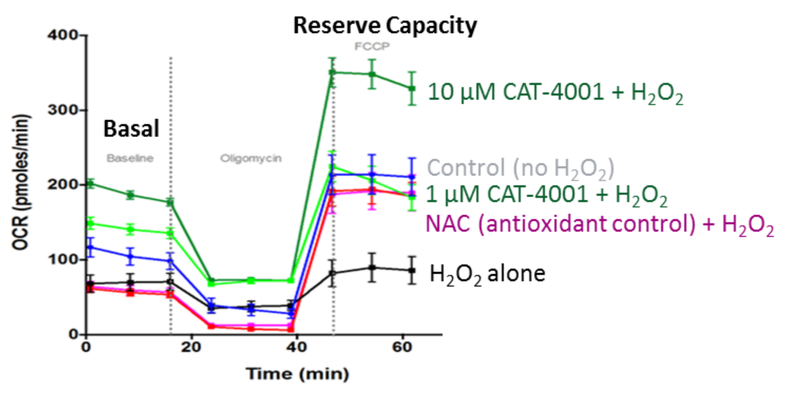


CAT-4001 Enhances Mitochondrial Respiration in C2C12 Cells

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 uncoupler FCCP to stimulate maximal respiration



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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