Pharmocological treatment with HDAC-6 Inhibitor (ACY-738) recovers Alzheimer's phenotype in APP/PS1 mice

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Background: Alzheimer's disease (AD) is a progressive, neurodegenerative disease that is currently the 6th leading cause of death in the United States. Although its pathological hallmarks of amyloid plaques and neurofibrillary tangles have been well characterized, targeted therapies have been met with limited success in clinical trials. Recent studies have suggested that microtubule-based axonal transport deficits can be detected *in* vivo in AD models prior to the onset of either plaques or tangles. In addition, autopsies of patients indicate that a microtubule modulator known as histone de-acetylase 6 (HDAC-6) is significantly increased in post-mortem samples from AD patients. However, commercially available HDAC-6 inhibitors have low specificity and brain bioavailability. We collaborated with Acetylon Pharmaceuticals to obtain a potent, highly selective, and bioavailable HDAC-6 inhibitor (ACY-738) to test *in vivo* in an AD model. *As a result, we hypothesized that treatment with ACY-738 would improve axonal transport, behavior, and AD pathology in the APP/PS1 mouse model.*

Methods: Mice were divided into two groups, early (treated for 21 days, evaluated at 3 months of age) and late treatment groups (treated for 90 days, evaluated at 6 months of age). Evaluations for axonal transport were conducted using the MEMRI protocol as previously described. Contextual fear conditioning and open field tests were used to assess learning and memory as previously described. Protein alterations of tau, tubulin, HDAC6, and were assessed using the Western blot technique. Amyloid pathology was assessed with enzyme-linked immunosorbant assays (ELISA) and immunohistochemistry for plaque visualization and quantification. Finally, the presence of ACY-738 was assessed with HPLC/MS/MS in both plasma and brain tissue of treated mice. All animals were handled in compliance with institutional and national regulations and policies.

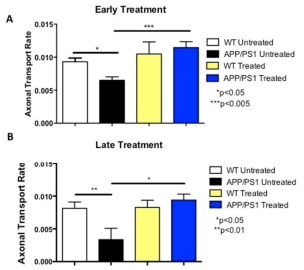

Figure 1: Axonal transport rates are improved in both early and late treatment groups. Quantification of axonal transport rate is displayed for mice treated for (A) 21 days prior to evaluation at 3 months of age for all groups and (B) for mice treated for 60 days prior to evaluation at 6 months of age.

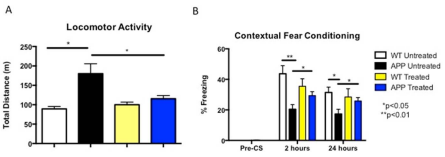

Figure 2: WT and APP/PS1 mice (male and female, 6-7 months old) were treated with normal or ACY-738 chow for 90 days prior to testing using an open field assay for 30 minutes and contextual fear conditioning paradigm. Results are reported for total distance traveled in the open field (A) and as % freeze as an indication of fear-associated memory recall. Graph displays pre-conditioned stimulus (Pre-CS), short-term recall (2 hours) and long-term recall (24 hours) (B). Groups tested include WT untreated (n=20), APP/PS1 untreated (n=13), WT treated (n=13), APP treated (n=15). *p<0.05, **p<0.01.

Imaging Protocol: All images were obtained using a 9.4T, Bruker Avance BioSpec Spectrometer with a 21cm horizontal bore (Bruker BioSpin, Billerica, MA) and a 35mm resonator. Mice were anesthetized using 5% isoflurane with oxygen and placed into the animal holder, where they were kept at 2% isoflurane for the rest of the imaging time. Mice were imaged using a Manganese Enhanced MRI (MEMRI) protocol to obtain T_1-weighted images. Imaging parameters used for MEMRI: TE=8.5 ms, TR=504 ms, FOV=3 cm, matrix size=128x128, taking 32 min, 15 seconds. Paravision 4.0 software (Bruker BioSpin, Billerica, MA). During imaging, body temperature was maintained at 37.0°C using an animal heating system (SA Instruments, Stony Brook, NY). ***Data Analysis:*** Obtained images were analyzed using Paravision 4.0 software. A region of interest (ROI) within the olfactory bulb was selected. T_1-signal intensity (SI) and times within these ROIs were measured and normalized to SI measured from a water phantom. Graphs and statistics from all data were generated using Prism (GraphPad Software, San Diego, CA).

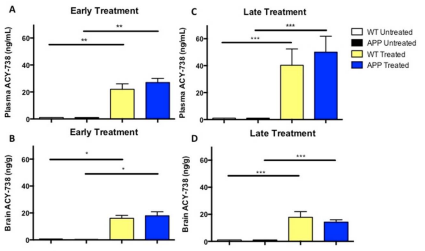

Figure 3: ACY-738 is present in the brain and plasma of treated mice in both early and late groups. Quantification of samples from plasma and brain tissue are displayed (A) for the early treatment group and (B) for the late treatment group.

Results: After treatment with ACY-738, we measured significant improvements in axonal transport rate (Fig. 1), hyperactivity and fear-associated learning and memory (Fig. 2) in APP/PS1 mice. We confirm that ACY-738 is present at therapeutic levels in the brain and the plasma of the treated mice (Fig. 3). In addition, these outcomes were accompanied by normalization of protein levels of acetylated tubulin and hyperphosphorylated tau. Finally, we observe a significant reduction in amyloid plaque pathology following 90 days of treatment with ACY-738 (Fig. 4).

Discussion: We have completed a pre-clinical proof of concept study with a novel, highly potent and selective HDAC-6 inhibitor ACY-738 in the APP/PS1 mouse model of Alzheimer's disease. After 90 days of treatment in chow formulation, we have demonstrated significant improvement in learning and memory, axonal transport, protein alterations, and amyloid pathology. In addition to these outcomes, we have validated MEMRI as a technique used to observe functional neuronal improvements with a compound given via oral administration. Our findings in this study validate and highlight the need for exploring HDAC-6 selective inhibitors for treatment in neurodegenerative diseases with axonal transport modulations in the future.

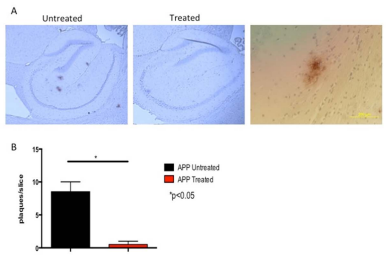

Figure 4: Representative images from APP untreated and APP treated mice from the late treatment group (A). Quantification of plaques is represented as plaque per slice (B).

References: **(1)** Inoue, et al. *Rev. Neurosci.* 2011; 22(6):675-94. **(2)** Majid, et al. *NeuroImage Clin.* 2014 Mar. 31; 4:711-7. **(3)** Jochems, et al. *Neuropsychopharmacology* 2014 Jan; 39(2): 389-400. **(4)** Cook, et al. *Hum. Mol. Genet.* 2014 Jan 1;23 (1): 104-16.