P4-440 DECLINE IN TAU LEVELS FROM ANTISENSE OLIGO TREATMENT: A POTENTIAL CURATIVE MECHANISM FOR ALZHEIMER'S DISEASE

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Background: Direct correlation between the density of hyperphosphorylated tau (tangles) and the extent of cognitive decline in Alzheimer's disease patients qualifies this microtubule-associated protein as an attractive therapeutic target. We propose that reducing total tau production would diminish free tau available for aggregation and potentially inhibit tangle formation. To this end, we investigated whether interference with splicing of tau-encoding gene (MAPT) can decrease tau levels. **Methods:** Morpholino antisense oligos (10 μM) designed to mask splice sites of constitutively spliced MAPT exons and/or the translation start codon, were nucleofected in in vitro models (human neuroblastoma cell lines, SH-SY5Y and IMR-32). In vitro studies were followed by injecting morpholinos in Mapt-/- transgenic mice expressing human tau protein. Exon 1-targeting oligos described above were injected either individually or in combination. **Results:** One of the most striking findings of our investigations was a significant decline in tau protein levels, by 80-90%, after 1-week treatment with antisense oligos masking the start codon and the 5'-splice site to exon 1. Total transcript expression was also reduced, by half of that evident in controls. PCR amplification confirmed that masking splice sites did indeed exclude morpholino-targeted exon from the final MAPT transcript. In vivo experiments: Effective drug delivery past the blood-brain barrier constitutes a major challenge. Therefore, for in vivo studies, we targeted intramuscular tau as a proxy for brain-localized tau. Gastrocnemius muscles were harvested at 1-day, 1-week or 2-weeks post-injection and changes in tau protein quantified by ELISA. Analysis of injected muscles revealed noteworthy decreases in tau levels at all time points, compared to controls. **Conclusions:** Similar outcomes in in vitro and in vivo models reflect the efficacy of splicing interference as a mechanism for decreasing tau production. Tau abnormalities constitute a common link across the broad spectrum of neurodegenerative diseases. Therefore, it is our assertion that tau knockdown can be a potential curative mechanism not only for Alzheimer's disease but also for related tauopathies.

P4-441 THE DIABETES DRUG LIRAGLUTIDE AMELIORATES INSULIN RESISTANCE IN THE HIPPOCAMPAL FORMATION OF ALZHEIMER'S DISEASE (AD) CASES

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Background: Insulin resistance, a risk factor for AD, retards Aß clearance, increases neuritic plaque load, decreases cerebral glucose metabolism, and impairs memory. Using an ex vivo stimulation paradigm, we recently showed that (1) insulin resistance occurs in the hippocampal formation (HF) of AD cases along with elevated basal levels of suppressed (i.e., serine phosphorylated) insulin receptor substrate-1 (IRS-1 pS), and (2) that HF levels of IRS-1 pS are strongly associated with episodic memory deficits. Since glucagon-like peptide 1 (GLP-1) analogs enhance insulin receptor (IR) sensitivity and cross the blood-brain barrier, we tested whether one such analogue, the FDA approved diabetes drug liraglutide, can alleviate brain insulin resistance. **Methods:** We adapted the method of Wang et al. (J. Neurosci. 29: 10961, 2009) to test ex vivo responses of the HF to 1 nM insulin in 8 pairs of AD and control cases matched for sex, age, and post-mortem intervals (PMI, mean = 6.7 h in AD, 9.2 h in controls). HF tissue incubated in 100 nM liraglutide for 30 min was tested for liraglutide-induced GLP-1 receptor activation assayed by cAMP production and GTPγS binding, insulin-induced IR activation assayed by IR pY1150/1151 levels, and (3) insulin-induced feedback inhibition assayed by IRS-1 pS616 levels. GLP-1 receptor levels were also studied with quantitative immunohistochemistry on an additional 22 pairs of sex-, age- and PMI-matched AD and control cases. **Results:** GLP-1 receptor density in AD cases was reduced 30.4% in the dentate gyrus inner molecular layer ($p = 0.028$), but not in CA1 pyramidal cells. Liraglutide potently activated these receptors in the HF of controls, but the effect was reduced by about 40% in AD cases ($p = 0.0001$). Compared to untreated AD samples serving as controls, however, liraglutide increased insulin-induced IR activation by 37% ($p = 0.0015$) and restored insulin-induced feedback inhibition of IRS-1 ($p = 0.027$). **Conclusions:** In AD brain tissue, liraglutide significantly reduces insulin resistance and normalizes an aspect of insulin signaling even in the setting of reduced GLP-1 receptor signaling. This may account for the demonstrated ability of liraglutide to reduce neuritic plaque density and memory deficits in AD mouse models.

P4-442 EFFECT OF APOAEQUORIN ON COGNITIVE FUNCTION

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Background: Calcium is important for many neuronal processes and essential for chemical signaling. As neurons age, their ability to regulate intracellular calcium is diminished. Loss of calcium homeostasis is a significant factor in age-associated neurodegeneration and the calcium hypothesis of Alzheimer's disease. Apoaequorin has previously demonstrated the ability to reduce calcium related neuronal cell death in vitro. Qualitative in vivo studies examining the effect of apoaequorin have shown improvements in cognitive functioning and participant reported quality of life. **Methods:** A double-blinded, placebo controlled study of apoaequorin in 222 healthy adults with self-reported memory complaints. Applicants who met the inclusion criteria and were not excluded by predetermined exclusion criteria were given the AD8 Dementia Screening Interview (AD8) and randomized prior to baseline testing. Over a three month study period, participants were tested five times. The primary outcome measures were changes on specific assessments of cognitive function. CogState Research (CogState Ltd.), a computerized cognitive testing system, was used to quantitatively measure changes in cognitive functioning. Statistical analysis was performed using IBM SPSS Statistics version 19 (IBM, Inc.). Analyses were based on a Mixed-Model-Repeated-Measures (MMRM) for changes between baseline (Day-0) and conclusion (Day-90) of the study. Individuals who scored less than two on the AD8 were segregated for analysis. **Results:** The apoaequorin group showed improvements in verbal and visual learning, memory and delayed recall that were not seen in the placebo group. After 90 days, the apoaequorin group showed statistically significant improvement on the International Shopping List (ISL) and the Groton Maze Learning Recall (GMR) compared to placebo. The ISL showed a significant difference between-arms from the baseline to Day-90 testing ($F(1, 50) = 4.33$, $p < .05$). Additionally, on the GMR, a significant difference between-arms and a large effect size from the baseline to Day-90 testing ($F(1, 50) = 4.93$, $p < .05$, and Cohen's $d = .76$] was seen. **Conclusions:** These results show a strong relationship between apoaequorin and improvements on several quantitative measures of cognitive function. Results suggest an important role and potential therapeutic utility for apoaequorin in delaying or modifying the decline in cognitive functioning associated with aging.

P4-443 CELL THERAPY PLUS VOLUNTARY RUNNING RESCUES MEMORY DYSFUNCTION IN A RODENT MODEL OF BRAIN AGEING

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Background: Age-related memory loss is characterized by degeneration and dysfunction of distributed synaptic networks. Both voluntary running (VR) and stem cell therapy have been studied as possible therapeutic strategies, but to date possible synergistic benefits from combining these approaches have not been investigated. Our aim was therefore to test the independent and combined effect of VR and cell therapy on older rats with specific deficits in hippocampally-dependent place recognition