EFFECTS OF T-TYPE CALCIUM CHANNEL MODULATOR AD101 ON THE ACCUMULATION OF BETA AMYLOID, TAU AND POLYUBIQUITINATED PROTEINS IN ANIMAL MODELS OF ALZHEIMER'S DISEASE

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Background:

- AD101 is a first-in-class small molecule with demonstrated positive effects on learning and memory function in animal models for both normal aging and Alzheimer's Disease (AD).
- While AD101 modulates T-type voltage gated calcium channels and thus stimulates the presynaptic release of acetylcholine in hippocampal neurons, further studies investigated the potential role of AD101 in protein processing and accumulation as potential contributors to its clinical effects.

Objectives:

- Summarize the effects of AD101 on Amyloid and Tau aggregation, and modulation of proteasomal and lysosomal protein degradation.

Methods:

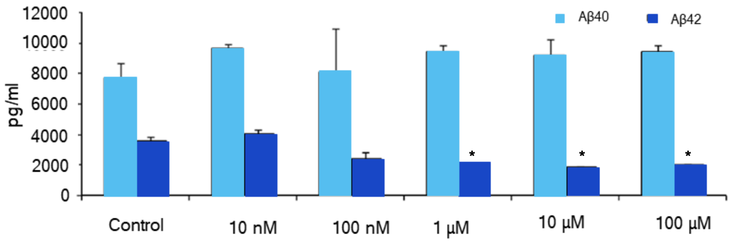
- Review of sponsored and previously unpublished in vitro and in vivo animal studies of AD models conducted as part of a collaboration with UC Irvine, led by Kim Green, Ph.D.
- In vitro studies were performed in Neuro2a neuroblastoma cells and primary neuronal cultures derived from transgenic mice overexpressing APP.
- Animal models used were:
  - **LaFerla triple transgenic mice (3xTgAD)** of varying age (12-24 months). These animals develop essential features of AD in an age-dependent fashion, with deficits in memory-related behavioral function, Aβ-plaque and neurofibrillary tangle pathology and synaptic dysfunction, including deficits in long-term potentiation.
  - **SAMP8 mice,** a mouse strain that, via a spontaneous gene mutation, develops age-related deficits in learning and memory along with accumulation of Aβ-like deposits in brain tissue in an accelerated manner as part of its normal maturation.

Results:

- In vitro studies using ELISA showed that AD101 reduces Aβ 1-42 in Neuro2a neuroblastoma cells (Figure 1) and both Aβ 1-40 and 1-42 in primary neuronal cultures derived from transgenic mice overexpressing APP (not shown).
- Similar results were seen in 3xTgAD mice for both soluble and insoluble Aβ (Figure 2).

Figure 1: AD101 reduces production of $A\beta_{42}$ in primary neuronal cultures (N2a cells).
* $p<0.05$ for $A\beta_{42}$ compared to control

A: $A\beta_{40}$ and $A\beta_{42}$ concentrations in pg/ml after treatment with control or AD101 for 24 hours.



B: ratio of Aβ 1-42 to Aβ1-40 concentration after 24 hours.

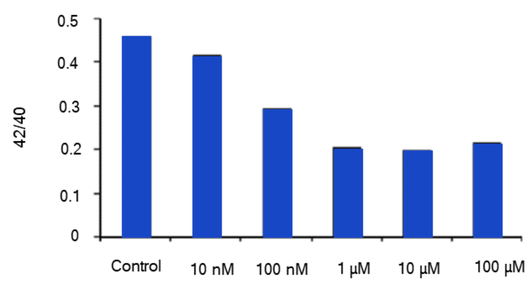


Figure 2: Concentrations of soluble and insoluble beta amyloid fragments 40 and 42 in 3xTG mice. Soluble and insoluble (70% formic acid extracted) Aβ40 and Aβ42 levels were measured from 3xTG-AD whole brain homogenates from animals treated for 2 months with AD101 (n=12) or vehicle (n=12). Significant reductions in soluble Aβ40 and Aβ42 and insoluble Aβ40 were seen between treatments.
* $p<0.05$ compared to control

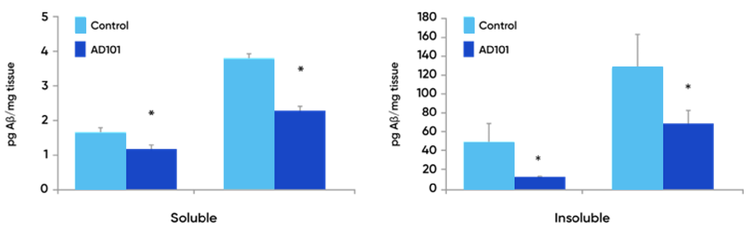


Figure 3: Performance of 3xTG mice on the Morris Water Maze.
Latency (seconds) at 24 and 72 hours after training. N=6 per group.
*$p<0.05$ compared with control mice

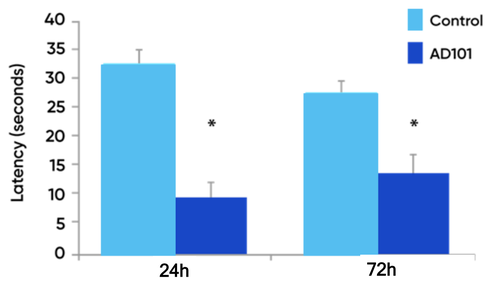
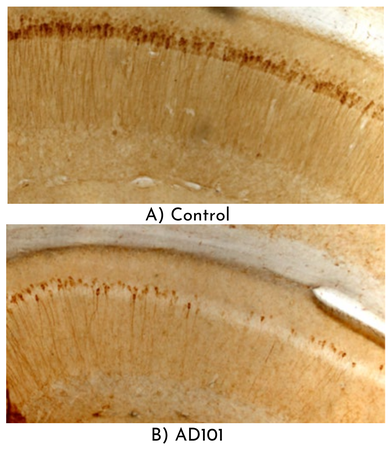


Figure 4: AD 101 effects on distribution and accumulation of somato-dendritic pathological tau in 3xTgAD mice.
Representative results from one animal after 2 months of treatment with 5 mg/kg/d AD101 (B) vs control animal (A). Hippocampal brain section of app. 12-month-old animals. Immunohistochemistry with H7 anti-Tau antibody.



A) Control

B) AD101

Figure 5: Western blot with antibody against ubiquitin (Dako Z0448).
C=control, A=AD101. Control samples show a typical high molecular weight "smear" representing a mixture of different polyubiquitinated proteins.

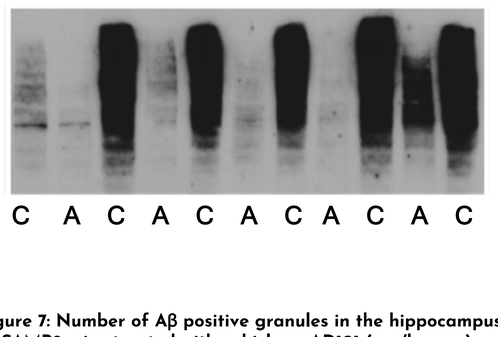


Figure 6: Exploratory Preference of SAMP8 mice in the Novel Object Recognition test after 16 weeks of treatment with AD101 (mg/kg, p.o.)
Numbers in the columns = animals/group. *$p<0.05$ **$p<0.01$

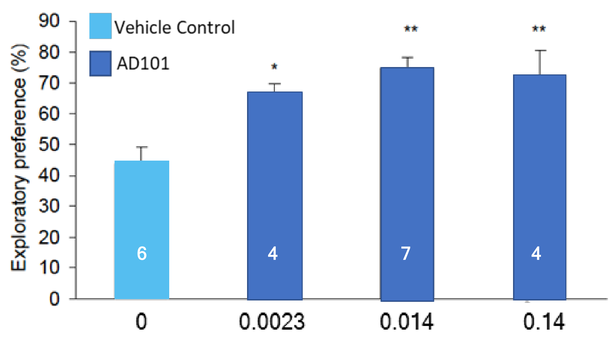
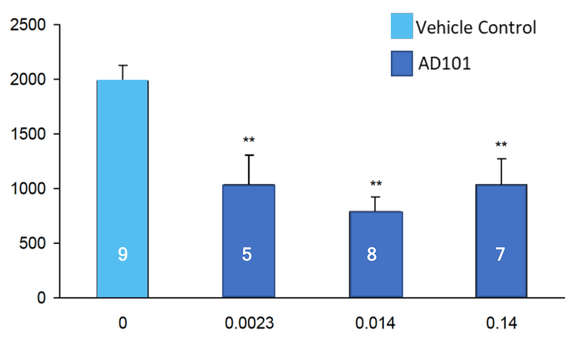


Figure 7: Number of Aβ positive granules in the hippocampus of SAMP8 mice treated with vehicle or AD101 (mg/kg, p.o.)
Numbers in the columns = animals/group. ** $p<0.01$ compared with control



- AD101 significantly decreased latency in the Morris Water Maze Performance after 2 months of AD101 treatment (Figure 3).
- This was accompanied by a reduction of pathological tau staining (Figure 4).
- Changes in the ubiquitin-proteasome pathway were assessed with Western-blot analyses using an anti-ubiquitin antibody: 12-month-old 3xTgAD mice that had been treated with 5/mg/kg/day AD101 over 2 months have shown a consistent and profound reduction of polyubiquitinated protein concentrations (Figure 5).
- While long term treatment with AD101 in younger 3xTgAD mice of 3 months demonstrated similar effects in the 1 mg/kg/day dose group after 10 months of AD101 administration, shorter treatments durations (3 months) and higher doses (5 mg/kg/day) showed negative results.
- Follow-up experiments showed that AD101 reduces ubiquitinated proteins in other models (C57 and SAMP8 mice) as well.
- In 12 months-old SAMP8 mice AD101 attenuated an expected decrease in Exploratory Preference (Figure 6). This was linked to a reduced Aβ-like immunoreactivity after 8 weeks of AD101 treatment (Figure 7).

Discussion:

- These series of studies demonstrate that AD101 can potentially target protein processing and degradation in neuronal cells.
- The reduction of polyubiquitinated proteins could result from increased rates of proteasomal degradation.
- Alongside the known effect of AD101 in increasing cholinergic neurotransmission, enhanced removal of nonfunctional proteins may contribute to the beneficial treatment effects of AD101 in AD.

References:

Green KN, Khashwji H, Estrada T, Laferla FM. ST101 induces a novel 17 kDa APP cleavage that precludes Aβ generation in vivo. Ann Neurol. 2011;69(5):831-44.

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