Oligomeric Aβ-induced neuronal toxicity is attenuated by ADDL-specific antibodies *in vivo*

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Questions and Aims

Alzheimer's disease (AD) is characterized by aggregation of amyloid-β (Aβ) and tau protein along with progressive dementia. Soluble Aβ oligomers (sAβo) / A β-derived diffusible ligands (ADDLs) have been shown to be toxic for neurons, and they are believed to be key factors in synaptic degeneration and memory loss in AD. We sought to investigate the molecular mechanisms that couple ADDLs to dysfunction of neuronal networks.

In this study, we applied an ADDL-specific antibody ACU-3B3, the murine precursor of humanized antibody ACU-193, to examine several questions:

1) Where do ADDLs bind in mouse and human brain tissues?

2) How do ADDLs affect intracellular calcium homeostasis?

3) Is ACU-3B3 capable of restoring ADDL-induced calcium dysregulation both *in vitro* and *in vivo*?

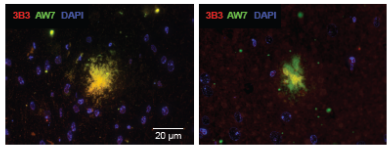
We first used array tomography, a technique that allows precise quantification of synapses based on ultrathin tissue sectioning and immunohistochemistry, to quantify the reductions of synaptic density in the presence of ADDLs in transgenic mice and human AD tissues.

In primary neuronal cultures, we tested if application of synthetic ADDLs lead to an increase in intracellular Ca^{2+} that can be prevented with immunodepletion using ADDL selective antibody ACU-3B3.

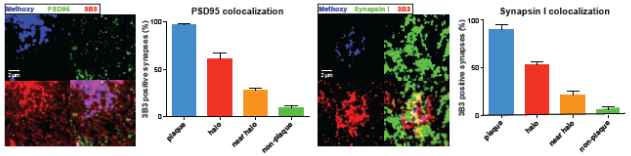
Furthermore, we used *in vivo* Ca^{2+} imaging in wildtype mice to interogate how ADDLs induce elevation in intracellular Ca^{2+} and whether the acute treatment with ACU-3B3 could inhibit ADDL-mediated responses in living animals.

i) Use array tomography to examine where 3B3 binds in mouse and human brain tissue.

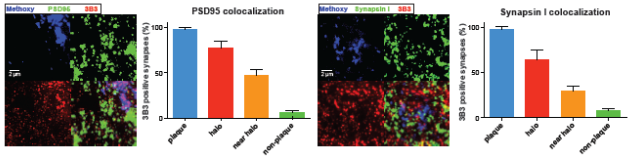
Type	Case#	Age	Notes
APP/PS1 mouse	6058-bcx1	8.5-month	Female; barrel cortex embedded
APP/PS1 mouse	6095-bcx1	8.5-month	Female; barrel cortex embedded
APP/PS1 mouse	9289-bcx1	15.4-month	Female; barrel cortex embedded
AD Human	1446-temp3	84 year-old	Male; B&B V/VI; probable by CERAD; ApoE 3/4
AD Human	1442-temp4	80 year-old	Female; B&B VI/VI; probable by CERAD; ApoE 4/4
AD Human	1418-temp9	84 year-old	Female; B&B VI/VI; definite by CERAD; ApoE 4/4



APP-PS1 mouse tissue

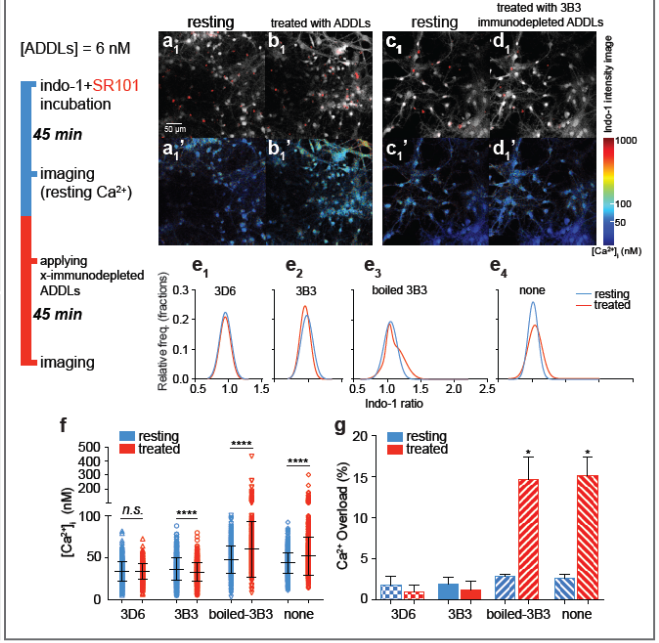


AD human tissue

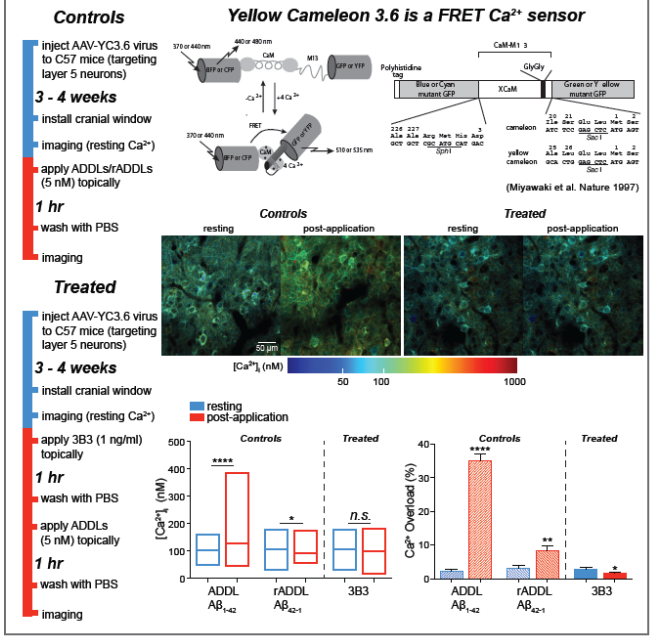


"plaque" refers to the dense core of the methoxy-XO4 labeling; "halo" is the areas surrounding the core; "near plaque" indicates areas extending out from the edge of the halo but within 10 μm; "non-plaque" areas are > 50 μm from the halo edge.

ii) Treat primary neuronal cultures with ADDLs or antibody immunodepleted ADDLs and use intracellular Ca^{2+} in neurons as readout.



iii) Treat wild-type mice with ADDLs topically applied to the brain or pretreat the animals with 3B3 before applying ADDLs and use intracellular Ca^{2+} in neurons as readout.



Conclusion

a) The array tomography data indicate that ADDLs bind to both pre- and postsynaptic areas in human and mouse brain and likely contribute to the decrease in synapses as neurotoxic species.

b) The neuronal culture experiments demonstrate that ADDLs acutely increase intracellular calcium concentration and lead to calcium overloads in neurons which contributes to neurotoxicity and aberrant signaling. This ADDL-mediated response can be prevented by immunodepletion with 3B3 antibody.

c) The *in vivo* calcium imaging results confirm the findings in primary neuronal cultures and show that 3B3 selectively inhibits the toxicity of ADDLs, and is able to prevent neuronal calcium elevation in live animals as well as preserve the calcium homeostasis.

Acknowledgements

Supported by Acumen Pharmaceuticals, Inc. and NIH grant EB000768.