Elucidating the mechanism of the protective effects of the botanical extract DA-9803 in Alzheimer's disease models

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Background

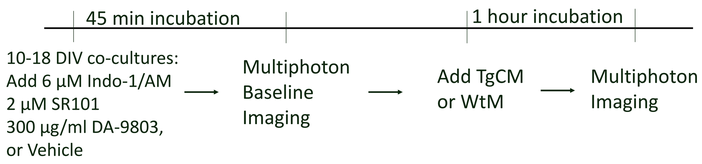
- Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by the existence of amyloid plaques, neurofibrillary tangles, and neuronal loss, currently without a cure.
- DA-9803* is a multimodal botanical extract that suppresses amyloid beta (Aβ) aggregation, blocks hyper-phosphorylation of tau, inhibits acetylcholinesterase activity and could have additional mechanisms of action.
- Two-month treatment of 6 month old APP/PS1 mice with DA-9803 halted plaque deposition and decreased the number of neurons with elevated intracellular calcium levels (calcium overload)[1].
- Intracellular calcium can be measured in primary neuron and astrocyte co-cultures using the ratiometric dye Indo-1. Elevated resting calcium levels (calcium overload) were present in a subset of cells, ~ 13% of neurons 12-14 days in-vitro (DIV) and ~20% of neurons 21 DIV, indicating aberrant calcium homeostasis[2].
- Amelioration of calcium overload can be used as a functional indicator of drug efficacy.

Question

How does DA-9803 prevent the Aβ oligomer mediated increase in intracellular calcium?

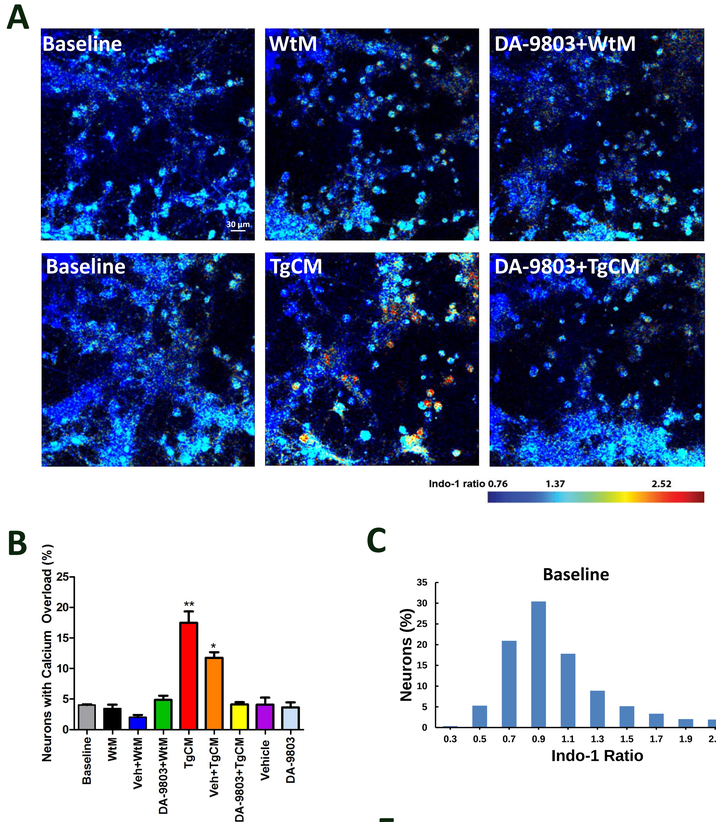
Methods

- Primary astrocyte and neuron co-cultures were prepared from E13-E16 CD1 wildtype embryo cortices, dissociated using a papain dissociation system. The cells were maintained in neurobasal medium with 2% B27 supplement, 2 mM Glutamax, 100 U/mL penicillin, and 100 g/mL streptomycin at 37 °C with 5% CO_2.
- The ratiometric calcium dye Indo-1 was used in 10-18 days in vitro (DIV) cultures to image cytosolic calcium. SR101 was added to specifically identify astrocytes. DA-9803 (300 µg/ml in HPMC) or vehicle (HPMC) alone was added to the cultures for 45 minutes.
- Aβ oligomers (transgenic conditioned media, TgCM, or wildtype media, WtM[3]) were added to the cultures for 1 hour.
- Cells were imaged before and after TgCM treatment on an inverted Zeiss LSM 510 multiphoton confocal live imaging system using a 25x water immersion objective, NA=0.8.
- Indo-1 was imaged using multi-photon microscopy. It was excited with 750 nm laser, using simultaneous non-descanned detectors at 390-465 nm and 480-522 nm. SR101 was imaged using 543 nm excitation with a 565-615 IR emission filter.
- Neurons were positive for Indo-1, not SR101. Astrocytes were positive for both Indo-1 and SR101.



Results

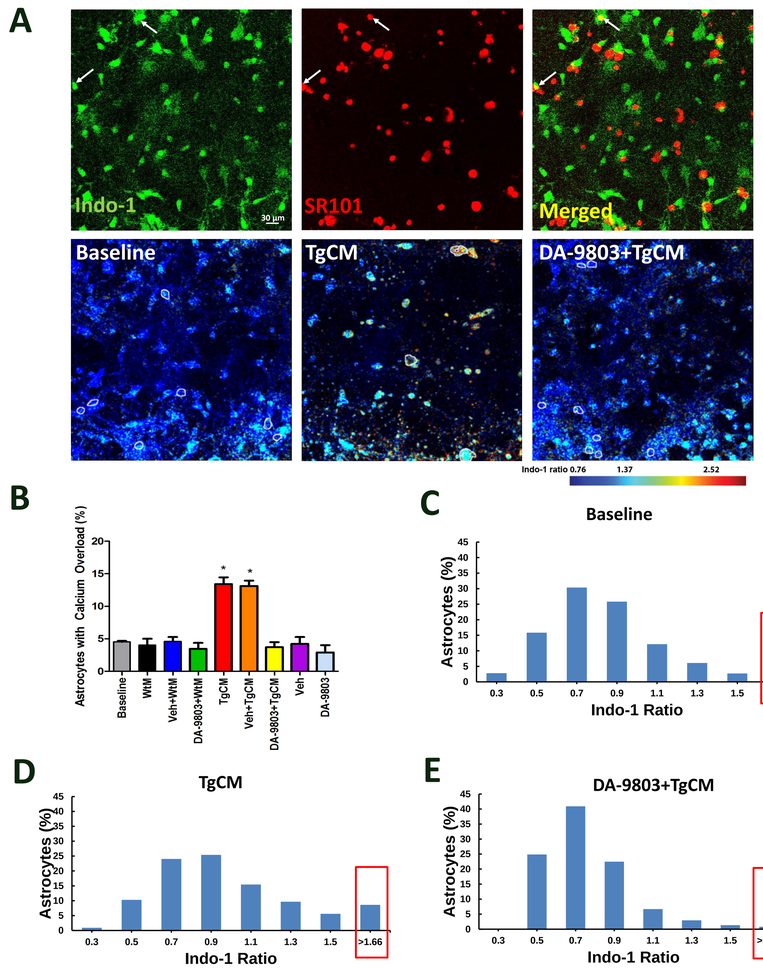
1. DA-9803 prevents Aβ mediated calcium overload in cultured neurons:



DA-9803 reduces TgCM-dependent calcium overload in cortical neurons.

A: Multiphoton microscopy images of neuron-astrocyte co-cultures pseudocolored according to intracellular calcium concentrations. B: Percentages of neurons that exhibit calcium overload (defined as a threshold of two standard deviations above the mean in baseline conditions) in each condition. N=6-56 wells, 1,804-19,854 cells/well. Mean±SEM. Kruskal-Wallis Test p<0.0001, and Dunn's Multiple Comparison Test *p<0.05 **p<0.01. C-E: Histogram distributions of neuronal calcium concentrations at baseline (C), after TgCM application (D), and DA-9803 application with TgCM (E). Neuron percentages with calcium overload are boxed in red. WtM, wildtype media; Veh, vehicle; TgCM, transgenic conditioned media.

2. DA-9803 prevents Aβ mediated calcium overload in cultured astrocytes:



DA-9803 reduces TgCM-dependent calcium overload in cortical astrocytes.

A: Multiphoton microscopy images of neuron-astrocyte co-cultures pseudocolored according to intracellular calcium concentrations. Astrocytes are outlined. B: Percentages of astrocytes that exhibit calcium overload in each condition. N=6-56 wells 374-5,964 cells. Mean±SEM. Kruskal-Wallis Test p<0.0001, and Dunn's Multiple Comparison Test *p<0.05. C-E: Histogram distributions of astrocytic calcium concentrations at baseline (C), after TgCM application (D), and DA-9803 application with TgCM (E). Astrocyte percentages with calcium overload are boxed in red.

Conclusions

- Soluble Aβ oligomers increase calcium overload in primary neurons and astrocytes.
- DA-9803 prevents Aβ dependent calcium overload in neurons and astrocytes.
- Due to DA-9803's strong, preventative effects it has great promise as a potential therapeutic for AD.

1. Pagnier G., Kastanenka K., et al.(2018). "Novel botanical drug DA-9803 prevents deficits in Alzheimer's mouse models." *Alzheimer's Research & Therapy*, 10:11.
2. Wang X,. Kastanenka K., et al.(2018). "An acute functional screen identifies an effective antibody targeting amyloid-β oligomers based on calcium imaging." *Scientific Reports, 8(1):4634.*
3. Wu, H. Y., et al.(2010). Amyloid beta induces the morphological neurodegenerative triad of spine loss, dendritic simplification, and neuritic dystrophies through calcineurin activation. *The Journal of neuroscience*, *30*(7), 2636-49.

***DA-9803 is now being developed as NB-02 by NeuroBo Pharmaceuticals Inc.**

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