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 presence 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).
• Intraplantar 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.
• 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% CO₂.
• 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) were added to the cultures for 3 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 490-465 nm and 480-522 nm. SR101 was imaged using 543 nm excitation with a 565 nm emission filter.
• Neurons were positive for Indo-1, but not SR101. Astrocytes were positive for both Indo-1 and SR101.

Results

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

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

   A. Multiphoton microscopy images of neuron-astrocyte co-cultures pseudocolored according to intracellular calcium concentrations. B: Percentages of astrocytes that exhibit calcium overload in each condition. N=6-56 wells 374-9,964 cells. Mean±SEM. Kruskal-Wallis Test p<0.0001, and Dunn’s Multiple Comparison Test *p<0.05, **p<0.01. C: 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.

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

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