BRAIN BETA-AMYLOID 42 IN MICE TREATED ORALLY WITH POSIPHEN TARTRATE IS SIGNIFICANTLY LOWER THAN IN VEHICLE CONTROLS

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Abstract

We have previously shown that (-)-phenserine tartrate (PT), which improves cognition in rodents and humans due to cholinesterase-inhibitory action, lowered amyloid beta-precursor protein (APP) and beta-amyloid (Aβ) levels by a ChE-independent mechanism. Posiphen™ [(+)-phenserine tartrate] is the positive enantiomer of PT, and has been shown to lower APP levels in human neuroblastoma cells (PNAS 98:7605-10, 2001). The current study examined the effect of orally administered Posiphen™ on beta-amyloid 42 (Aβ42) levels in mouse brain.

Male C57BL/6 mice were treated orally either for 7 or 21 days with different doses of Posiphen™ (0 [vehicle] through 75 mg/kg/day). Plasma and brain samples were collected approximately 2 hours after the last dose on the scheduled collection day. Brain extracts were analyzed for Aβ42 using a sandwich ELISA. Plasma Posiphen™ concentrations were determined by LC/MS/MS. On day 7, the mean brain Aβ42 levels in the 50 and 75 mg/kg Posiphen™ groups were significantly lower than in the vehicle control, by 34.7% and 44.6%, respectively. On day 21, the mean brain Aβ42 levels in the 25, 50, and 75 mg/kg. Posiphen™ groups were significantly lower than in the vehicle control by 52.3%, 56.9%, and 60.2%, respectively. Mean Posiphen™ plasma concentrations at the time of sample collection were lower than Posiphen™ plasma concentration in a recent clinical pharmacology study of Posiphen™ in healthy men and women, suggesting that the therapeutic ratio may also be favorable in humans.

Introduction

- Alzheimer's disease (AD) is characterized by synaptic loss and amyloid-β peptide (Aβ) protein deposition[1,2,3]
- Phenserine [(-)–phenserine tartrate] is a physostigmine analogue and acetylcholinesterase (AChE) inhibitor that lowers amyloid beta-precursor protein (APP) and Aβ levels in cell culture and animal models[4-6]
- Posiphen™ [(+)–phenserine tartrate] (**Figure 1**) is the positive enantiomer of phenserine and is significantly less potent at inhibiting acetylcholinesterase activity than (-)-phenserine,[5,6] but has been shown to be approximately equal in potency to (-)-phenserine at lowering the levels of beta-amyloid-related molecules in neuronal cell cultures[4]

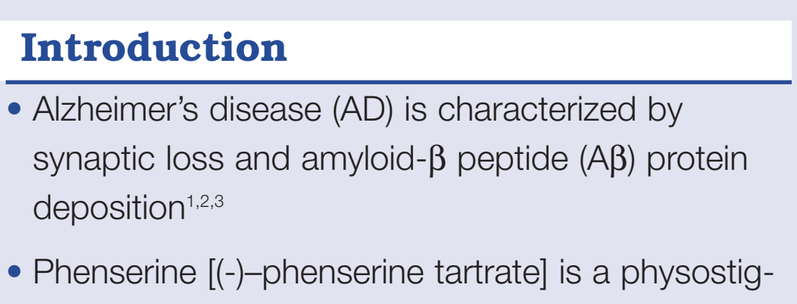

Figure 1. Posiphen™ [(+)–phenserine tartrate]

Objective

- To investigate the effects of orally administered Posiphen™ on brain levels of Aβ42 in an animal model

Materials & Methods

Animals

- 55 adult male mice (C57Blk/6nHsd) weighing 20.2 to 24.8 g

Interventions

- Posiphen™ (25 mg/kg, 50 mg/kg, or 75 mg/kg in reverse-osmosis water) or vehicle (reverse-osmosis water) administered orally (via ball-tipped gavage needle) once daily for 7 or 21 days

Assays

- Plasma and brain samples were collected approximately 2 hours after the last dose on the scheduled collection day (Day 7 or Day 21)
  - Blood samples were collected via cardiac puncture; blood was placed on wet ice, in a chilled Kryorack, or stored at approximately 5°C until centrifuged to obtain plasma and cellular fraction
  - Brains were collected and separated into right and left cerebral hemispheres and cerebellum; brain tissues were rinsed with water, blotted dry, and stored on dry ice
  - Plasma and brain samples were stored at approximately -70°C prior to analysis
- Plasma Posiphen™ concentrations were determined by LC/MS/MS
- Cerebral hemispheres were homogenized and cell lysates were prepared according to manufacturer's instructions for enzymatic assay kits (R&D Systems, Inc, Minneapolis, MN)
- The protein concentration in each sample was estimated according to biuret–derived assay
- Brain extracts were analyzed for Aβ42 using a sandwich ELISA
  - Sensitive sandwich ELISAs were performed using IBL kits to quantitatively assay Aβ42 levels as previously described[7]; results were adjusted to the experimentally determined concentration of protein in each brain tissue extract

Statistical analysis

- Results were analyzed by One-Way Analysis of Variance (ANOVA) using GraphPad Prism Version 4.0 (GraphPad Software, Inc., San Diego, CA). The significance of difference from concurrent control was determined by Dunnett's test

Results

Day 7

- Mean (SD) brain Aβ42 levels were 124.0 (39.4) pg/mg protein, 81.0 (17.1) pg/mg protein and 68.7 (12.3) pg/mg protein in the vehicle, Posiphen™ 50 mg/kg/day, and Posiphen™ 75 mg/kg/day groups, respectively (**Figure 2**). Differences from control were significant in both Posiphen™-treated groups ($p \leq .05$)
- Relative to vehicle control, Posiphen™ 50 mg/kg/day and 75 mg/kg/day reduced mean brain Aβ42 levels by 34.7% and 44.6%, respectively

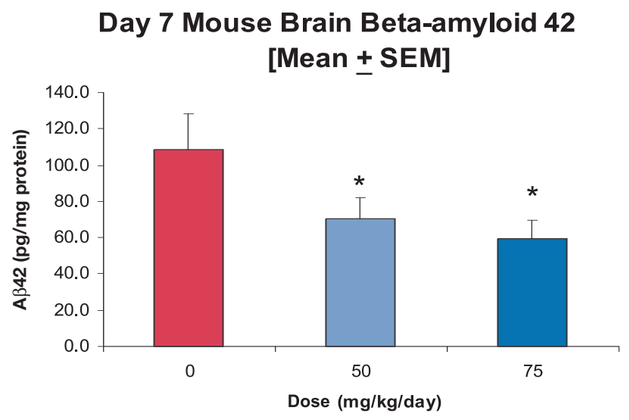

Figure 2. Mouse brain Aβ42 after 7 days of Posiphen™ tartrate administration

*$p \leq .05$ relative to vehicle control (0 mg/kg/day; Dunnett's test)

Day 21

- Mean (SD) brain Aβ42 levels were 190.2 (74.2) pg/mg protein, 90.6 (20.0) pg/mg protein, 82.0 (22.0) pg/mg protein and 75.6 (17.2) pg/mg protein in the vehicle, Posiphen™ 25 mg/kg/day, Posiphen™ 50 mg/kg/day, and Posiphen™ 75 mg/kg/day groups, respectively (**Figure 3**). Differences from control were significant in all three Posiphen™-treated groups ($p \leq .01$)
- Relative to vehicle control, Posiphen™ 25 mg/kg/day, 50 mg/kg/day, and 75 mg/kg/day reduced mean brain Aβ42 levels by 52.3%, 56.9%, and 60.2%, respectively
- Mean Posiphen™ plasma concentrations at the time of brain sample collection were lower than maximum Posiphen™ plasma concentration in a recent clinical pharmacology study of Posiphen™ in healthy men and women (**Figure 4**),[8] suggesting that the therapeutic ratio may also be favorable in humans

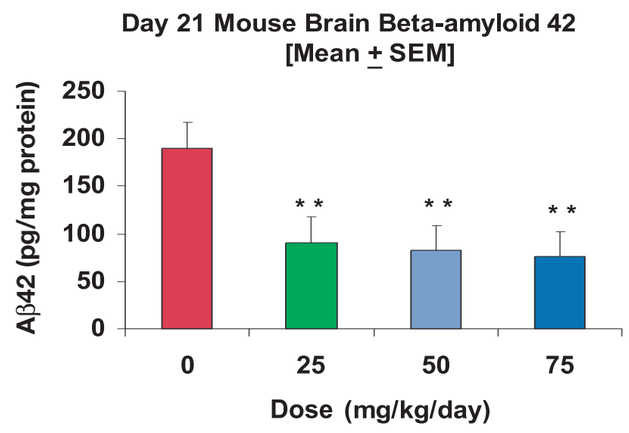

Figure 3. Mouse brain Aβ42 after 21 days of Posiphen™ tartrate administration

**$p \leq .01$ relative to vehicle control (0 mg/kg/day; Dunnett's test)

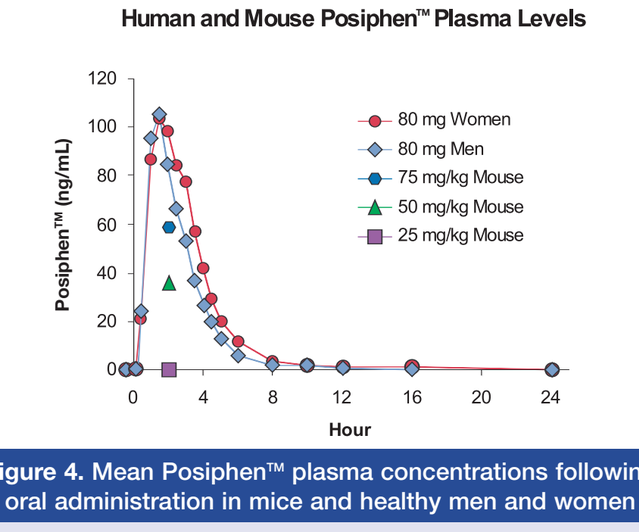

Figure 4. Mean Posiphen™ plasma concentrations following oral administration in mice and healthy men and women

Conclusion

- In mice, orally administered Posiphen™ lowered brain Aβ42 levels at plasma concentrations that are attainable with oral dosing in humans

Acknowledgements

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References

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