Development of Cognitive Clarity™: A Unique Dietary Supplement for Reduction of Brain "Plaques and Tangles" and Enhancement of Memory, Focus and Concentration

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ABSTRACT

β-amyloid (Aβ) and tau are key proteins in the aging and Alzheimer's brain that are linked to memory loss and cognitive decline. There is currently no pharmaceutical drug that has been approved for reducing and removing both Aβ-containing "plaques" and tau protein-containing "tangles" in the brain. Cognitive Clarity™ may be the first nutraceutical product with the potential to reduce both "plaques and tangles", two pathological hallmarks of brain aging, memory loss and cognitive decline. Cognitive Clarity™ is a specific polyphenol-enriched combination of 1) a proprietary extract from the Amazon rain forest woody vine *Uncaria tomentosa* (cat's claw), and 2) a specific Oolong tea extract identified by screening for direct activity against "plaques and tangles". A number of *in vitro* studies including Thioflavin T fluorometry, Congo red binding, Congo red and Thioflavin S staining, circular dichroism spectroscopy, and negative stain electron microscopy, all demonstrated that Cognitive Clarity™ (and its major components) are effective reducers and disaggregators of Aβ and tau protein "plaques and tangles". The studies with a transgenic "amyloid plaque" mouse model demonstrate that the main plant extract ingredients of Cognitive Clarity™ cause a reduction and clearance of Aβ plaques, leading to marked improvement in memory (as assessed by water maze testing and probe trials). A randomized, double-blind, placebo-controlled human trial is currently underway to confirm the effects of Cognitive Clarity™ on improving memory, concentration, and focus in subjects with age-associated memory impairment.

INTRODUCTION

Cognitive Clarity™: An Oral Nutraceutical Agent for the Prevention and Treatment of "Plaques and Tangles" and Cognitive Decline

- Based on over 15-years of scientific research studies to identify a nutraceutical that acts as both potent inhibitor/reducer of both Aβ and tau protein aggregation in brain.
- **Cognitive Clarity™** is a proprietary extract from the Amazon rain forest woody vine *Uncaria tomentosa* (known as PTI-00703®), in combination with a specific Oolong tea extract identified by screening for direct activity against both "plaques" and "tangles".
- **Cognitive Clarity™** is being developed as an oral nutraceutical for memory emhancement to be the world's first nutraceutical product to inhibit and reduce "plaques and tangles" in the aging brain. Prevention and/or reduction of brain "plaques and tangles" is believed to lead to cognitive and memory improvements.

RESULTS

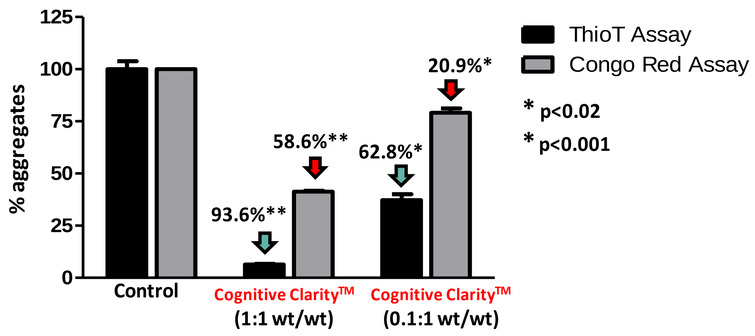


Figure 1: Disaggregation of Aβ1-42 by Cognitive Clarity™ as Measured by Thioflavin T (Thio T) Fluorometry and Congo Red (CR) Binding Assays. Aβ1-42 peptide at 88 μM (rPeptide) was incubated at 37°C in the absence or presence of Cognitive Clarity™ for 72 hrs. The CR binding assay measures large Aβ1-42 aggregates/fibrils retained on a 0.22μm filter, while the Thio T fluorometry assays determines the binding of the Thio T reagent to aggregated Aβ1-42 fibrils. Both assays consistently demonstrated that incubation with Cognitive Clarity™ caused significant (p<0.02-0.001) reductions in pre-formed Aβ1-42 fibrils by up to 62.8, and 93.6% at Cognitive Clarity™: Aβ1-42 (wt/wt) ratios of 0.1:1, and 1:1, respectively. The results indicate that Cognitive Clarity™ is an effective disrupter/reducer of pre-formed Aβ1-42 fibrils.

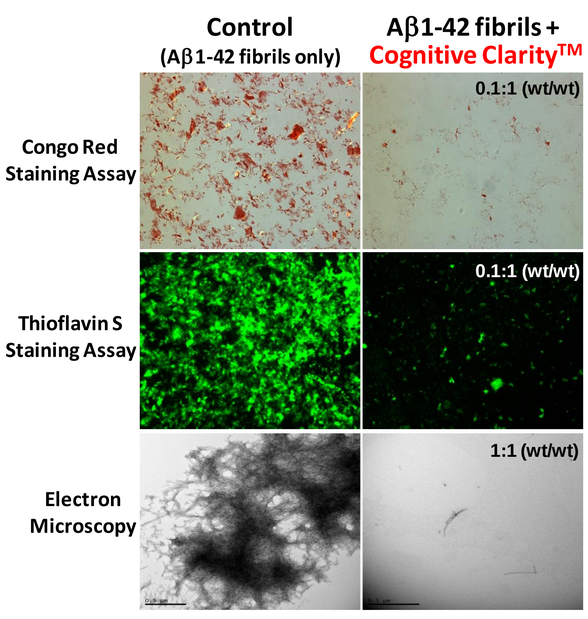


Figure 2: Disaggregation/Reduction of Aβ1-42 Fibrils by Cognitive Clarity™ Confirmed Visually by Congo Red Staining, Thioflavin S Fluorescence, and Electron Microscopy. The reactions were set up as described in Fig. 1. Congo red (under polarized light; top panels), and Thio S fluorescence (middle panels) staining demonstrated marked disaggregation of pre-formed Aβ1-42 fibrils by Cognitive Clarity™ at a ratio of 0.1:1 (wt/wt) with Aβ1-42. Negative stain electron microscopy demonstrated a complete loss of the Aβ1-42 fibril architecture (bottom panels) by Cognitive Clarity™ at a ratio of 1:1 (wt/wt) with Aβ1-42.

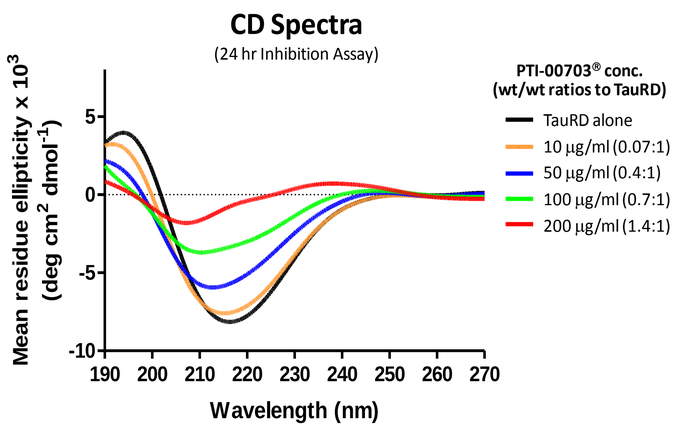


Figure 3: A Major Component of Cognitive Clarity™ (PTI-00703®) Inhibits Tau Protein to Form β-Sheet Fibrils. The recombinant tau repeat domain (TauRD) protein (10 μM) was induced to form β-sheet fibrils by incubation with 10 μM heparin with shaking at 37°C for 24 hrs, in the absence or presence of PTI-00703® (a major component of Cognitive Clarity™). Samples were then collected for CD spectroscopic analysis. The results demonstrated that increasing concentrations of PTI-00703® led to a dose-dependent reduction in formation of tau β-sheet secondary structure, as seen by a reduction and flattening of the signature β-sheet spectrum with negative minima at 218 nm.

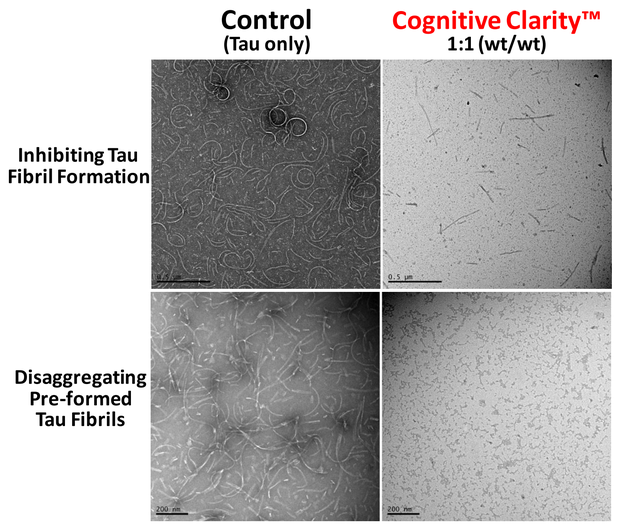


Figure 4: Inhibition of Tau Protein Fibril Formation, and Disaggregation of Pre-Formed Tau Fibrils by Cognitive Clarity™ Confirmed Visually by Electron Microscopy. Inhibition of tau fibril formation experiments was performed as described in Fig 3. The results demonstrated that Cognitive Clarity™ at a 1:1 (wt/wt) ratio markedly inhibited tau protein fibril formation as visualized by negative stain microcopy (top panels). Similarly, when incubated with pre-formed tau fibrils at a 1:1 (wt/wt) ratio for 24 hrs, Cognitive Clarity™ also disaggregated pre-formed tau fibrils (bottom panels). Time-course studies indicated that the disaggregation effect occurred rapidly: it could be seen within 15 minutes after incubation of Cognitive Clarity™ with pre-formed tau fibrils.

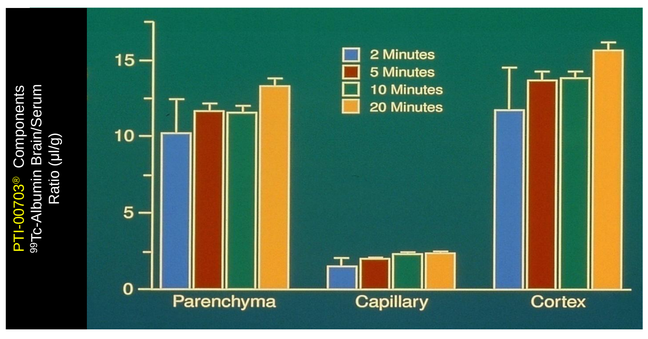
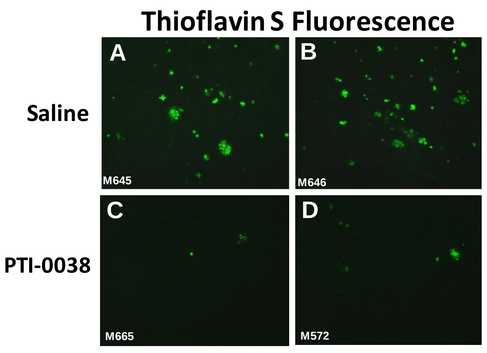
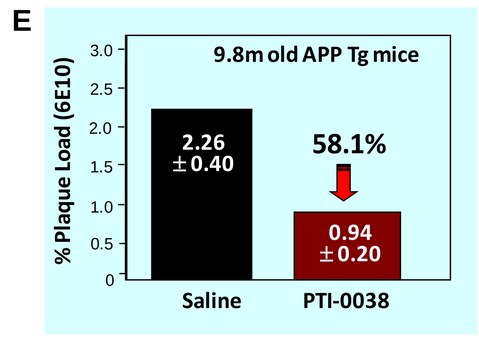


Figure 5: The Major Component of Cognitive Clarity™ Rapidly Crosses the Blood-Brain-Barrier (BBB) and Enters the Brain Parenchyma. ³H-PTI-00703® (a major component of Cognitive Clarity™) was tested in rodents (using the capillary depletion method) for its ability to cross the BBB, entering the brain parenchyma. A proprietary method of ³H-radiolabeling (Moravek Biochemicals) was used to radiolabel PTI-00703®. Radioactive counts in the capillary fraction only represented a negligible amount of total radioactive counts, whereas the majority of the radio-labeled PTI-00703® components were detected in the brain parenchyma. The results suggest that the PTI-00703® components have the ability to cross the blood-brain-barrier, entering the brain parenchyma within minutes after a single *i.v.* injection.

PTI-0038 Causes a Marked Reduction in Brain Amyloid Load

PTI-0038 Causes Marked Improvements in Memory (Spatial Acquisition)





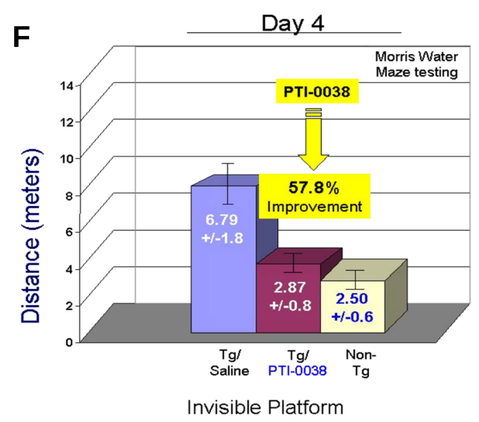


Figure 6: Marked Reduction of Brain Plaque Load and Improved Memory in APP Transgenic Mice Following Administration of the Major Component of Cognitive Clarity™. The major polyohenolic-rich component of Cognitive Clarity™ (known as PTI-0038) was tested in an APP Tg mouse model (TASD-41, bearing London/Swedish mutations). PTI-0038 was administered peripherally at 50 mg/kg/day for 90 days (until mice were 9.8-months old). Brain plaque load was determined by Thioflavin S fluorescence staining **(A-D)**, and anti-Aβ mAb 6E10 immunostaining **(E)**, followed by quantitative imaging analysis. The treatment with PTI-0038 for 3 months led to a significant (p<0.01) 58.1% reduction in amyloid plaque load **(E)**. Short-term memory was also assessed by Morris water maze testing prior to sacrifice **(F)**. The results indicated that PTI-0038 treatment caused a significant 57.8% improvement in short-term memory (i.e. spatial acquisition), close to the level found in non-Tg mice. Similar results were also observed at day 1 of the invisible platform.

CONCLUSIONS

1) **Cognitive Clarity™ is a specific polyphenol-enriched combination of a proprietary extract (known as PTI-00703®) from the Amazon rain forest woody vine, *Uncaria tomentosa* (cat's claw), and a specific Oolong tea extract identified by screening for direct activity against "plaques and tangles".**

2) **Cognitive Clarity ™ is a potent inhibitor of β-amyloid and tau protein aggregate/fibril formation. Extensive studies also demonstrate that Cognitive Clarity™ and/or its major components are effective disrupters/disaggregators of β-amyloid plaques and tau tangles.**

3) **Transgenic "amyloid plaque" mouse model studies demonstrate that the main plant extract ingredients of Cognitive Clarity™ (i.e. PTI-00703® and PTI-0038) cause a mark reduction and/or clearance of brain β-amyloid plaques, leading to improvement in memory.**

4) **A randomized, double-blind, placebo-controlled human trial is currently underway to confirm the effects of Cognitive Clarity™ on improving memory, concentration and focus in subjects with age- associated memory impairment.**

5) **Cognitive Clarity™ is anticipated to represent the world's first nutraceutical oral supplement for memory enhancement by inhibition and reduction of brain "plaques and tangles."**

Funded by ProteoTech Inc.

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