Exebryl-1®: A Novel Small Molecule Drug that Reduces Amyloid Plaque Load and Improves Memory Enters Human Clinical Trials

Alan D. Snow, Joel A. Cummings, Thomas P. Lake, Luke A. Esposito, F. Michael Hudson, Qubai Hu, Judy A. Cam, Jocelyn R. Aker and Steve Runnels

ProteoTech Inc., Kirkland, Washington, USA

Introduction

During the last 8 years, ProteoTech Inc. and its world-renowned investigators of amyloid disease research, have worked on the development of a disease-modifying small molecule therapeutic specifically targeting Aß accumulation and fibrillogenesis for the treatment of Alzheimer's disease. One compound, known as **Exebryl-1®**, was identified from a unique synthetic compound and analog library established at ProteoTech focused on targeting specific amyloid proteins. In early studies molecular modeling and structure-activity-relationship (SAR) studies identified a pharmacophore that led to the design, synthesis and testing of over 70 small molecule analogs for Aß inhibitory activity using *in vitro* and transgenic animal models. Exebryl-1® had impressive effects of Aß fibrillogenesis in a number of different *in vitro* assays and transgenic animal models, and was found to specifically reduce Aß fibril formation, deposition, accumulation and induce clearance (as described below).

In vitro assays including use of Thioflavin T fluorometry, Congo red binding, circular dichroism spectroscopy, x-ray diffraction and negative stain electron microscopy all demonstrated that Exebryl-1® had the ability to halt formation of Aß 1-40 into forming beta-sheet amyloid fibrils, as well as cause a disruption/disaggregation of pre-formed Aß 1-42 fibrils. Circular dichroism spectroscopy demonstrated Exebryl-1®'s ability to cause a marked reduction of the beta-sheet secondary structure of Aß fibrils (believed to be due to reduction of H bonding), while not having a similar effect on other beta-sheet proteins.

Methods and Results

A number of animal studies involving the use of Alzheimer's APP (beta-amyloid precusor protein) over-expressing-transgenic mice demonstrate that Exebryl-1® causes a marked reduction in brain Aß plaque load while causing marked improvements in memory impairments following only after 3 months of peripheral or oral administration.

Double Transgenic (London and Swedish Mutation) Studies

In one APP transgenic mouse model study (using double transgenic mice expressing the London and Swedish mutations; implemented in the laboratory of Dr. Eliezer Masliah, Univ. of California-San Diego), Exebryl-1® following 90 days of daily i.p. injections in older Alzheimer's transgenic mice (i.e. 9.8-months old and 8.58% of their cortex occupied by Aß deposits) demonstrated a significant (p<0.01) 44.9% reduction in Aß load and 40% reduction in plaque number (**Figs. 1 and 3**). A similar marked reduction of Thioflavin-S (**Fig. 2**) and Congo red positive amyloid plaques (not shown) were observed following 90 days of Exebryl-1® treatment. This was accompanied by a 69.5% improvement in spatial learning/memory as determined by Morris water maze testing. (**Fig. 4**).

Tg2576 Transgenic Studies

In another APP transgenic mouse model study (using Tg2576 single mutation transgenic mice), 14-month old transgenic mice received **oral treatment** of Exebryl-1® or vehicle (saline) for 3 or 6 months. Within 3 months, Exebryl-1® oral treatment caused a significant (p<0.01) 46.6% reduction of Aß amyloid load and a significant 39.4% (p<0.05) reduction in amyloid plaque number (per sq mm)(**Fig. 5**). Marked reduction in amyloid plaque load ranging from 30-41% was observed using 3 different methods of image analysis and quantitation including Thioflavin T fluorescence, Congo red staining (as viewed under polarized light) and Aß immunostaining (using 6E10 antibody). Marked reduction of brain soluble Aß 42/40 ratios were also observed suggesting that Exebryl-1® contributes to not only the disruption of Aß 42 fibrils, but also to the brain clearance and/or removal of soluble Aß.

In addition, Exebryl-1® treatment in APP transgenic mice resulted in a marked reduction in astrocytosis (as determined by GFAP immunofluorescence and image analysis) and microgliosis (at later stages as determined by MHC-II immunofluorescence and image analysis), suggesting that Exebryl-1® has anti-inflammatory secondary characteristics, which would be beneficial to brain inflammation which is also known to occur in AD.

Pharmacokinetics

A PK study was implemented at the University of Otago (New Zealand) to determine the PK parameters of Exebryl-1® in Sprague-Dawley rats following oral and i.v. administration. Both an intraveneous (25 mg/kg) and single oral dose (100mg/kg) of ^{3}H-Exebryl-1® demonstrated that Exebryl-1® entered the brain (cortex) rapidly. Within 30 minutes following a single oral dose a cortex to plasma ratio of 1:7.2 was observed (**Fig. 6**). At 4 hours following a single oral dose of Exebryl-1®, brain tissue levels remained elevated, and exceeded that of plasma levels. By 24 hours following a single oral dose of Exebryl-1®, the parent compound was still detectable in the brain. The Exebryl-1® parent compound was identified in brain tissue and plasma by extraction methods, followed by thin layer chromatography, scintillation counting and LC/MS/MS.

Mechanism of Action

Evidence suggests that Exebryl-1® penetrates the blood-brain-barrier within 30 minutes of oral administration, binds to beta-amyloid protein and fibril deposits, and causes a disruption/solubilization of beta-amyloid protein fibrils and oligomers, enhancing the microglia's ability to clear beta-amyloid deposits from brain.

Pre-IND Studies

All pre-IND enabling studies have been completed and Exebryl-1® represents an exciting new small molecule compound anticipated to demonstrate disease-modifying effects for the treatment of Alzheimer's disease and related disorders. ProteoTech Inc. filed an IND application with the FDA in April 2008 and has received clearance to initiate human clinical trials for Exebryl-1® as a new treatment for mild-to-moderate Alzheimer's disease. Human phase 1 clinical trials for Exebryl-1® have been initiated.

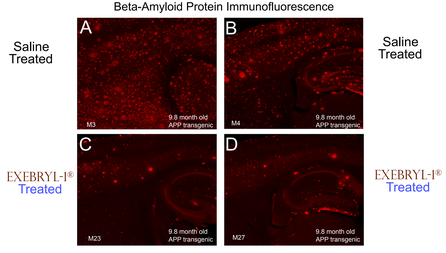

Figure 1: Exebryl-1® Causes a Marked Reduction in Brain Beta-Amyloid Protein Deposits in APP Transgenics (Double Mutation) as Demonstrated by Beta-Amyloid Protein Immunofluorescence.

A & B) Example of 2 APP transgenic mice (London and Swedish mutation) treated with saline for 90 days (from mice 6.5-7.0 months of age at start of treatment) and stained for beta-amyloid protein deposits using 6E10 antibody and immunofluorescence. **C&D)** Examples of 2 APP transgenic mice treated for 90 days (i.p. at 50 mg/kg/day) with Exebryl-1® demonstrating a marked reduction of brain beta-amyloid protein deposits in brain (compare to Figs. A & B). All figs. X100.

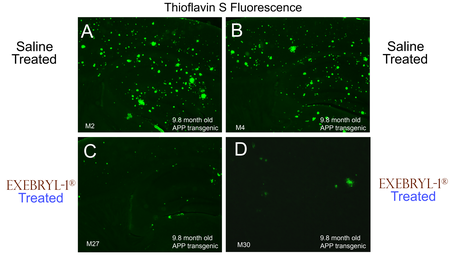

Figure 2: Exebryl-1® Causes a Marked Reduction in Brain Amyloid Plaque Deposits in APP Transgenics (Double Mutation) as Demonstrated by Thioflavin S Fluorescence.

A&B) Examples of 2 APP transgenic mice (London and Swedish mutation) treated with saline for 90 days (from mice 6.5-7.0 months of age at start of treatment) and stained with Thioflavin S to detect fibrillar amyloid deposits. **C & D)** Examples of 2 APP transgenic mice treated for 90 days (i.p. at 50mg/kg/day) with Exebryl-1® demonstrating a marked reduction of brain amyloid plaques (compare to Figs. A & B). All figs. X100.

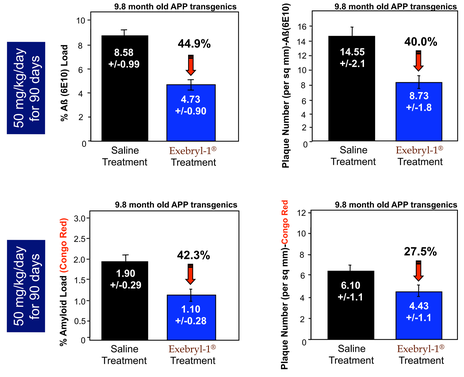

Figure 3: Exebryl-1® Causes a Marked Reduction in Brain Amyloid Load and Plaque Number as Assessed by Quantitative Image Analysis.

Upper Panels: Exebryl-1® treatment (90 days, i.p. injections at 50 mg/kg/day) significantly (p<0.01) reduce brain Aβ (6E10) load by 44.9%, and plaque number by 40% as determined by Aβ immunofluorescence and image analysis.

Lower Panels: Exebryl-1® treatment (90 days, i.p. injections at 50mg/kg/day) significantly (p<0.001) reduces fibrillar brain amyloid plaques by 42.3% and plaque number by 27.5% as determined by Congo red staining and image analysis.

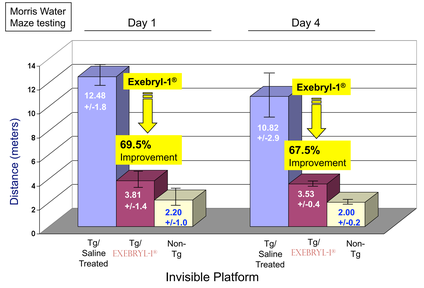

Figure 4: Exebryl-1® Causes a Marked Improvement in Hippocampal Dependent Memory (Spatial Acquisition as Determined by Morris Water Maze Testing.

Following 90 days of treatment with saline or Exebryl-1®, APP transgenic mice and non-transgenic control littermates (Non-Tg) were tested in a Morris water maze to determine effects on hippocampal-dependent memory (i.e. spatial acquisition). Exebryl-1® treatment (Tg/Exebryl-1®) caused marked improvement in hippocampus dependent memory (by 69.5% on day 1 of the invisible platform and by 67.5% on the 4th day of the invisible platform). This was detected by both path length (meters) as shown, and latency (sec)(not shown). No change in swimming speed (m/sec) between all groups was found (not shown). Exebryl-1® treated APP mice had remarkable improvements in spatial acquisition approaching those levels observed in non-transgenic animals (Non-Tg). On the last day of training, all groups easily found the visible platform demonstrating that all animals had no motor abnormalities.

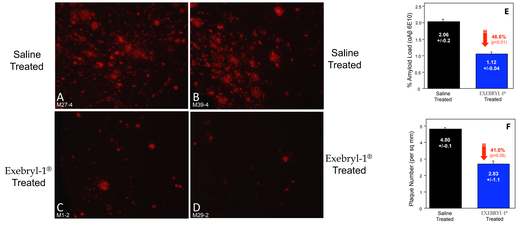

Figure 5: Exebryl-1® Causes a Marked Reduction in Brain Amyloid Load and Plaque Number in APP Tg2576 (Single Mutation) Transgenic Mice Within 3 Months of Oral Administration as Determined by Beta-Amyloid Protein Immunofluorescence and Image Analysis.

14-month old Tg2576 transgenic mice were treated for 90 days with saline or Exebryl-1® (100-150mg/kg/day) following daily oral gavage.

A & B): Examples of 2 APP Tg2576 transgenic mice treated for 90 days with saline and stained for beta-amyloid protein deposits using 6E10 antibody and immunofluorescence. Image analysis and quantitation revealed that 2.06% of the cortex is occupied by beta-amyloid protein deposits with 4.8 plaques per sq mm in saline treated animals (see Figs. E & F, saline treatment). **C & D):** Examples of 2 APP Tg2576 transgenic mice treated for 90 days by oral gavage with Exebryl-1® demonstrated a marked reduction of beta-amyloid deposits in cortex. **E & F)** Image analysis and quantitation revealed that Exebryl-1® for 3 months caused a significant (p<0.01) 46.6% reduction in Aß load, and a significant (p<0.05) 41.0% reduction in plaque number (per sq mm).

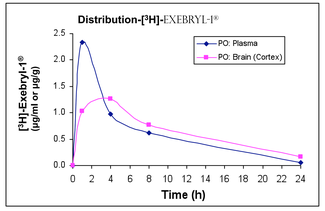

Figure 6: Evidence for Blood-Brain-Barrier Penetration by Exebryl-1® (Parent Compound) Following Oral Adminnistration.

^{3}H-Exebryl-1® (parent compound) was identified in cortex following a single oral dose (100mg/kg) in Sprague-Dawley rats as verified by thin layer chromatography/scintillation counting and LC/MS/MS. Exebryl-1® enters the brain rapidly after oral dosing (after 30 minutes) as shown by the cortex:plasma ratio of 1:2.3 at 1 hour. By 4 hours, Exebryl-1® in brain (cortex) exceeds that in plasma (cortex:plasma ratio of 1.3:1) and remains higher than plasma over 24 hours.

Exebryl-1® for the Treatment of Alzheimer's Disease

- **Exebryl-1® is a Small Molecule Compound**
- **Causes Marked Reduction of Beta-amyloid Protein Deposits in APP Transgenic Mouse Brain Following Oral Administration**
- **Inhibits Beta-amyloid Protein-Proteoglycan Interactions**
- **Potent Anti-inflammatory Agent (Reduces Astrocytosis and Microgliosis)**
- **Causes Improvements in Memory Deficits**
- **I.P. Injections and Oral Administration Show Good Safety Profiles**
- **Brain Penetration within 30 minutes with Peak Levels at ~4 hours after Single Oral Dose**
- **IND Enabling Studies Completed**
- **IND Application Cleared May 2008**
- **Phase I Human Clinical Trials Started July 2008**

Supported by ProteoTech

For Further Information please go to: www.proteotech.com (website updated July 2008) or contact:

Steve Runnels
CEO
ProteoTech Inc.
runnels@proteotech.com

Alan D. Snow, Ph.D.
Chief Scientific Officer
ProteoTech Inc.
snow@proteotech.com