Blood-brain-barrier Penetration of a Novel Disease-Modifying 7-mer Peptide (DP-74) that Reduces Brain Amyloid Load and Improves Memory in a Transgenic Mouse Model of Alzheimer's Disease

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Introduction

Our previous work identified a region within the globular domain of laminin A chain that binds effectively to the beta-amyloid protein (Aβ) of Alzheimer's disease (AD), inhibits Aβ fibril formation, and disrupts pre-formed Aβ fibrils. Screening of over 300 synthetic peptides (12- and 13-mers) derived from globular domain regions of various laminin A chains resulted in the identification of 19 peptides with marked Aβ fibril disrupting activity. Novel 6-9mer peptide analogs were then designed that were derived from their parent 12-13mer peptide sequences, and confirmed to maintain Aβ fibril disrupting ability by a number of different screening assays.

Methods

Transgenic Mouse Model Studies:

In the present study the effects of four of the lead peptides on potential reduction of brain amyloid load and improved memory was assessed in a transgenic mouse model of AD. Groups of 6.0-6.5-month old APP mice (containing the London and Swedish mutation) were injected daily i.p. (at 50mg/kg/day) for 90 days with either saline, or four different novel 6-9mer peptides (as described above). The animal studies were implemented in the laboratory of Dr. Eliezer Masliah at University of California-San Diego.

Kinetics and Blood-Brain-Barrier Penetration of DP-74

A number of studies were implemented to study the kinetics and potential blood-brain-barrier penetration of ^{3}H-DP-74. The influx rate of ^{3}H-DP-74 was determined in anesthetized CD-1 mice at different time points 1-20 mins after i.v. injection of 2μCi of ^{3}H-DP-74. To determine the relative distribution of ^{3}H-DP-74 in brain parenchyma rather than trapped in cerebral vasculature, capillary depletion analysis was performed 10 minutes after an i.v. injection of ^{3}H-DP-74. A series of HPLC analyses were also implemented to determine the stability of ^{3}H-DP-74 in the circulation and whether it was detected in the brain homogenate. The potential of intranasal delivery of ^{3}H-DP-74 was also assessed by analyzing specific brain regions, olfactory bulb, and spinal cord.

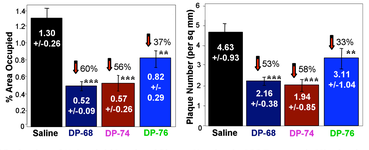

Figure 1: Marked Reduction of % Amyloid Load and Plaque Number in APP Transgenic Mice by the 7mer Peptides DP-74 and DP-68, as Determined by Thioflavin S Fluorescence and Image Analysis.

Thioflavin S fluorescence and image analysis were used to quantify the effects of three 7-8mer peptides (DP-68, DP-74 and DP-76) on brain amyloid load in 9-9.5 month old (at sacrifice) APP transgenic mice following 90 days of daily injection (i.p. at 50mg/kg/day). DP-68 and DP-74 were effective in reducing % amyloid load by 60% and 56%, respectively, and in reducing plaque number by 53% and 58%, respectively. DP-76 was less effective but still reduced % amyloid load by 37% and plaque number by 33%. **p<0.01; ***p<0.001.

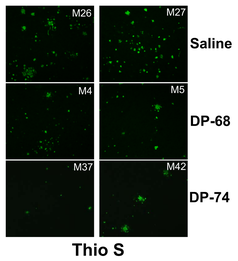

Figure 2: Marked Reduction of Thioflavin S Fluorescent Plaques in APP Transgenic Mice by Treatment with DP-74 and DP-68 Peptides.

Shown are representative Thioflavin S fluorescent images of cortex sections from 9-9.5 month old (at sacrifice) APP transgenic mice treated with either saline (2 different mice referred to as M26, M27), DP-68 (M4, M5) or DP-74 (M37, M42). A marked reduction of Thio S positive plaques is observed with DP-74 and DP-68. All figures are at X100.

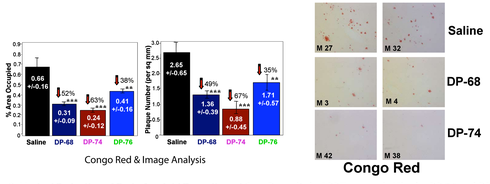

Figure 3: Marked Reduction of Brain Amyloid Deposits and Core Deposits in APP Transgenic Mice by DP-74 and DP-68 as Determined by Congo red Staining and Image Analysis.

A) Congo red staining and image analysis quantified the effects of three 6-7mer peptides on brain amyloid load in 9-9.5 month old (at sacrifice) APP transgenic mice following 90 days of daily injections (i.p. at 50 mg/kg/day). DP-68 and DP-74 were effective in reducing amyloid load by 52% and 63%, respectively, and in reducing plaque number by 49% and 67%, respectively. DP-76 was less effective but still reduced % amyloid load by 38% and plaque number by 35%. ***p<0.01. **B)** Shown are representative Congo red stained images of cortex sections from APP transgenic mice treated with either saline (2 different mice referred to as M27, M32), DP-68 (M3, M4) or DP-74 (M42, M38). A marked reduction of Congo red stained plaques is observed with DP-74, and to a lesser extent DP-68.

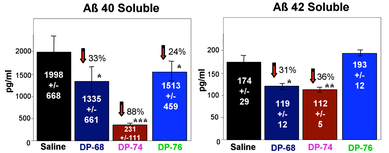

Figure 4: Marked Reduction of Soluble Aβ40 and Aβ42 Levels in Brains of APP Transgenic Mice Following Treatment with DP-74 and (to a Lesser Extent) DP-68.

Biochemical analyses was performed on brain tissue of 9-9.5 month old APP mice (at sacrifice) following 90 days of i.p. injections (50mg/kg/day). This figure shows Aβ40 and Aβ42 soluble levels (i.e. TBS extract) determined by ELISA (Biosource) analysis. DP-74 was extremely effective by significantly reducing Aβ 40 levels by 88% and Aβ 42 levels by 36%. DP-68 was less effective but still significantly reduced Aβ 40 levels by 33% and Aβ 42 levels by 31%. DP-76 was less effective. ***p<0.001; **p<0.01; *p<0.05.

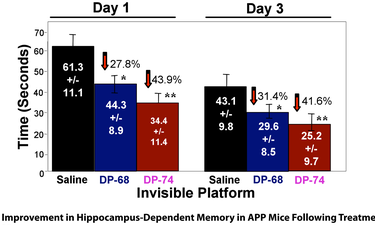

Figure 5: Marked Improvement in Hippocampus-Dependent Memory in APP Mice Following Treatment with DP-74 and DP-68.

Morris water maze was implemented in saline vs. DP-peptide treated APP mice following 90 days of daily i.p. injections (at 50mg/kg/day). Shown is the latency (secs) data for day 1 and day 3 of the invisible platform. Mice treated with DP-74 showed significant improvements in spatial acquisition and retention as demonstrated by a 43.9% (p<0.01) improvement in latency on day 1 of the invisible platform, and a 41.6% (p<0.01) improvement on day 3 of the invisible platform. DP-68 was also somewhat effective, causing a 27.8% (p<0.05) improvement in latency on day 1, and a 31.4% (p<0.05) improvement on day 3 of the invisible platform. **P<0.01; *p<0.05.

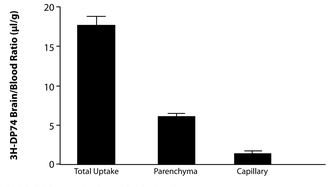

Figure 6: Penetration of ^{3}H-DP-74 Across the Blood-Brain-Barrier

A capillary depletion study was implemented to determine if ^{3}H-DP-74 entered the brain parenchyma. One group of CD-1 mice was sacrificed 10 min. after i.v. injection of ^{3}H-DP-74, whereas another group of animals received intracardial perfusion of 25ml of saline prior to sacrifice (i.e. capillary depletion). The results demonstrate that the apparent uptake (brain radioactivity without vascular perfusion) was composed mainly of parenchymal permeation, by contrast with capillary entrapment. Therefore, DP-74 following i.v. injection crosses the blood-brain-barrier and enters the brain parenchyma, with little radioactivity in capillaries.

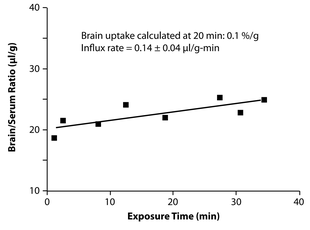

Figure 7: Kinetics of ^{3}H-DP-74 Permeation from Blood to Brain

The influx rate of ^{3}H-DP-74 determined in anesthetized CD-1 mice at different time points (1-20 mins.) after i.v. injection of 2μCi of ^{3}H-DP-74, was 0.14+/-0.04μl/g-min. The initial volume of distribution was 20.2 +/-0.9μl/g. The initial high volume of distribution indicated relatively rapid uptake of ^{3}H-DP-74 from blood to the brain. Mice were anesthetized and ^{3}H-DP-74 was then injected i.v. into the jugular vein at time 0. At different time points (1-30 min. later) mice were sacrificed. Brain/blood ratio of radioactivity was plotted against exposure to obtain the influx rate and the initial volume of distribution.

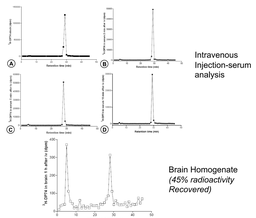

Figure 8: Stability of ^{3}H-DP-74 in Circulation and in Brain

In the next set of studies, the stability of ^{3}H-DP-74 in circulation and in brain homogenates was determined. A series of HPLC analyses determined that ^{3}H-DP-74 was completely stable in circulation following at least the first 30 minutes (**Figs A-D** represent up to 15min analyses), following i.v. injection. At 30 minutes following i.v. injection, intact ^{3}H-DP-74 representing approximately 45% of total radioactivity was recovered in the brain homogenate. This finding demonstrates that DP-74 can cross the BBB in intact form.

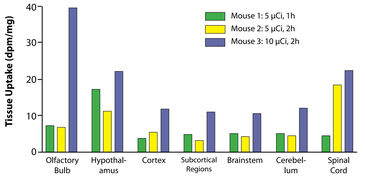

Figure 9: Intranasal Delivery of ^{3}H-DP-74

In a subsequent set of studies, it was determined whether intranasal delivery might also be a preferred route for this peptide. In a first set of experiments, the time course of ^{3}H-DP-74 distribution following intranasal delivery was determined in CD-1 mice. There was found to be a dose- and time-dependent effect. As expected the olfactory bulbs showed the highest effect. The hypothalamus and spinal cord also showed high levels if uptake compared with other brain regions. These studies do suggest that the intranasal route of administration may be effective for this and related peptides.

Conclusions

1. **Two different and novel 7-mer D-amino acid peptides (DP-74 and DP-68) have been designed and tested and found to be potent inhibitors/ disrupters of Aβ deposits and fibrils.**
2. **DP-74, our lead peptide therapeutic, following 3-months of peripheral administration (i.e. daily i.p. injections) in APP transgenic mice cause:**
   - **a 56-63% reduction in brain fibrillar amyloid load (quantified by Thio S and Congo red staining and image analysis),**
   - **a 58-67% reduction in brain plaque number,**
   - **a 60-66% reduction in total Aβ deposits (diffuse and fibrillar deposits),**
   - **an 88% clearance of soluble Aβ40 and a 33% clearance of soluble Aβ42 brain levels,**
   - **improved hippocampus-dependent memory by 41-44% as assessed by Morris water maze testing).**
3. **DP-74 demonstrates brain penetration following i.v. and intranasal administration.**
4. **DP-74 is a novel (new chemical entity) promising peptide therapeutic agent due to its relative stability, permeation across the blood-brain-barrier, and its remarkable efficacy *in vitro* and in APP transgenic animal studies. It is currently being developed as a nasal spray for AD treatment.**
5. **The identification of a novel 7-mer D-amino acid peptide shows promise for the development of a disease-modifying peptide therapeutic for the prevention and treatment of Alzheimer's disease and related disorders.**

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

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