Peptides of Presenilin-1 offer a novel and specific therapeutic approach to reduce β-amyloid in Alzheimer’s Disease

Nazneen N. Dewji, S. J. Singer, Eliezer Masliah, Edward Rockenstein, Mihyun Kim, Martha Harber and Taylor Horwood

University of California, San Diego and Cenna Biosciences Inc., La Jolla, CA
β-amyloid (Aβ) Considered to be the Major Toxic Species in Alzheimer’s Disease

Two major forms: Aβ 40 and Aβ 42

Aβ Accumulates in the Brain in

- Plaques
- Cerebrovascular walls
Cleavage of Aβ from β-APP
Atomic structure of human γ-secretase.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Inheritance</th>
<th>Onset (years)</th>
<th>% FAD</th>
<th>% All</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-APP</td>
<td>21</td>
<td>Autosomal Dominant</td>
<td>45-65</td>
<td>2-3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>PS-1</td>
<td>14</td>
<td>Autosomal Dominant</td>
<td>30-60</td>
<td>70-80</td>
<td>5-10</td>
</tr>
<tr>
<td>PS-2</td>
<td>1</td>
<td>Autosomal Dominant</td>
<td>40-70</td>
<td>~ 20</td>
<td>2-3</td>
</tr>
</tbody>
</table>
Reduction of Aβ 40 and 42 in vitro in the presence of the entire amino-terminal domain of PS-1 fused to FLAG

Dewji NN et al. (2006) PNAS 103:1540-1545
Effect of Peptides P1-P3 on Aβ 40 and Aβ 42 Production in Vitro
Effect of Peptides P4-P10 on Aβ 40 and Aβ 42 Production in Vitro

Aβ 40

Aβ 42
Effects Of Peptides P1 Through P10 On Aβ40 and 42 Production In Vitro.


http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0122451
Peptide Treatment in Transgenic Mouse Model Of Alzheimer’s Disease

- Infusates delivered directly into lateral ventricles by osmotic minipump at a flow rate of 0.25 µl per hour (100 µl total volume) for two weeks

- Mice maintained for an additional two weeks

- Mice sacrificed one month after surgery

6 Month old male and female Bl6/DBA/SW APP transgenic Mice (n=9 per treatment).
Immunohistochemical Analysis of Aβ Deposits in Transgenic Mouse Brain Sections

Immunolabeling with antibodies against Aβ 1-16, followed by FITC-conjugated anti-mouse IgG

Non Tg vehicle  APP Tg vehicle  APP Tg P4  APP Tg P8  APP Tg P9
Immunohistochemical Analysis of Aβ Load in Neocortex and Hippocampus of Treated Mice

N = 9-12 per treatment

** P < 0.005 vs PBS
* P < 0.05 vs PBS
Aβ40 and 42 Levels in Tg Mouse Brains Treated with Peptides P4-P10
Distance between the two reflecting surfaces $= \ell$

Intensity $\lambda = f(\lambda, \ell)$

Monitoring the Interference Pattern on Real Time Provides Kinetic Data on Molecular Interactions
Binding Affinity Measurements Between Soluble APP and Select Peptides by Biolayer Interferometry

Soluble APP purified from conditioned media of sf9 insect cells infected with recombinant baculovirus expressing human APP695

Purified by heparin-affinity, anion-exchange and gel filtration chromatographies.

**Purity:** >95% purity as assessed by densitometry of SDS-PAGE/Coomassie.

Biotinylated peptides immobilized on Strepavidin biosensors
**Assay Conditions For The Interaction Of Each Peptide With APP Or IgG.**

**Instrument:** Octet Red 96

**Affinity measurements in 1x KB**

Typically:
- **Pre-equilibration:** 120s
- **Peptide loading:** Optimized concentration and time
- **Quenching:** 10µg/ml biocytin in KB for 180s
- **Brought to baseline:** 120s
- **Transferred to wells containing serially diluted APP or IgG (1-200nM)**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Loaded Peptide concentration (µg/mL)</th>
<th>APP titration (nM)</th>
<th>IgG titration (nM)</th>
<th>Association time (s)</th>
<th>Dissociation time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1.00</td>
<td>3.13–200</td>
<td>-</td>
<td>120</td>
<td>200</td>
</tr>
<tr>
<td>SP1</td>
<td>1.00</td>
<td>3.13–200</td>
<td>-</td>
<td>120</td>
<td>200</td>
</tr>
<tr>
<td>P4</td>
<td>0.40</td>
<td>3.13–200</td>
<td>3.13–200</td>
<td>360</td>
<td>360</td>
</tr>
<tr>
<td>P5</td>
<td>1.00</td>
<td>3.13–200</td>
<td>3.13–200</td>
<td>360</td>
<td>360</td>
</tr>
<tr>
<td>P7</td>
<td>1.00</td>
<td>3.13–200</td>
<td>3.13–200</td>
<td>360</td>
<td>360</td>
</tr>
<tr>
<td>P8</td>
<td>0.78</td>
<td>1.1–12.5</td>
<td>1.1–12.5</td>
<td>120</td>
<td>200</td>
</tr>
</tbody>
</table>


http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0122451
Binding Kinetics calculated using the ForteBio Data Analysis V7.1 software.

Association ($k_{on}$) and dissociation ($k_{off}$) rate constants were Obtained by fitting the association and dissociation data to a 1:1 Model.

Binding affinity, $K_D$, was calculated as $k_{off}/k_{on}$.
Binding Affinities of Select Peptides

APP Ectodomain

Control IgG

<table>
<thead>
<tr>
<th>Peptide</th>
<th>$K_D$ (M)</th>
<th>$k_{on}$ (M$^{-1}$s$^{-1}$)</th>
<th>$k_{off}$ (s$^{-1}$)</th>
<th>Full $X^2$</th>
<th>Full $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1.43E-08</td>
<td>1.20E+05</td>
<td>1.72E-03</td>
<td>0.004</td>
<td>0.991</td>
</tr>
<tr>
<td>P4</td>
<td>1.61E-08</td>
<td>8.36E+04</td>
<td>1.34E-03</td>
<td>0.065</td>
<td>0.995</td>
</tr>
<tr>
<td>P7</td>
<td>1.01E-08</td>
<td>1.04E+05</td>
<td>1.05E-03</td>
<td>0.041</td>
<td>0.978</td>
</tr>
<tr>
<td>P8</td>
<td>3.45E-09</td>
<td>8.86E+05</td>
<td>3.06E-03</td>
<td>0.537</td>
<td>0.985</td>
</tr>
</tbody>
</table>
Summary of results:

1. Biotinylated peptides P8, P4, P7 and P1 bound to purified APPs in a concentration dependent manner.

2. Binding profile of biotinylated P8 to APPs demonstrated a strong, biologically relevant and specific affinity (KD) of 3.45 nM and a high goodness of fit to a 1:1 binding model.

3. P8 showed the strongest affinity for APPs, almost 5 times stronger than that demonstrated for P4.

4. Specificity of peptide binding to APPs was demonstrated by a lack of significant binding to identical concentrations of mouse IgG, used in these experiments as a control irrelevant protein.
Specific binding of P4 and P8 to Cell-surface APP by Confocal Microscopy
β- and γ-secretase Activities Are Unaffected By Peptides P4 and P8

Analysis of Neocortex of Treated Tg Mice

** P < 0.005 vs PBS
Fig 8. Dose-dependent Effects Of Peptides P4, P8 and Scrambled P1 On NICD Levels And on BACE-1 Activity In Vitro.

http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0122451
Summary

Two small non-overlapping PS-1 Peptides, P4 and P8

Reduce Aβ 40 and 42 levels *In-Vitro* by greater than 50%

Reduce Aβ 40 and Aβ 42 levels in a Transgenic Mouse Model of Alzheimer’s Disease by 40-60%

Show strong, specific binding with the APP ectodomain by biolayer interferometry and confocal microscopy

Do not inhibit or modify the activity of β- or γ-secretase and offer a novel, early and effective approach for the treatment of Alzheimer’s disease
Acknowledgements

S.J. SINGER
ELIEZER MASLIAH
MIHYUN KIM
EDWARD ROCKENSTEIN
MARTHA HARBER
TAYLOR HORWOOD

NIH GRANTS 5RO1 NS055161, 5RO1 NS44768,
5RO1 AG17888, 1R43AG043278 and
Alzheimers Drug Discovery Foundation
TO NND