Microglia Activation in the Alzheimer and Parkinson Brain; Normalization by Posiphen®

WCP June 2012
Microglia Activation by Fibrillar Aβ and α-Synuclein
Activated Microglia in Primary Culture

Primary rat P2-3 microglia/astroglia (2-3 week old)

Primary rat E14 mesencephalic neuron (7 day old)

\( \alpha \)-Synuclein

- Measure Neurotoxicity
- Measure Microglia activation

Wei Zhang et al. FASEB 19, 2005
Size Exclusion Column of Aged aSYN

- α-Synuclein aged for 0 day
- α-Synuclein aged for 7 days
- α-Synuclein aged for 14 days
Activated Microglia in Primary Culture

aSYN induced dopaminergic neurotoxicity in a dose dependent fashion, while having no effect on GABA uptake.

aSYN kills TH-ir neurons in a dose dependent fashion, while having no effect on total neurons.
A. aSYN treatment of neuron cultures enriched with different percentages of astroglia – astroglia is neuroprotective

B. aSYN treatment of neuron cultures enriched with different percentages of microglia – microglia is detrimental

C. aSYN treatment of neuron-glial cocultures compared to microglia depleted cultures – microglia is detrimental
aSYN induced microglia activation

Levels of $\text{O}_2^-$ and iROS were measured 25 min and 2 hrs after aSYN treatment – there is a 300% increase in reactive oxygen species.

Levels of $\text{PGE}_2$ were measured 24 hours after aSYN treatment – there is a 250% increase in prostaglandin $\text{E}_2$.
Fibrillar Aβ Stimulates Growth of Microglia

Microglia cultures were incubated for 24 hours with 1 µM fibrillar (stored at 37°C for 7 days) or soluble Aβ40 (stored at -20°C). Microglia cultures were counted and expressed as % of number at time 0 (dashed line).

Fibrillar, but not soluble Aβ promotes microglial cell growth.
Fibrillar Aβ Stimulates Release of TNF-α and IL-1β

Fibrillar but not soluble Aβ stimulates microglia to release TNF-α and IL-1β

Inflammatory Cycle Hypothesis

In this inflammatory cycle activated microglia release:
- reactive oxygen species/cytokines/chemokines

1. Activates APP/aSYN
2. activates heme oxygenase
3. releases iron
4. damages mitochondrial and stimulates APP/aSYN
Common Behavior of Aggregating Neurotoxic Proteins
Aggregates in Neurodegenerative Diseases

**AD**: plaques and tangles

**PD**: Lewy bodies

**HT**: Huntingtin inclusions

**TSE**: prion amyloid plaque

**ALS**: superoxide dismutase inclusions
### Neurotoxic Aggregating Proteins and Associated Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Old Knowledge</th>
<th>New Knowledge</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>APP, tau</td>
<td>APP, tau, SNCA, prions</td>
</tr>
<tr>
<td>PD</td>
<td>SNCA</td>
<td>SNCA, APP, tau</td>
</tr>
<tr>
<td>DS</td>
<td>APP, tau</td>
<td>APP, tau, SOD, prions</td>
</tr>
<tr>
<td>FTD &amp; others</td>
<td>Tau</td>
<td>in most neurodegenerative disorders, ?</td>
</tr>
<tr>
<td>ALS</td>
<td>SOD</td>
<td>SOD, TDP43 ?</td>
</tr>
<tr>
<td>CJ</td>
<td>Prions</td>
<td>Prions, ?</td>
</tr>
</tbody>
</table>
Summary of Neurotoxic Protein Regulation

Neurotoxic aggregating proteins, such as APP, aSYN, tau, SOD and prions display the same behavior, from gene activation, to protein synthesis to folding, misfolding, toxicity and aggregation:

1. Transcription is regulated by Cu/Zn/cytokines
2. Translation is regulated by Fe
3. At low concentrations they have a normal function
4. At high concentrations they form toxic oligomers
5. Oligomers can infect other cells in the brain and spread
6. They are degraded by the proeasome
7. The cell sequesters these toxic oligomers into aggregates to neutralize them

Posiphen Inhibits APP, tau and aSYN Synthesis
(2. Translation)
Posiphen®
Posiphen Overview

- **First in class**, small molecule with novel MOA for disease modification
  - Inhibits APP, tau, aSYN protein synthesis
  - Disease modifying

- Orally available with good blood brain-barrier permeability

- Effective in cell cultures, transgenic AD, trisomic DS and transgenic PD animal models and in humans

- Three Phase I studies conducted in over **120 subjects** (healthy volunteers and MCI patients) with clean safety profile and statistically significant biomarker data

- **Issued and newly filed IP**
Posiphen’s Mechanism of Action on 5’UTR of APP, aSYN and tau mRNA

IRP-1

Kd = 35pM

Iron Responsive Element
RNA stem loop

IRP-2

IRP-1 binding to APP IRE inhibits APP and SNCA translation

IRP-1

Kd = 15pM

+ Posiphen (PS)

Posiphen increases IRP-1 binding to the 5’UTR region of APP and SNCA mRNA

40S

Kd = 35pM

AUG APP/aSYN/tau

5’

3’

IRP – iron binding protein

IRE – iron responsive element

Posiphen is a Well Tolerated Translation Inhibitor of APP and aSYN

Posiphen

- 0.1 1 5 10 µM

APP

ACTIN

SNCA

Consensus Loop (from IRE stemloop)  CAGTGN

H-chain  5’  53  CGGGTTTCTGCTTTCAACAGTGGTTGACCAAACCGCCGCTCGT  3’  99
L-chain  5’  21  GTCTGTCTCTTGCTTTCAACAGTGGTTGACCAAACGAGATCCGGGA  3’  67
ASYN    5’  1   GGAGTGCCATTCCAGGACACAGGTGGTGTAAGGAATTCATATGCCC  3’  50
APP      5’  64  CGGGGTGGCGCCGCGCAGCAGAGGAGCGGCGGATCCCACTC  3’  103

Jack Rogers et al. JNT 2010
Posiphen Inhibition of Neural aSYN Expression is Potentiated by Iron

Posiphen decreased aSYN and APP levels dose-dependently in dopaminergic SH-SY5Y cells; iron potentiates the effect: The 5’UTRs of both APP & aSYN share 50% homology. Quantitative Western blotting established the efficacy of Posiphen on aSYN expression (IC-50 < 1 M); after standardization for β-actin

Rogers et al., JNT 2010; Friedlich et al., 2007
Posiphen Inhibits Levels of APP, Aβ42 and tau in APPswe/PS1 Mice

The 5’UTRs of APP, aSYN and tau share 50% homology and Posiphen inhibits their synthesis by enhancing binding of IRP-1 to the IRE stem look in the 5/UTR of their mRNAs
Proof of Mechanism Study in MCI Patients

- Treat 5 MCI patients with Posiphen for 10 days, oral dose at 240 mg/ day (4 x 60mg/day)
- CSF and plasma drawn for 12 hrs on day 0 and day 11
- PK: Posiphen and metabolites
  - Brain levels of Posiphen are 8 times higher than plasma
  - Approximately 96% binding to brain protein
  - $T_{1/2}^{\text{plasma}} = 5 \text{ hrs, } T_{1/2}^{\text{CSF/brain}} = 12 \text{ hrs}$
- PD: Reduced
  - sAPP and tau back to levels found in normal healthy volunteers
  - Inflammatory factors
## AD Biomarkers after 10 days on Posiphen

<table>
<thead>
<tr>
<th>Human Biomarker</th>
<th>CSF % of Time 0</th>
<th>Standard Error</th>
<th>p-Value</th>
<th>Assay</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>sAPP α</td>
<td>-34.1%</td>
<td>0.659</td>
<td>0.0661</td>
<td>MSD</td>
<td>MY Chan / QR Pharma</td>
</tr>
<tr>
<td></td>
<td>-59.9%</td>
<td>0.231</td>
<td>0.0006</td>
<td>AlphaLisa</td>
<td>V. John / Buck Institute</td>
</tr>
<tr>
<td>sAPP β</td>
<td>-34.0%</td>
<td>1.516</td>
<td>0.0901</td>
<td>MSD</td>
<td>MY Chan / QR Pharma</td>
</tr>
<tr>
<td></td>
<td>-57.7%</td>
<td>0.361</td>
<td>0.0001</td>
<td>AlphaLisa</td>
<td>V. John / Buck Institute</td>
</tr>
<tr>
<td>Aβ42</td>
<td>-45.2%</td>
<td>1.726</td>
<td>0.0995</td>
<td>AlphaLisa</td>
<td>V. John / Buck Institute</td>
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<td></td>
<td>-51.4%</td>
<td>1.119</td>
<td>0.0533</td>
<td>Innogenetics</td>
<td>C. Pan / Inarian</td>
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<tr>
<td>Tau</td>
<td>-46.2%</td>
<td>0.538</td>
<td>0.0020</td>
<td>AlphaLisa</td>
<td>V. John / Buck Institute</td>
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<td></td>
<td>-74.1%</td>
<td>0.259</td>
<td>0.0150</td>
<td>Innogenetics</td>
<td>C. Pan / Inarian</td>
</tr>
<tr>
<td>pTau</td>
<td>-61.0%</td>
<td>0.195</td>
<td>0.0005</td>
<td>Innogenetics</td>
<td>C. Pan / Inarian</td>
</tr>
</tbody>
</table>

Maccecchini et al. JNNP 2012
Comparison of Posiphen Treated MCI Patients with Healthy Volunteers

Posiphen given for 10 days to MCI patients lowers their APP and tau levels back to the levels found in healthy volunteers.
## Inflammatory Markers after 10 days on Posiphen

<table>
<thead>
<tr>
<th>Human Inflammatory Protein</th>
<th>CSF % of Time 0</th>
<th>Standard Error</th>
<th>p-Value</th>
<th>Assay</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement C3</td>
<td>-86.9%</td>
<td>0.139</td>
<td>0.0007</td>
<td>Millipore</td>
<td>C. Pan / Inarian</td>
</tr>
<tr>
<td>MCP-1</td>
<td>-87.5%</td>
<td>4.813</td>
<td>0.0007</td>
<td>MSD</td>
<td>H. Zetterberg / U.Goteborg</td>
</tr>
<tr>
<td>YKL40</td>
<td>-72.7%</td>
<td>2.2</td>
<td>0.0113</td>
<td>R&amp;D Systems</td>
<td>H. Zetterberg / U.Goteborg</td>
</tr>
<tr>
<td>sCD14</td>
<td>-26.1%</td>
<td>1.7</td>
<td>0.1159</td>
<td>R&amp;D Systems</td>
<td>H. Zetterberg / U.Goteborg</td>
</tr>
<tr>
<td>Factor FH</td>
<td>23.7%</td>
<td>1.237</td>
<td>0.4988</td>
<td>Millipore</td>
<td>C. Pan / Inarian</td>
</tr>
</tbody>
</table>
In CNS 10 days of Posiphen treatment lowers the levels of:

- Chitotriosidase: YKL-40
- Chemokine/chemoattractant: MCP-1
- C3, complement C3

Posiphen did not lower levels of two factors that are not elevated:

- Factor H, complement control protein
- sCD14, differentiation factor
Inhibition of Inflammatory Cycle

Breaking of Cycle

Posiphen inhibits iron activation of APP and α-Synuclein translation and inhibits microglia activation.