Vitamin A, Phenolic compounds and Alzheimer’s disease

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Brain atrophy of Alzheimer’s disease

In AD, parieto-temporal atrophy, especially HC and para HC atrophies are specific.
Alzheimer disease: AD

Ten % aged 60 and over develop the dementia whose main symptoms are cognitive decline and character change. AD is most common disease in the dementia.

It is thought that it happens by the accumulation of amyloid β-protein (Aβ) with abnormal structure in extracellular space.
Drug development in Alzheimer’s disease (AD)

Disease modifying drugs

Symptomatic therapy

Genetic factors
Environmental factors

Aβ protein
Senile plaque
Immune response
Neurite degeneration
Cell degeneration
Transmitter abnormality
Symptom onset

Tau protein
Neurofibrillary tangle (NFT)
Change in acetylcholine (ACh)

Brain atrophy
This hypothesis proposes a series of pathogenic events leading to AD. Cerebral Aβ protein accumulation is the primary factor in AD, and the rest of the disease process results from an imbalance between Aβ production, accumulation, and Aβ clearance.
Aggregation of β-amyloid protein (Aβ) has been considered a critical step in AD pathogenesis. The most potent neurotoxic assemblies appear to be oligomers rather than fibrils (Haass & Selkoe, 2009; Ono & Yamada, 2011).
Understanding Aβ assembly is difficult

- Aβ folding *in vivo* begins from an undefined structure.
- Aβ *in vitro* is unstructured.
- Oligomerization occurs rapidly (<1 min).
- Oligomers are unstable.
- Oligomers exist in many forms that inter-convert among each other.
- X-ray crystallography/NMR may not be used.
Photo-induced cross-linking of unmodified proteins (PICUP)
(Photocatalyzed reaction using photocatalyst: Ru)

- PICUP is useful method for the characterization of oligomer size distribution in vitro.
- PICUP is applied to quantitative study of metastable amyloid protein assemblies, including Aβ, prion, and α-synuclein.

Bitan et al., PNAS, 2003; Ono et al., PNAS, 2009
Vitamin A has anti-oligomerization effects on amyloid β-protein.
1st Introduction

- Patients with AD were reported to have low serum and plasma concentrations of vitamin A and β-carotene (Sinclair et al., 1998; Bourdel-Marchasson et al., 2001).

- We previously reported that vitamin A, β-carotene, α-lipoic acid and coenzyme Q10 inhibit fibrillation of Aβ40 and Aβ42 in vitro (Ono et al., 2004; Ono et al., 2005; Ono et al., 2006).

\[
\text{all-trans retinoic acid} \quad \text{all-trans retinol} \quad \text{all-trans retinal} \quad \beta\text{-carotene}
\]
Vitamin A (i.e. retinol) inhibited Aβ fibril formation (Ono et al. Exp Neurol, 2004).

Scale bars are 100 nm.
Vitamin A (i.e. retinoic acid ) inhibited low-n order oligomer formation.

Aβ40

Aβ42

Vitamin B2 retinoic acid retinol retinal β-carotene

M_r (kDa)

3.5 6.0 14.4 21.5 31.0 36.5 55.4 66.3


Oligomer
Comparison of oligomerization by Atomic Force Microscopy

<table>
<thead>
<tr>
<th>Aβ40</th>
<th>uncross-linking</th>
<th>cross-linking</th>
<th>cross-linking with retinoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.20 ± 0.03 (31)</td>
<td>0.88 ± 0.17 (30)</td>
<td>0.34 ± 0.05 (38)</td>
</tr>
<tr>
<td>Aβ42</td>
<td>0.31 ± 0.09 (46)</td>
<td>1.09 ± 0.56 (49)</td>
<td>0.36 ± 0.10 (38)</td>
</tr>
</tbody>
</table>

100nm
Retinoic acid decreased cellular toxicity by inhibition of Aβ oligomerization.

* \( p < 0.01 \)
Retinoic acid inhibited aggregation of \( \text{A}\beta_{25-35} \), but not of \( \text{A}\beta_{1-16} \).
Retinoic acid attenuated Aβ deposition and rescues memory deficits in vivo (Ding Y et al., J Neurosci, 2008).

A robust decrease in brain Aβ deposition and tau phosphorylation was reported in a study of APP/PS1 transgenic mice that were treated for 8-weeks with retinoic acid. The retinoic acid-treated APP/PS mice showed decreased activation of microglia and astrocytes, attenuated neuronal degeneration, and improvements in spatial learning and memory compared with vehicle-treated APP/PS mice.
1. Vitamin A inhibit aggregation of Aβ40 and Aβ42 in vitro and in vivo.

2. Retinoic acid decreased cellular toxicity by inhibition of Aβ42 oligomerization.

3. Specific binding of retinoic acid may be the C-terminal portion of Aβ.
Phenolic compounds Prevent Amyloid β-protein Oligomerization and Synaptic Dysfunction by Site-specific Binding.
2nd Introduction

- French and Danish epidemiological studies suggesting that moderate wine drinking may protect against AD (Orgogozo et al., 1997; Truelsen et al., 2002).

- Phenolic compounds, such as myricetin (Myr), curcumin (Cur), its analog rosmarinic acid (RA), nordihydroguaiaretic acid (NDGA), and ferulic acid (FA) inhibit the formation of fAβ in vitro (Ono et al., 2003; Ono et al., 2004; Ono et al., 2005).
Phenolic compounds inhibited low-n order oligomer formation.

Aβ40

Aβ42

Ono et al., *J Biol Chem* 2012
Comparison of oligomerization by Atomic Force Microscopy

<table>
<thead>
<tr>
<th></th>
<th>un-cross-linking</th>
<th>cross-linking</th>
<th>cross-linking with Myr</th>
<th>cross-linking with RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ40</td>
<td>0.23±0.03 (45)</td>
<td>0.90±0.05 (34)</td>
<td>0.32±0.03 (53)</td>
<td>0.42±0.04 (63)</td>
</tr>
<tr>
<td>Aβ42</td>
<td>0.33±0.02 (89)</td>
<td>1.39±0.09 (54)</td>
<td>0.32±0.02 (80)</td>
<td>0.33±0.01 (88)</td>
</tr>
</tbody>
</table>

Scale bars are 100 nm.
Myr and RA decreased cellular toxicity by inhibition of Aβ oligomerization.
Myr and RA decreased Aβ oligomer-induced LTP suppression.

**p < 0.01.

Ono et al., *J Biol Chem* 2012
Myr and RA decreased Aβ oligomer-induced LTD facilitation.

* $p < 0.05$, ** $p < 0.01$.

Ono et al., *J Biol Chem* 2012
In vivo effects of phenolic compounds on Aβ oligomerization (1)

Phenolic Compounds Prevent Alzheimer’s Pathology through Different Effects on the Amyloid-β Aggregation Pathway

Tsuyoshi Hamaguchi, Kenjiro Ono, Atsushi Murase, and Masahiro Yamada
From the Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

and behavioral changes. One of the pathological hallmarks of AD is extracellular deposits of aggregated amyloid-β protein (Aβ) in the brain parenchyma (senile plaques) and cerebral blood vessels (cerebral amyloid angiopathy (CAA)). Deposition of high levels of fibrillar Aβ in the AD brain is associated with loss of synapses, impairment of neuronal functions, and loss of neurons.

Control

Myricein-treated

Human trials for AD patients

Hamaguchi and Ono et al., Am J Pathol, 2009
In vivo effects of phenolic compounds on Aβ oligomerization (2)

Brief Communications

Grape-Derived Polyphenolics Prevent Aβ Oligomerization and Attenuate Cognitive Deterioration in a Mouse Model of Alzheimer’s Disease

Jun Wang,1 Lap Ho,2,3 Wei Zhao,1 Kenjiro Ono,1 Clark Rosensweig,3 Linghong Chen,4 Nelson Humala,1 David B. Teplow,1 and Giulio M. Pasinetti1,2,3

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Heterogeneity in Red Wine Polyphenolic Contents Differentially Influences Alzheimer’s Disease-type Neuropathology and Cognitive Deterioration

Lap Ho4,5,6,7, Ling Hong Chen4,5, Jun Wang6, Wei Zhao6, Stephen T. Takahashi, Kenjiro Ono4, David Teplow6, Nelson Humala4, Alice Cheng4, Susan S. Perlović6, Mario Ferruzzi6, Elsa Janie4, Dara L. Dickstein6, and Giulio Maria Pasinetti4,5,6,7

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Expanded HSQC spectra of uniformly $^{15}$N-labeled Aβ42.
A representative NMR/MD structural model of Aβ42 that shows binding locations with myricetin (M yr).

Mechanism of polyphenolic inhibition of Aβ aggregation
Grape derived polyphenols also exhibit anti-tau effects. (Wang, ….., Ono et al., J Alzheimers Dis, 2010).
**In vitro effect**
Grape derived polyphenols inhibit secondary structure transition (random coil $\rightarrow$ $\beta$-sheet) and aggregation of tau.

**In vivo effect**
Grape derived polyphenols inhibit aggregation of phosphorylated tau.
Final Conclusion

1. Vitamin A and phenolic compounds dose-dependently inhibited oligomerization of Aβ40 and Aβ42.

2. MTT, LTP and LTD assays established that vitamin A and phenols inhibit Aβ oligomer-induced cellular and synaptic toxicities.

3. Myricetin promoted significant NMR chemical shift changes of monomeric Aβ.

4. Vitamin A and phenolic compounds are worthy therapeutic candidates for Alzheimer's disease.
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Thank you for your attention!