Amyloid β-Protein Oligomers and Alzheimer Disease

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Alzheimer’s Disease (AD):

The most common form of progressive senile dementia primarily affecting individuals over the age of 65. The disease leads to the inevitable destruction of neurons, and ultimately death within 7 to 10 years.

Actual and estimated number of new Alzheimer disease cases in the US through the year 2050.

Actual and estimated financial costs of Alzheimer disease in the US

Outline

◆ Background
  1. The hallmarks of Alzheimer Disease
  2. Amyloid Hypothesis

◆ Oligomer Toxicity

◆ Approaches to Study the Oligomers

◆ Summary
The Hallmarks of AD: Plaques and Tangles

- Amyloid β plaques: dense deposits of protein that accumulate outside and around nerve cells
- Neurofibrillary tangles: twisted fibers that build up inside the nerve cell
Amyloid precursor protein (APP) is membrane protein that sits in the membrane and extends outward. It is thought to be important for neuronal growth, survival, and repair.

1. APP sticks through the neuron membrane.

2. Enzymes cut the APP into fragments of protein, including beta-amyloid.

3. Beta-amyloid fragments come together in clumps to form plaques.

**APP Protein:**

β- and γ-secretase cut APP protein, giving:

- Aβ40 Fragment
- Aβ42 Fragment

Aβ40: Aβ42=10:1 in vivo
Aβ42 forms fibrils more rapidly and more neurotoxic.
Microtubules are like railroad tracks that transport nutrition and other molecules. Tau-proteins act as “ties” that stabilize the structure of the microtubules. In AD, tau proteins become tangled, unstabilizing the structure of the microtubule. Loss of axonal transport results in cell death.
Accumulation of Aβ in the brain is the primary influence driving AD pathogenesis.

1. Some humans without symptoms of AD have many critical A-beta deposits.
2. Transgenic mice expressing Aβ has revealed neurological deficits prior to amyloid deposition.

Soluble oligomers of A-beta are the proximate neurotoxins in AD.

Oligomer Toxicity

- Interact with membrane

amphipathic peptide: DAEFRHDSGYEVHOKLVFFAEDEVGSNKGAIGLGMVGVVIA

I. Disrupt membrane integrity.
II. Increase membrane permeability.

Ion channel formation.
Membrane thinning.

facilitate charge translocation across the bilayer, release of membrane components (including cholesterol, phospholipids)

Oligomer Toxicity

- **Bind with metals**
  1. Disrupt the metal homeostasis
  2. Produce radical, reactive oxygen species, oxidative stress

  Catalyze Fenton reaction:

  \[ M^{n+} + H_2O_2 \rightarrow M^{(n-1)+} + HOO \cdot + H^+ \]

- **Mitochondrial dysfunctions**

  Tau hyperphosphorylation, disrupting proteasome (蛋白酶体) and mitochondrial (线粒体) function, and triggering calcium and synaptic dysfunction.

Oligomer

Fundamental Questions:

1. What’s the structures and relative abundance of small oligomers?
2. Which types of oligomers are involved in the disease?
3. How these oligomers are assembled?

Difficulties in studying oligomers:

1. Aβ is “natively unfolded” and preferentially form amyloid fibrils rather than protein crystals. X-ray diffraction ×

2. Aβ oligomers often are metastable and comprise structurally heterogeneous populations in equilibrium with monomers and fibrils. Pure populations of conformers: CD ×, FT-IR ×, SDS-page ×

(3) Some conformations of Aβ oligomers are rarely-populated and variations of conformations can occur under different experimental conditions.

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UCLA Alzheimer's Disease Research Center
Brain Research Institute
UCLA Health System Research
UCSF California Alzheimer's Disease Centers

In hydro Studies---provide information about oligomers in solution

1. photoinduced cross-linking of unmodified proteins (PICUP)

Study of Oligomer

**In Vacuo Studies:**

1. ESI–MS has been shown to be capable of preserving non-covalently bound species
2. Detect rarely-populated conformers of highly dynamic proteins
3. IMS–MS is now accepted as a powerful method to determine conformational properties of unfolded and partially folded species, as well as natively folded proteins
4. IMS–MS determinates oligomer mass and shape and studies of self-association kinetics

In Aβ42 an ‘open’ tetramer promotes the formation of the planar hexamer (paranucleus) and the stacked dodecamer.

For Aβ40 and other studied alloforms the key structure is the tetramer that resists further monomer or dimer addition.

Study of Oligomer

- **In Silico Studies:**
  1. Provide structural information at atomic resolution.
  2. Provide information of assembly dynamics for oligomers.

- **In silico study of amyloid β-protein folding and oligomerization**

  **Method:** discrete MD (DMD) algorithm with a coarse-grained protein model (four-bead protein model)

  1: Oligomer Size Distributions of Aβ 40 and Aβ 42.

Study of Oligomer

2: Intramolecular contacts in pentamers.

The Aβ42 contact map contains a significantly greater number of contacts.

These additional contacts do not involve only the Ile–Ala residues but result from the involvement of residues in the 1–40 region establishing contacts where none existed before.

Met35 contacts the Aβ C-terminus in Aβ42 but not in Aβ40.

Oxidation of Met35 in Aβ42 blocked paranucleus formation and produced oligomers indistinguishable in size and morphology from those produced by Aβ40.

Study of Oligomer

3: Geometrical Characteristics of Aβ Pentamers.

**Commonalities**: globular, C termini is in the assembly core and N termini on the surface.

**Difference**: the N termini of the Aβ42 pentamers are more extended and less structured.

the N-terminal β-strand in Aβ40 may shield the hydrophobic core of the oligomer, hinder the intermolecular interactions among hydrophobic cores of multiple oligomers, thus hinder higher-order association reactions.

Summary

- Soluble oligomers of Aβ are the proximate neurotoxins in AD.

- Study of oligomers is difficult task.

- Approaches to study oligomers:
  1. in hydro studies---PICUP method:
     provide information in solution and a standard to MS and MD methods.
  2. in vacuo studies---IMS-MS method:
     determinates oligomer mass and shape, detect rarely-populated conformers
  3. in silico studies---MD simulations:
     provide high resolution structural information and information of assembly dynamics
Thanks for your attention!
Amyloid β-Protein Assembly

Therapy of AD

<table>
<thead>
<tr>
<th>Drug</th>
<th>Manufacturer</th>
<th>Mechanism of action</th>
<th>Launched</th>
<th>US sales (2005)</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donepezil (Aricept)</td>
<td>Eisai Inc.</td>
<td>Cholinesterase inhibitor; prevents the breakdown of acetylcholine in the brain</td>
<td>1997</td>
<td>$1.1 billion</td>
<td>Nausea, diarrhea, insomnia, vomiting, muscle cramps, fatigue, anorexia</td>
</tr>
<tr>
<td>Memantine (Namenda)</td>
<td>Forest Pharmaceuticals</td>
<td>NMDA receptor antagonist; blocks toxic effects associated with excess glutamate and regulates glutamate activation</td>
<td>2003</td>
<td>$498 million</td>
<td>Dizziness, headache, confusion, constipation</td>
</tr>
<tr>
<td>Rivastigmine (Exelon)</td>
<td>Novartis Pharmaceuticals Corporation</td>
<td>Cholinesterase inhibitor; prevents the breakdown of acetylcholine and butrylcholine in the brain</td>
<td>2000</td>
<td>$226 million</td>
<td>Nausea, vomiting, loss of appetite, indigestion, weakness/ lack of energy, dizziness, diarrhea, headache, stomach pain</td>
</tr>
<tr>
<td>Galantamine (Razadyne)</td>
<td>Ortho-McNeil Neurologics Inc.</td>
<td>Cholinesterase inhibitor; prevents the breakdown of acetylcholine and stimulates nicotinic receptors to release more acetylcholine</td>
<td>2001</td>
<td>$223 million</td>
<td>Nausea, vomiting, diarrhea, anorexia, weight loss</td>
</tr>
<tr>
<td>Galantamine (Razadyne ER)</td>
<td>Ortho-McNeil Neurologics Inc.</td>
<td>Cholinesterase inhibitor; prevents the breakdown of acetylcholine and stimulates nicotinic receptors to release more acetylcholine</td>
<td>2005</td>
<td>$24 million</td>
<td>Nausea, vomiting, diarrhea, anorexia, weight loss</td>
</tr>
</tbody>
</table>

Source: IMS Health

Weak weapons: The drugs available for Alzheimer disease have shown only modest benefits
### Target practice: Most candidates being tested for Alzheimer disease are based on the amyloid hypothesis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Manufacturer</th>
<th>Mechanism of action</th>
<th>Stage of development</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New drugs</strong></td>
<td></td>
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<tr>
<td>3APS (Alzhemed)</td>
<td>Neurochem, Inc.</td>
<td>inhibits amyloid-beta aggregates, binds and reduces soluble amyloid-beta</td>
<td>Phase 3</td>
<td>Nausea, vomiting</td>
</tr>
<tr>
<td>MPC-7869 (Flurizan)</td>
<td>Myriad Pharmaceuticals</td>
<td>NSAID derivative; inhibits amyloid-beta aggregates and reduces their levels of amyloid-beta with little or no anti-inflammatory effect</td>
<td>Phase 3</td>
<td>None disclosed</td>
</tr>
<tr>
<td>AAB-001</td>
<td>Elan Pharmaceuticals</td>
<td>monoclonal antibody binds to and clears amyloid-beta, is designed to directly deliver antibodies to amyloid-beta</td>
<td>Phase 2</td>
<td>None disclosed</td>
</tr>
<tr>
<td>Neramexane</td>
<td>Forest Laboratories</td>
<td>NMDA receptor antagonist; blocks the effects of excessive glutamate at the receptor</td>
<td>Phase 3</td>
<td>None disclosed</td>
</tr>
<tr>
<td><strong>Drugs for other conditions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Simvastatin (Zocor)</td>
<td>Merck</td>
<td>Statin; reduces cholesterol-carrying protein that promotes amyloid-beta aggregation</td>
<td>Phase 3</td>
<td>None disclosed for the trial, but Zocor has been known to cause nausea, diarrhea, abdominal pain and muscle cramps</td>
</tr>
<tr>
<td>VP4896</td>
<td>Voyager Pharmaceutical</td>
<td>Hormone drug leuprolide acetate; decreases amount of luteinizing hormone in body, might prevent brain cell death</td>
<td>Phase 3</td>
<td>None disclosed</td>
</tr>
<tr>
<td>Valproate</td>
<td>Manufacturer not disclosed</td>
<td>Anticonvulsant drug; neuroprotective properties may delay clinical progression of Alzheimer disease</td>
<td>Phase 3</td>
<td>None disclosed</td>
</tr>
<tr>
<td><strong>Dietary supplements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gingko biloba</td>
<td></td>
<td>Antioxidants neutralize free radicals and may reduce or prevent the damage they cause in brain cells</td>
<td>Phase 3</td>
<td>Headache, upset stomach, allergic reactions</td>
</tr>
<tr>
<td>Vitamin E Selenium</td>
<td></td>
<td>Antioxidants neutralize free radicals and may reduce or prevent the damage they cause in brain cells</td>
<td>Phase 3</td>
<td>None disclosed</td>
</tr>
</tbody>
</table>
New strategy: Immunotherapy

<table>
<thead>
<tr>
<th>Model</th>
<th>Antibody or antigen</th>
<th>Route of Immunization</th>
<th>Effect</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pheochromocytoma cells</td>
<td>Amyloid-β-specific antibody</td>
<td>In vitro</td>
<td>Inhibition and solubilization of fibrils of amyloid-β peptide through antibody recognition of the amino-terminal Glu-Phe-Arg-His epitope</td>
<td>61-64</td>
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<tr>
<td>APP(V717F)-transgenic (PDAPP) mice</td>
<td>Amyloid-β specific antibody</td>
<td>Subcutaneously with adjuvant</td>
<td>Reduced amyloid-β plaques, neuritic dystrophy and astrogliosis</td>
<td>65</td>
</tr>
<tr>
<td>APP(V717F)-transgenic (PDAPP) mice</td>
<td>Amyloid-β specific antibody</td>
<td>Nasally</td>
<td>Reduced cerebral amyloid burden</td>
<td>66</td>
</tr>
<tr>
<td>APP(KS70N, M671L, Y717F)-transgenic (CRND8) mice and APP(K570N, M671L), PSEN1 (M146L) double-transgenic mice</td>
<td>Amyloid-β specific antibody</td>
<td>Subcutaneously with adjuvant</td>
<td>Reduced behavioural impairment and amyloid plaque deposition</td>
<td>14.15</td>
</tr>
<tr>
<td>APP(KS70N, M671L)-transgenic (Tg2576) mice</td>
<td>A non-fibrillar amyloid-β homologous peptide</td>
<td>Subcutaneously with adjuvant</td>
<td>Reduced Alzheimer’s disease-associated pathology</td>
<td>70</td>
</tr>
<tr>
<td>APP(V717F)-transgenic mice</td>
<td>Filamentous phage displaying amyloid β-s (Glu-Phe-Arg-His epitope)</td>
<td>Intraperitoneally</td>
<td>Reduced amyloid-β plaques and behavioural impairment</td>
<td>79.80</td>
</tr>
<tr>
<td>APP(KS70N, M671L)-transgenic (Tg2576) mice</td>
<td>Recombinant adeno-associated virus vector expressing amyloid-β</td>
<td>Orally</td>
<td>Reduced amyloid-β plaques</td>
<td>77</td>
</tr>
<tr>
<td>APP(KS70N, M671L)-transgenic (Tg2576) mice</td>
<td>Amyloid-β encoding DNA vaccine</td>
<td>Intramuscularly</td>
<td>Decreased amyloid burden due to antibodies induced by DNA vaccination</td>
<td>76</td>
</tr>
<tr>
<td>APP(V717F)-transgenic (PDAPP) mice</td>
<td>Amyloid-β₇₅ and amyloid-β₃₅ specific antibodies</td>
<td>Passive</td>
<td>Reduced amyloid-β plaques</td>
<td>67</td>
</tr>
<tr>
<td>APP(V717F)-transgenic (PDAPP) mice</td>
<td>Amyloid-β₇₅-specific antibody</td>
<td>Passive</td>
<td>Reversion of memory deficits without reduction of brain amyloid-β burden</td>
<td>68.69</td>
</tr>
<tr>
<td>APP(KS70N, M671L, V717F)-transgenic (CRND8) mice</td>
<td>Amyloid-β₇₅ and amyloid-β₃₅ specific antibodies</td>
<td>Passive</td>
<td>Attenuation of amyloid deposition</td>
<td>82</td>
</tr>
<tr>
<td>APP(V717F)-transgenic (PDAPP) mice</td>
<td>Amyloid-β₇₅-specific antibody</td>
<td>Passive</td>
<td>Reduced amyloid-β plaques</td>
<td>83</td>
</tr>
<tr>
<td>APP(KS70N, M671L)-transgenic (Tg2576) mice</td>
<td>Oligomeric amyloid-β₇₅-specific antibody</td>
<td>Passive</td>
<td>Reduced amyloid-β plaques, improvement in learning and memory</td>
<td>81</td>
</tr>
</tbody>
</table>

Amyloid-β-specific antibody as a mediator of amyloid clearance following active or passive immunization