The Fight against rogue proteins in Alzheimer’s disease

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Alzheimer’s disease

- What is Alzheimer’s disease?
- Who gets it?
- Why do they get it?
- What can be done about it?
- What research is being done at KU?
10 warning signs of Alzheimer’s

1. Memory loss that disrupts daily life
2. Challenges in planning or solving problems
3. Difficulty completing familiar tasks at home, at work or at leisure
4. Confusion with time or place
5. Trouble understanding visual images and spatial relationships
6. New problems with words in speaking or writing
7. Misplacing things and not being able to retrace steps
8. Decreased or poor judgment
9. Withdrawal from work or social activities
10. Changes in mood and personality

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OVERVIEW OF ALZHEIMER’S DISEASE

A. Alzheimer

A. Deter
1906

ALZHEIMER’S DISEASE IS THE MOST COMMON TYPE OF DEMENTIA.

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Alzheimer’s Pathology
Pathological Hallmarks of Alzheimer’s disease

**Aβ Senile Plaques**
- Extracellular
- Composed of cleaved Beta Amyloid
- Highly elevated in Alzheimer’s disease

**Neurofibrillary Tangles**
- Intracellular
- Composed of Microtubule-Associated Protein tau
- Elevated in affected regions of Alzheimer’s disease

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Beta-Amyloid Processing

• The Amyloid Precursor Protein (APP) is a transmembrane protein that is cleaved by proteases to release a peptide in the extracellular spaces.

• β- and γ- secretases generate the Beta-Amyloid 1-42 amino acid fragment, while the α-secretase generates a relatively harmless 1-40 amino acid peptide.

• Familial mutations leading to Alzheimer’s disease involve either the Amyloid Precursor Protein or the secretases (also called “presenilins”).

• Beta-Amyloid 1-42 peptides aggregate together to form oligomers, fibrils and eventually lead to Senile Plaques.

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Formation of Tau Pathology

- Tau normally binds to and stabilizes microtubules in neurons creating a stable cytoskeleton that can be used for transport.

- In disease, tau is modified by unknown mechanisms that could include phosphorylation, truncation or other changes.

- The modified tau releases from microtubules, destabilizing them.

- Tau self-associates into oligomers, fibrils and eventually into intracellular Neurofibrillary Tangles.
Stages of Alzheimer’s disease

Early (Mild cognitive impairment):
• Memory & Learning
• Thinking & Planning

Mild to moderate (Possible Alzheimer’s):
• Language problems
• Sense of your surroundings

Severe (Probable Alzheimer’s):
• Lose ability to communicate
• Lose ability to care for themselves
• Lose ability to recognize friends and family

Aβ Plaques elevated everywhere in brain

Tau Neurofibrillary Tangles elevated in affected brain regions
Alzheimer’s Risk Factors

- Advancing Age
- Family History
- Apolipoprotein E ε4 gene
- Mild Cognitive Impairment
- Cardiovascular Disease Risk Factors
- Less Education
- Decreased Social and Cognitive Engagement
- Severe Traumatic Brain Injury, Mild Repetitive TBI (concussions)

- There are currently no pharmacologic treatments that slows or stops the progression of Alzheimer’s disease symptoms
- Currently approved drugs that may have some benefit:
  1. Donepezil (Aricept)*
  2. Galantamine (Razadyne)*
  3. Rivastigmine (Exelon)*
  4. Tacrine (Cognex)*
  5. Memantine (Namenda)**

*Cholinesterase inhibitors
**NMDA receptor agonist
• Approximately 1 in 8 people over the age of 65 has Alzheimer’s disease

• ~1 in 3 people 85 and older has AD

• An estimated 5.2 million Americans have AD

• By 2050, it is estimated that AD occurrence could almost triple to ~13.8 million cases in the US
• 83,494 people died from AD in 2010

• This is a 68% increase from the year 2000

• According to the Chicago Health and Aging Project, an estimated 400,000 people died with AD although there was a different direct cause of death
• Estimated value of unpaid care was $216 Billion dollars in 2012

• Caregivers for AD and other related dementias need to provide more assistance with getting in and out of bed, dressing, getting to bathroom, bathing, managing incontinence and feeding than is needed from caregivers of other older people.

• Providing care to AD patients can have a negative impact on caregiver emotional and physical well-being.
The per-person average health care costs for patients with AD and other dementias was approximately $46,000 compared to $14,500 for Medicare beneficiaries age 65 and older without AD.

Medicare payments were ~3 times higher and Medicaid payments were ~19 times higher for AD patients compared to non-AD patients in 2012.
TARGETS FOR FUTURE DRUGS

- Reducing β amyloid
  - Inhibiting proteases
  - Antibody clearance
- Tau protein
  - Kinase inhibitors
  - Anti-aggregation
  - Antibody clearance
- Inflammation
  - Reducing inflammation
  - Reducing oxidation
- Metabolism
  - Insulin resistance
  - Mitochondrial function

My laboratory is very interested in identifying inhibitors of Tau Protein Aggregation

THERE ARE ONLY 5 AVAILABLE DRUGS FOR AD, AND THEY HAVE LIMITED FUNCTIONALITY
Compounds previously identified with tau-aggregation inhibition activity*

Variations of rhodanine based inhibitors

Core structure of thiazolylhydrazide Inhibitors

N-Phenylamine-derived compounds

Benzothiazole derivatives

Phenothiazines, Porphyrins, and Polyphenols

Anthraquinone-derived compounds

Aspergillus Nidulans

- Filamentous fungus
- Fungi have historically been a rich source of biomedically useful compounds
  - Penicillin
  - Lovastatin
  - Cyclosporin
- Many of these compounds are secondary metabolites, or are not made under normal conditions
Mining the *Aspergillus* Metabolome

- Using advanced genetic techniques, the fungi can be engineered to produce compounds that they normally would only produce under special circumstances.
- They produce large amounts that can be readily purified and identified.

Dr. Berl Oakley
Irving S. Johnson Distinguished Professor of Molecular Biology
Department of Molecular Biosciences
A. *Nidulans* 2° metabolites screened for tau anti-aggregation properties

**Multicyclic Aromatics**

A. Emericellin

![Image of Emericellin](image)

B. Variecoxanthone

![Image of Variecoxanthone](image)

C. Emodin

![Image of Emodin](image)

D. 2,α-dihydroxyemodin

![Image of 2,α-dihydroxyemodin](image)

E. Endocrocin

![Image of Endocrocin](image)

F. Sterigmatocystin

![Image of Sterigmatocystin](image)

G. F8775 A

![Image of F8775 A](image)

H. F9775 B

![Image of F9775 B](image)

I. Asperthecin

![Image of Asperthecin](image)

J. Chrysophanol

![Image of Chrysophanol](image)

K. Aloe emodin

![Image of Aloe emodin](image)

L. Shamixanthone

![Image of Shamixanthone](image)

M. Demethylsterigmatocystin

![Image of Demethylsterigmatocystin](image)

N. α-hydroxyemodin

![Image of α-hydroxyemodin](image)

O. Monodictyphenone

![Image of Monodictyphenone](image)

P. 3′-hydroxyversiconol

![Image of 3′-hydroxyversiconol](image)

**Monocyclic Aromatic**

Q. Asperbenzaldehyde

![Image of Asperbenzaldehyde](image)
Biochemical Model of Alzheimer’s disease: Fatty Acids Induce Tau Aggregation

• The filaments resemble authentic filaments from AD
  • morphologically
  • immunologically
  • structurally
• We can screen compounds to determine whether they affect tau aggregation

Synthetic tau filaments induced by arachidonic acid (ARA)
Filter Trap Assay of Tau Aggregation with *A. Nidulans* Compounds

Statistically Significant Inhibition by:
- 2,ω-dihydroxyemodin
- Asperthecin
- Chrysophanol
- Asperbenzaldehyde
Electron Microscopy of Tau Aggregation with Effective Compounds

- Asperthecin reduces the number of longer filaments and increases the number of smaller filaments and aggregates below 100nm in length.
Chrysophanol has only a small effect on filaments larger than 200nm
Asperbenzaldehyde reduces long (>200nm) filaments and increases the number of aggregates in the 30-50nm range.
2,ω-dihydroxyemodin reduces the number of longer filaments and increases the number of smaller filaments and aggregates below 100nm in length.
Effective Concentrations of Inhibition

- Compounds were added over a wide range of concentrations
- All 3 compounds show a dose-dependent decrease in tau aggregation
- Under these conditions, Asperthecin is the most potent compound
Effect of Compounds on Tau’s Normal Function

• Tau’s normal function is to stabilize microtubules

• Tau was mixed with tubulin without compound and with compounds at 2 different concentrations

• Tubulin polymerization into microtubules was monitored by a fluorescence assay

• Tau retained its ability to stabilize microtubules in the presence of all three compounds
Conclusions

• A. Nidulans produces 2° metabolites that have tau anti-aggregation properties
• The success rate of 3 compounds out of 16 from a targeted search is much improved over high-throughput approaches that may find a few compounds out of hundreds of thousands of compounds
• Asperbenzaldehyde represents a novel structural class of compounds for the inhibition of tau aggregation
• The compounds inhibit tau aggregation but allow it to retain its normal function, making them good candidates for further development
Future plans

• We are exploring 2nd generation compounds from *Aspergillus*

• We have brought in a medicinal chemist to make new compounds for structure/activity modifications

• We are generating animal models to test the compounds

Tau-GFP in *C. elegans* neurons; courtesy of Dr. Brian Ackley
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