Metabolic and Biochemical Alterations in Alzheimer Disease: Facts and Fictions
Prof. Mark A. Smith
Case Western Reserve University

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The brain and Alzheimer disease
Alzheimer disease attacks nerve cells in several regions of the brain
A. Cerebral Cortex: involved in conscious thought and language
B. Basal forebrain: has large numbers of neurons containing acetylcholine, a chemical important in memory and learning
C. Hippocampus: essential to memory storage
The earliest signs of Alzheimer’s are found in the nearby entorhinal cortex (not shown)
What causes selective neuronal loss in AD?

- Almost every physiological/pathological mechanism that a cell can elicit has been implicated!
- And most are probably involved…
- At some point…

The usual suspects

Proposed chronology of changes in AD Tauist and BAPtist

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Our hypothesis

- Tau phosphorylation (Neurofibrillary tangles)
- Amyloid-β deposition (Senile plaques)

Fundamental disease mechanisms

Neurofibrillary tangles

Clues to disease mechanism

Pathological lesions are very insoluble, possibly crosslinked?

Since the prevalence of AD is strictly age-dependent, cause must also be

Oxidative stress/modifications?

Oxidative modifications affect all cellular macromolecules

- Lipid peroxidation/protein adduction (4-HNE)
- Protein oxidation (free carbonyl groups)
- Nucleic acids (8-OH-guanosine)
- Glycoxidation (carboxymethyllysine)
What is relationship?
Pathology versus oxidative stress

<table>
<thead>
<tr>
<th>Causes</th>
<th>Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tau phosphorylation</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>[neurofibrillary tangles]</td>
<td></td>
</tr>
<tr>
<td>Amyloid-β deposition</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>[senile plaque]</td>
<td></td>
</tr>
</tbody>
</table>

Amyloid deposition occurs after oxidative stress!
Tau phosphorylation/NFT occur after oxidative stress!

Any theory on AD has to account for NFT and amyloid-β.

In other words, if they are not the cause, they may be the consequence.
Oxidative stress induces Tau phosphorylation


Stress-activated protein kinases and phospho-\(\tau\): a complete overlap

p-p38 p-\(\tau\)
p-JNK p-p44

Oxidative stress mediates amyloid-\(\beta\) production

Both \(\beta\)APP and amyloid-\(\beta\) increased 3-4 fold after oxidative insult

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Amyloid-targeted approaches have been unsuccessful (so far...ever?)

<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>Study1</td>
<td>Method1</td>
<td>Reference1</td>
</tr>
<tr>
<td>2010</td>
<td>Study2</td>
<td>Method2</td>
<td>Reference2</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Reasons: Too late! Not strong enough! Wrong target!

The American Journal of Geriatric Pharmacotherapy (2009) M.N. Sabbagh

Is amyloid an antioxidant?

Neuronal oxidative stress decreases following amyloid-β deposition

\[ R = -0.97, p < 0.0001 \]

Ascorbate radicals Hydroxyl radicals
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“Al mutations significantly increased Aβ42/Aβ40 in vitro by significantly decreasing Aβ40 with accumulation of APP C-terminal fragments, a sign of decreased PSEN activity.”

But what is the mechanism?

APP/PS* → [Aβ42/Aβ40] → AD

Or

APP/PS* → Aβ → ? → AD
Genetic factors of AD are associated with increased oxidative stress or elevated vulnerability to oxidative stress

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Transgenic/knock in mice</th>
<th>Postmortem brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>AβPP gene mutation</td>
<td>Eckert et al., 2001</td>
<td>Smith et al., 1998</td>
</tr>
<tr>
<td></td>
<td>Iwatsubo et al., 2003</td>
<td>Takahashi et al., 2000</td>
</tr>
<tr>
<td>PS1 gene mutation</td>
<td>Go et al., 1997</td>
<td>Go et al., 1997</td>
</tr>
<tr>
<td></td>
<td>Launder et al., 2000</td>
<td>Launder et al., 2000</td>
</tr>
<tr>
<td>PS2 gene mutation</td>
<td>Hashimoto et al., 2002</td>
<td></td>
</tr>
<tr>
<td>APOE4 gene polymorphism</td>
<td>Miyata and Smith, 1996</td>
<td></td>
</tr>
</tbody>
</table>

Therapeutics

Potential mechanism of reactive oxygen species generation in Alzheimer disease

Redox active metals

Hormonal changes leptin/LH

Mitochondria/metabolic alterations
Oxidative modifications affect all cellular macromolecules

Lipid peroxidation: protein adduction (4-HNE)

Protein oxidation (free carbonyl groups)

Nucleic acids (8-OH-guanosine)

Glycoxidation (carboxymethyllysine)

Causes?

Mitochondrial abnormalities: 5Kb-deletion

Mitochondria/redox metal abnormalities predict sites of oxidative damage

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Oxidative damage directly correlates with mtDNA

Abnormal mitochondrial distribution in AD brains

PET SCAN-profound metabolic decrease as an early indicator of AD
However, oxidative damage is apparently restricted to cytoplasm.

Hydrogen peroxide generates oxidative radicals.

Alzheimer disease (+ H2O2)
Alzheimer disease (- H2O2)
Aged-matched control
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In situ oxidation of 3,3' diaminobenzidine (chelation)

- Alzheimer disease
- 10mM DFX
- 10mM DTPA

Iron histochemistry

- AD
- Ctl
- DFX
- DFX/Fe

Redox active metals are also in cytoplasm

Control cases lacking endogenous redox active metals...

... have metal (Fe²⁺/Fe³⁺) binding sites that produce ROS if supplied with excess H₂O₂.

Control case untreated and after addition of Fe²⁺/Fe³⁺


Summary: oxidative stress

Proliferation

Degradation

Heme oxygenase-1

Redox metals

ROS

H₂O₂

What causes these changes?

Predictions

• Therapeutics/preventatives (antioxidants)
• Models (cell/animal)
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Antioxidant diet is protective

<table>
<thead>
<tr>
<th>Nutrients per 1000 kilocalories</th>
<th>Alzheimer disease cases n=104</th>
<th>Controls n=223</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (RE)</td>
<td>855</td>
<td>983</td>
<td>0.001</td>
</tr>
<tr>
<td>α-carotene (mcg)</td>
<td>294</td>
<td>389</td>
<td>0.001</td>
</tr>
<tr>
<td>Β-Carotene (mcg)</td>
<td>1521</td>
<td>2370</td>
<td>0.003</td>
</tr>
<tr>
<td>Pro-Β-carotene (mcg)</td>
<td>2231</td>
<td>2809</td>
<td>0.001</td>
</tr>
<tr>
<td>Lutein (mcg)</td>
<td>972</td>
<td>1214</td>
<td>0.016</td>
</tr>
<tr>
<td>Lycopene (mcg)</td>
<td>666</td>
<td>927</td>
<td>0.001</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>74.6</td>
<td>86.7</td>
<td>0.007</td>
</tr>
<tr>
<td>Vitamin E (a TE)</td>
<td>5.6</td>
<td>5.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

Servings per day

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Alzheimer disease cases</th>
<th>Controls</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow, green vegetables</td>
<td>2.0</td>
<td>2.3</td>
<td>0.022</td>
</tr>
<tr>
<td>Vitamin C fruits, vegetables</td>
<td>2.4</td>
<td>2.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Vitamin E and AD

Rather than capturing radicals, a better therapeutic strategy would be to reduce their production
Only one non-amyloid drug in phase III: 2010

- IVIg or Intravenous immunoglobulin (Baxter)
- Monoclonal Anti-AB Antibody (Elan-Wyeth; Lilly & Co.)
- Semagacestat or LY-450139 (Lilly & Co.)
- Dimebon (Medivation)
  - Neuroprotective agent, thought to work by protecting mitochondria in brain cells

Oxidative stress: cellular/animal models

- Cell death!!
- Selective cell death!! e.g., CA1 neurons
- Oxidative stress increases amyloid-β
  - (e.g., Beal SOD/APP)
- Antioxidants decrease amyloid-β
  - e.g., Papolla w/ melatonin
  - Cole w/ curcumin
  - But at best partial models….certainly not AD

Perhaps

- Oxidative stress is necessary, but not sufficient
- So what else is necessary?
What causes selective neuronal loss in AD?

- Almost every physiological/pathological mechanism that a cell can elicit has been implicated!
- And most are probably involved....
- At some point...

Fundamental mechanisms

- Luteinizing hormone
  - Higher in AD/DS
  - High LH = poor cognition (receptor-dependent)
  - Agonist rescues OVX-mediated declines
  - Menopause is major risk factor for AD
- Leptin
  - Low in AD
  - *In vitro* leptin reduces phospho-tau through GSKβ
  - *In vitro/vivo* (CNDR8) leptin reduces amyloid/improves cognition
- Cell cycle alterations

Cell cycle related proteins in AD

- Cell cycle markers
- Telomeres/telomerase
- Bi-nucleation
- Chromosome instability

Causes or consequences?

Neurobiology of Aging, 21:173-176, 2000
Establishment of a new animal model

CaMKII-MYC mice

MYC is specifically induced in cerebral cortical and hippocampal neurons by depletion of doxycycline.

C-MYC (now referred to as MYC) is a member of a family of proto-oncogenes comprising C-MYC, N-MYC, and L-MYC. MYC encodes a transcription factor that, as part of a heterodimeric complex with MAX, regulates the expression of a multitude of genes involved in regulating cellular proliferation and growth. Overexpression of MYC is commonly associated with tumorigenesis. Where MYC exerts its neoplastic function by inducing autonomous cellular proliferation and cellular growth, blocking differentiation, and inducing genomic destabilization.

Cell cycle re-entry in CaMKII-MYC mice

PCNA
NeuN
Ki-67

Cell cycle re-entry in CaMKII-MYC mice (2)
Selective neurodegeneration in CaMKII-MYC mice

Selective neurodegeneration in CaMKII-MYC mice (2)

Cognitive deficits in CaMKII-MYC mice

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Cell cycle abnormalities

Proliferation: cancer
Cell death: Alzheimer disease

Models and/or Tx????
- Oxidative stress
- Mitochondrial alterations
- Cell cycle alterations
- Hormonal imbalances (leptin/gonadotropins)

Work well as preventatives, not as treatments
Why, why< why?

Back to the drawing board?

ALZ-50

Normal neuron
Pre-NFT
NFT
E-NFT

Oxidative stress
Cell cycle changes

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Alzheimer disease

Phospho-ERK
Mitogenic signal

Phospho-JNK
Oxidative signals

Phospho-p38


Oxidative stress and cell cycle changes occur earlier than Tau or amyloid-β

ALZ-50

Normal neuron

Pre-NFT

I-NFT

E-NFT

Oxidative stress

Cell cycle changes

But which one is first?


Control cases (pre-Alzheimer?)

Control group 1

Phospho-ERK
Mitogenic signal

Phospho-JNK

Phospho-p38
Oxidative signals

Control group 2


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Alzheimer disease

Phospho-ERK Phospho-JNK Phospho-p38
Mitogenic signal Oxidative signals

Two hit hypothesis

Are there more than two hits?

Risk
Age (Age in years) lbs
Mutations (50lbs)
Meno
ApoE4 (20lbs/Apoe4 allele)
Head injury (10-20lbs)
Smoking (5lbs)
Atherosclerosis (15lbs)

Protection
Reserve (100lbs)
Male gender (10lbs)
Exercise (10lbs)
Education (10lbs)
Antioxidant (10lbs)
ERT (10lbs)

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“The way to get things done is not to mind who gets the credit for doing them”
Benjamin Jowett (1817-1893), British theologian and classicist

Collaborators

- George Perry (Pathology)
- Lawrence M. Squire (Chemistry)
- Xiongwei Zhu (Pathology)
- Gemma Casadesus (Neurosciences)
- Hyung-gon Lee (Pathology)
- Robert P. Friedland (Neurology)
- Rudy Castellani (Pathology)
- Luke Swope (Physiology/Biophysics)
- Alfred Rimm (Epidemiology)
- Bruce Lamb (Genetics)
- Grace Petot (Nutrition)
- Paul Carey (Biochemistry)
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- Shigeru Chiba

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- University of Barcelona
- University of California, San Diego
- University of South Alabama
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- Thomas Jefferson University
- Kiyoshi Hiroki
- Tohoku University
- School of Medicine
- Atsushi Takeda
- University of California
- San Diego
- Donald Cleveland
- University of South Alabama
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- University of Genova
- Massimo Tabaton

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"If you don't have anything smart to say, say it with an English accent"