Mitochondrial dysfunction, common final pathway for aging and Alzheimer's disease: Therapeutical aspects

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The failure of Aβ directed treatment strategies leaves all hopes for better antidementia drugs behind.

The failures
1. Tarenflurbil, Semagacestat (LY-450139), Avagacestat (γ-secretase inhibitors)
2. Tramiprosate (inhibits Aβ aggregation)
3. Bapineuzumab and others (Aβ antibodies)
4. Aβ vaccination

Our hope
Drugs which protect mitochondria and/or improve their function
Mitochondrial dysfunction, the common player

PS1- Mutations → APP- Mutations → Trisomy 21

- Altered APP processing
  - Oligomeric, intracellular Aβ
  - Fibrills/ Extracellular aggregation
  - Neuritic Plaques

- Tau → Neurofibrillary tangles

- Reduced mitochondrial membrane potential
  - reduced ATP levels
  - elevated ROS production
disturbed calcium regulation

- Enhanced Apoptosis
  - Mitochondrial and synaptic dysfunction

Complex I dysfunction leads to Aβ generation in vivo

**FIG. 7.** Enhanced Aβ levels in a neuronal cell model and two animal models of complex I dysfunction. (A) SH-SY5Y cells were treated with rotenone (0.25, 2.5, and 25 μM) for 24 h and ATP and MMP as well as soluble Aβ1-40 levels (B) were determined \( n = 6 \pm \text{SEM} \), unpaired *t*-test, (A) \(*p < 0.05\), \(**p < 0.01\), ATP ctrl against rot, \(#p < 0.01\), \(***p < 0.001\) MMP against rot. (B) \(*p < 0.01\) ctrl against rot 2.5 μM, \(***p < 0.001\) ctrl against rot 25 μM. (C) APP transgenic animals received rotenone via i.p. application (10 mg/kg/ body weight) or vehicle for 3 days and soluble Aβ1-40 levels in brain homogenates were measured. \( n = 6 \pm \text{SEM} \) per treatment group unpaired *t*-test \(*p < 0.05\). (D) In homozygous knockout (KO) Ndufs4 mice, Aβ1-40 levels are significantly increased compared to wild-type (wt) and heterozygous (HET) mice. Ndufs4 mice wt \( n = 5 \pm \text{SEM} \), HET \( n = 5 \pm \text{SEM} \), KO \( n = 12 \pm \text{SEM} \); unpaired *t*-test; \(*p < 0.05\) wt against KO, \(#p < 0.05\) HET against KO.
Aβ accumulation and CSF turnover

Graphs of the concentration of Aβ40 and Aβ42 in cerebral cortex plus hippocampus, and CSF turnover plotted against age. Aβ40 (dashed line, diamonds) and Aβ42 (solid line, squares) in pg/mg total protein concentrations are plotted on the left ordinate (3 months, n= 8; 12 months, n = 8; 20 months, n = 8; 30 months, n = 7). CSF turnover (dotted line, circles) is plotted on the right ordinate. Error bars for Aβ are SEM. CSF turnover is calculated from the means of CSF production and volume measurements. Note that Aβ40 and Aβ42 concentrations increase before there is a significant decrease in CSF turnover. Only later (after 12 months) in the lifespan of the F344/BN rat is there an inverse relationship between Aβ accumulation and the CSF turnover rate.

Chiu, C. et al., Fluids and Barriers of the CNS 9 (3), 2012
Mitochondrial dysfunction, the common player

PS1- Mutations     APP- Mutations     Trisomy 21

Neuritic Plaques

Tau

Neurofibrillary tangles

Altered APP processing

Oligomeric, intracellular Aβ

Fibrills/ Extracellular aggregation

Neuritic Plaques

- Reduced mitochondrial membrane potential
  - reduced ATP levels
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Enhanced Apoptosis

Mitochondrial and synaptic dysfunction

Membrane fluidity, ROS

Complex I II III IV V

Aging

1. Mitochondrial cascade hypothesis of sporadic AD (Swerdlow): Interaction of individually high baseline Aβ production and individually high oxidative stress due to aging trigger the switch between „normal brain aging“ and the beginning of AD decades before clinical onset

´Swerdlow et al., 2010, 2012
1. Doubling of Aβ production leads to several mitochondrial defects (e.g. MMP, dynamics) but also to partial compensation e.g. enhanced glycolysis, and enhanced neuritogenesis by elevated sAPP production.

2. Additional complex I dysfunction (induced by rotenone) interferes with the compensation mechanisms and causes very pronounced defects (largely enhanced Aβ1-42, less sAPP, further reduction of neuritogenesis).

Stockburger et al. JAD in press
Enhanced sAPP production and increased neuritogenesis of SY5YAPPwt cells: Compensation gets lost following complex I dysfunction

Stockburger et al. JAD in press
Complex I inhibition in SY5YAPPwt cells: Aβ1-42 goes up and sAPP goes down

Stockburger et al. JAD in press
Conclusions I

1. Most sporadic (aging), histopathological (Aβ, Tau), and genetic (ApoE₄) risk factors of AD synergistically lead to mitochondrial dysfunction

2. Mitochondrial dysfunction explains most of the functional deficits of AD brains (impairment of energy metabolism, synaptic dysfunction and deficits of information transfer, loss of synapses and synaptic contacts, loss of neurons by apoptosis

3. Mitochondrial improvement should be a relevant therapeutic approach
Mitochondrial protection, which is the best choice?

- Flavonoids
- Cholesterol derivatives
- $\omega$-fatty acids
- Statins
- Piracetam
- Levetiracetam
- Ginkgo (EGB761)
- Curcumine
- Methylene Blue
- Dimebon
- Complex I and IV
- Membrane
- PPAR/sAPPα
- Bcl-2
- ROS
- MMP/AB
- Neuritogenesis
- MMP/ROS
- MMP
- ROS

The three antidementia drugs: Pharmacological profiles

1) Ginkgo biloba extract EGb761®
Antioxidant and radical properties, mitochondrial protection, PAF antagonism

2) Piracetam
Improvement of membrane fluidity leads to improvement of neuronal and mitochondrial function

3) Dimebon
Multiple receptor antagonism, Antagonism of PTP, mitochondrial improvement
Dimebon, no effect in control cells, but significant restoration of mitochondrial dynamics in HEK\textsubscript{sw} cells

**Fig. 2.** In HEK\textsubscript{sw} cells mitochondria are highly fragmented. Incubation with Dimebon for 6 h significantly shifts mitochondrial morphology in HEK\textsubscript{sw} cells to tubular shape.

A HEK\textsubscript{ut} cells were incubated with Dimebon for 6 h. Mitochondria were labelled with Mito Tracker CMXRos, fixed with PFA, and mitochondrial lengths were quantified with Image J. B HEK\textsubscript{ut} cells were incubated with Dimebon for 6 h. Mitochondria were labelled with Mito Tracker CMXRos, fixed with PFA, and mitochondrial lengths were quantified with Image J. C Representative images of mitochondria in HEK cells in the presence and absence of Dimebon. Data represent the means ± SD with at least 100 measured mitochondria per experiment, n = 8-9, Two-way ANOVA with Bonferroni post tests, **p<0.01, ***p<0.001.

**Dimebon increases Oxphos activity in HEK\textsubscript{sw} but not in HEK\textsubscript{ut} cells**

A HEK\textsubscript{ut} cells were incubated for 6 h with dimebon (0.1 µM). Respiration was measured using protocol one. B HEK\textsubscript{sw} cells were incubated for 6 h with dimebon (0.1 µM). Respiration was measured using both protocols A and B. Using the Oxygraph-2k, oxygen consumption in different mitochondrial stages was measured by injecting several substrates and inhibitors. CI+II\textsubscript{Leak}, respiration on CI+II substrates to compensate for proton leak, is considered as state L; CI\textsubscript{OXPHOS}, CI dependent oxidative phosphorylation; CI\textsubscript{OXPHOS}; CII \textsubscript{OXPHOS}, CII dependent oxidative phosphorylation; CI+II\textsubscript{OXPHOS}, oxidative phosphorylation providing CI and CII substrates, is considered as state P; CI+II\textsubscript{ETS}, non-coupled respiration with CI and CII substrates, is considered as maximum capacity of the ETS – state E; CII\textsubscript{ETS}, non-coupled CII dependent respiration. CIV\textsubscript{ETS}, non-coupled respiration with CIV substrates. Values represent the means ± SD from n = 6-9 experiments per protocol. Two-way ANOVA with Bonferroni post tests, *p<0.05, **p<0.01, ***p<0.001. C Ratios

Eckert et al., J Alzheimer's Dis (2012)
Activity of the mPTP is reduced by cyclosporin A and dimebon (calcium induced swelling of mouse brain mitochondria)

## Piracetam – previous studies on mitochondrial protection

<table>
<thead>
<tr>
<th>Model</th>
<th>Effects</th>
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<tbody>
<tr>
<td>NMRI mice</td>
<td>• SOD, GR u. GPx↓ in aged animals</td>
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<tr>
<td></td>
<td>• Mitochondrial membrane potential↑ in aged animals</td>
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<tr>
<td></td>
<td>• Mitochondrial membrane potential↑ after H\textsubscript{2}O\textsubscript{2}, Aβ\textsubscript{1-42} or SNP insult</td>
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<tr>
<td>Tg AD mice</td>
<td>• Aβ↓</td>
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<tr>
<td></td>
<td>• ATP production↑</td>
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<tr>
<td></td>
<td>• Mitochondrial membrane potential↑</td>
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<tr>
<td>HEK APP\textsubscript{wt}</td>
<td>• Aβ↓</td>
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<tr>
<td></td>
<td>• Aβ↓ after SNP insult</td>
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<td>• Mitochondrial membrane potential↑</td>
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<tr>
<td></td>
<td>• Mitochondrial membrane potential↑ after SNP insult</td>
</tr>
<tr>
<td>PC12 cells</td>
<td>• ATP production↑ after serum deprivation and or SNP insult</td>
</tr>
<tr>
<td></td>
<td>• Mitochondrial membrane potential↑ after serum deprivation and Aβ\textsubscript{1-42} or SNP insult</td>
</tr>
<tr>
<td></td>
<td>• Neurite outgrowth↑ after Aβ\textsubscript{1-42} or SNP insult</td>
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<tr>
<td></td>
<td>• Caspase 9 activity ↓ after SNP insult</td>
</tr>
<tr>
<td>PC12 APP\textsubscript{wt/sw} cells</td>
<td>• Neurite outgrowth↑</td>
</tr>
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Piracetam improves impaired neuritic outgrowth in PC12 cells expressing very low or intermediate (APPsw) levels of human Aβ.

Piracetam restores mitochondrial morphology in a concentration dependent manner

Piracetam inhibits PTP following calcium or atracyloside activation

Stockburger et al., unpublished
Conclusions II

1. Mitochondrial protection by piracetam, Ginkgo extract, and dimebon not only improves mitochondrial function in models of brain aging and AD but also ameliorates major consequences of mitochondrial damage like enhanced Aβ production and deficits of synaptic plasticity.
Mitochondriale Biologie

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Safety and Tolerability of the γ-Secretase Inhibitor Avagacestat in a Phase 2 Study of Mild to Moderate Alzheimer Disease
Vladimir Coric et al.
Arch. Neurol. 2012. 69(11): 1430-1440

Outcome after 24 weeks

- Worsening of ADAScog by about 3 points
- Worsening of MMSE by 2 points
- Many severe side effects (GI, skin, cardiovascular etc.)
Mitochondrial dysfunction (Oxphos activity) increases with aging in Thy1-APP mice

State 3 respiration and the respiratory control ratio are decreased in 17 months old but not in 6 months old Thy1-APP mice relative to control animals

Hauptmann et al., (2009) Neurobiology of Aging
The three antidementia drugs: Evidences of clinical efficacy

1) Ginkgo biloba extract EGb761®
   Positive effects on cognition in AD - patients in recent metaanalyses

2) Piracetam
   Positive effects on cognition over several groups of patients with geriatric memory disorders

3) Dimebon
   Positive effects on NPI over several studies
Treatment with piracetam (500 mg/kg, 14 days) normalised reduced mitochondrial membrane potential (MMP) and ATP levels in dissociated brain cells of APP transgenic mice.

Kurz et al., *British Journal of Pharmacology* 2010
Mice transgenic for mutant human APP (swedish mutation) and non-transgenic littermates were treated for 14 days with piracetam (500mg/day orally).

Soluble Aβ₁₋₄₀ was determined in brain homogenates by Elisa.

Kurz et al., British Journal of Pharmacology 201
Mitochondrial dysfunction in Alzheimer’s disease. The important mutual interaction with brain aging

Our experimental model systems:

1. NMRI mice (3, 12, 22 months of age)
2. PC12 cells, SY5Y cells, or HEK cells transgenic for human APP or the swedish APP double mutation
3. SY5Y cells transgenic for human APP
4. Mice transgenic for the swedish APP double mutation (Thy1-APP)
5. Mice transgenic for the human Tau mutant P301L
6. Freshly isolated lymphocytes from non-demented aged controls, patients with minimal cognitive impairment (MCI), and Alzheimer patients (AD)
Mainly complex I function is impaired during aging

Complex I activity and mitochondrial function significantly reduced in aged NMRI mice.

Impaired mitochondrial function in Alzheimer cell and animal models

Pronounced mitochondrial dysfunction associated with reduced complex IV activity at an age of three months in AD animal model.