Neuroprotective Strategies in Neurodegenerative Disorders

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Neuroprotection

DAAergic Cell Function
Age

Appearance of symptoms

Neurorescue

DAAergic Cell Function
Age
GRAND CANYON EFFECT
Neuroprotection-Neurorescue In Neurodegenerative Diseases

• Healthy food
  Green tea
  Red wine
  Blueberries

• Exercise

• Intellectual activities
GRAND CANYON EFFECT
Neuroprotection-Neurorescue In Neurodegenerative Diseases

- Healthy food
  - Green tea
  - Red wine
  - Blueberries
- Exercise
- Intellectual activities

Neuroprotection

Neurorescue

NEUROPROTECTIVE DRUG

NEURORESCUE DRUG
GRAND CANYON EFFECT
Neuroprotection-Neurorescue In Neurodegenerative Diseases

- Healthy food
  Green tea
  Red wine
  Blueberries

- Exercise

- Intellectual activities
Neurodegeneration

Stroke:
- Oxygen deprivation
- Glucose deprivation
- Glutamate/neurotoxins release

Traumatic brain and spinal-cord injury:
- Physical damage
- Glutamate/neurotoxin release
- Ischemia

Aging

Malnutrition

Alzheimer’s disease:
- β-amyloid
- Presenilins
- Apolipoprotein E

Parkinson’s disease:
- α-synuclein, Parkin, LRRK2 etc
- Toxins (rotenone, iron)

Huntington’s disease:
- Huntingtin (polyglutamine)

Amyotrophic Lateral Sclerosis (ALS)

Oxidative stress
Metabolic impairment
Ion dyshomeostasis
DNA damage

mitochondrial membrane permeabilization

synaptic dysfunction
neuritic degeneration
neuronal death

Inflammation
Accumulation of iron
Increase in reactive oxygen and nitrogen species
Glutamatergic excitotoxicity
Mitochondrial (complex I deficiency)
Ubiquitin-proteasome system dysfunction
Abnormal protein folding and aggregation
Decline in growth factors levels
Therapeutic Approaches in Alzheimer’s disease
The main potential therapeutic approaches to multifactorial AD

1. **Cholinesterase inhibitors** - Boosting residual cholinergic neurotransmission would reduce symptoms of the illness. Cholinesterase inhibitors are thought to accomplish this by inhibiting acetylcholinesterase.

2. **Anti-excitotoxic strategies** - Under pathologic conditions, excessive activation of receptors by glutamate kills cells, and there is evidence that the pathologic cascade of AD includes an excitotoxic component. **Memantine**, a non-competitive NMDA antagonist blocks glutamate-mediated excitotoxicity (in case of over-activation of the receptors) without alteration of the physiological activation of the NMDA receptor during neurotransmission.

3. **Anti-inflammatory agents** - Inhibit chronic inflammatory processes in the AD brain. There is abnormal activity of several aspects of immune function in AD (e.g., reactive microglia surround amyloid plaques, astrocyte proliferation, increased inflammatory cytokines and free radicals). Epidemiological evidence suggests that use of nonsteroidal anti-inflammatory medication earlier in life may reduce the risk of developing AD.
4. **Antiamyloid Strategies** - According to the amyloid hypothesis, inhibition of $\beta$- or $\gamma$-secretase could reduce $A\beta$ production and diminish subsequent pathologic consequences of its abnormal regulation.

5. **Amyloid vaccine** - immunization with aggregated $A\beta$ induces antibody response. Elicited antibody binds to and facilitates clearance of $A\beta$.

6. **Metal complexing agents and antioxidants** - There are theoretical reasons as well as clinical data to suggest that free radical damage may cause neuronal degeneration in a range of conditions including aging and AD. Studies have found evidence of increased levels of oxidative damage to neurons, proteins, DNA, and lipids in AD as well as accumulation of iron at sites where neurons degenerate in AD. Thus, treatment with iron-chelators will:

1. Abstract iron, copper and zinc from $A\beta$ plaques.
2. Inhibit iron and copper-dependent neurotoxicity.
3. Facilitate amyloid plaque disaggregation.
The ClinicalTrials.gov (http://clinicaltrials.gov/) currently contains around 30 registered clinical trials in PD and AD with antioxidants and nutritional supplements (e.g. vitamins E and C, alpha-lipoic acid, creatine, melatonin, omega-3 polyunsaturated fatty acids, CoQ10, curcumin, resveratrol, glucose, malate), individually or in cocktail formulation that are supported by The National Institute of Health (NIH; USA), other federal agencies and private industry.
Therapeutic approaches targeting amyloid β-protein production and oligomerization

Amyloidogenic derivatives:
- sAPPβ
- Aβ
- β–CTF

Non-amyloidogenic derivatives:
- sAPPα
- α–CTF
Therapeutic approaches targeting amyloid β-protein production and oligomerization.
Pathologically AD is defined by:

1. Neuronal loss
2. Extracellular insoluble deposition of amyloid or senile plaques (composed mainly of Aβ)
3. Intracellular lesions: neurofibrillary tangles (composed mainly of hyperphosphorylated microtubule associated protein, tau)
Targeting Aβ oligomers

- γ-secretase modulators
- The non-steroidal anti-inflammatory drugs (NSAIDs) R-flurbiprofen (Flurizan), which lacks cyclo-oxygenase inhibitory activity,

Does not block the γ-secretase cleavage but rather shift its cleavage site from the rapidly aggregating 42-residue variant to the far less amyloidogenic 38-residue form (shaded amino acids are in the transmembrane domain). This shift will not affect potential signalling functions of γ-secretase substrates.

Myriad Genetics: Results of U.S. Phase 3 Trial of Flurizan™ in Alzheimer's Disease:

Did not achieve statistical significance on either of its primary endpoints -- cognition and activities of daily living.
Targeting Aβ oligomers

• γ-secretase modulators

Semagacestat (Eli Lilly & Co.)
Halted in August when preliminary results of an ongoing phase 3 study showed that the drug failed to slow disease progression among more than 2,600 patients with mild to moderate AD, and actually worsened their cognitive decline and ability to perform activities of daily living.

In addition, semagacestat was associated with an increased risk of skin cancer.
Targeting Aβ oligomers

• Active and passive immunization against amyloid-beta (Abeta) are employed to clear and reduce cerebral Abeta towards treatment of AD patients.

• Limitation: A Phase 2 trial of an Aβ1–42 vaccine in patients with AD immunization with Aβ42 (AN1792, Elan Pharmaceuticals) in September, 2000 was associated with the development of a T-cell-mediated, autoimmune meningoencephalitis in 6% of patients, leading to cessation of dosing.
Long-term effects of Aβ₄₂ in Alzheimer's disease: follow-up of a randomised phase I trial

Clive Holmes, Delphine Boche, David Wilkinson, Ghassan Yaqoot, James W Neal, Elina Zlotova, James A R Nicoll

Summary
Background Immunisation of patients with Alzheimer's disease involves amyloid plaques from the brain. Our aim was to assess the effect of plaque removal and long-term clinical outcomes.

Methods In June 2003, consent for long-term clinician follow-up was sought from 80 patients (or their carers) of immunisation with Aβ₄₂ (AN1792, Elan Pharmaceuticals) in September 2006. Plaques were assessed in terms of plaque burden and in terms of characteristic histology until severe dementia or death was assessed.

Findings 20 participants—15 in the AN1792 group and further 22 participants—19 in the AN1792 group, died. The remaining participants in the AN1792 group were immunised with Aβ₄₂. Case 1 died 4 months after the first immunisation and showed an early stage of plaque clearance. Cases 2-8 survived 20-64 months after the first immunisation. Cases 9-16 died 8-16 months after the first immunisation. Cases 17-22 showed an intermediate range of plaque clearance. Cases 7 and 8 showed very extensive (case 9) and nearly complete (case 8) removal of Aβ plaques throughout the cerebral cortex. All the long-term survivors (cases 2-8) continued to have progressive dementia with cognitive function declining to an unrecordable level (i.e., MMSE<0) before death.

Interpretation Although immunisation with Aβ₄₂ in Alzheimer's disease, this clearance did not prevent the progression of dementia.

Funding Alzheimer's Research Trust, Medical Research Council, Department of Health, and the Wellcome Trust.
Amyloid-β Immunotherapy continues with more than 13 therapies in clinical trials (http://www.clinicaltrials.gov)

Can Alzheimer Disease Be Prevented by Amyloid-β Immunotherapy?
Cynthia A. Lemere; Eliezer Masliah
Nat Rev Neurol. 2010;6(2):108-119
Targeting Aβ oligomers

- Active and passive immunization against amyloid-beta (Abeta) are employed to clear and reduce cerebral Abeta towards treatment of AD patients. **Limitation**: A Phase 2 trial of an Aβ1–42 vaccine in patients with AD was associated with the development of a T-cell-mediated, autoimmune meningoencephalitis in 6% of patients, leading to cessation of dosing.

- Inhibition of Abeta production via **antibodies against the beta-secretase cleavage site** of the amyloid precursor protein (APP). Solomon B (Tel Aviv univ), anti-APP beta-site antibodies to Tg2576 transgenic mice improved mouse cognitive functions associated with a reduction in both brain inflammation and the incidence of microhemorrhage. Furthermore, antibody treatment did not induce any peripheral autoimmunity responses.
Targeting Aβ oligomers

- Selective degradation of oligomers and fibrils and destabilization of Aβ oligomers.
  Proteoglycans and their constituent glycosaminoglycans are associated with amyloid plaques in AD brain tissue and might stabilize the aggregates and make them more resistant to proteolysis.

- One compound ("Alzhemed", tramiprosate,) designed to prevent Aβ from interacting with glycosaminoglycans and proteoglycans

**Failed at Phase 3 AD trial**
Targeting amyloid-beta

## Progress

<table>
<thead>
<tr>
<th>Mechanism of action</th>
<th>Product</th>
<th>Company</th>
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<tbody>
<tr>
<td>Aβ Secretase Inhibitors</td>
<td>R-flurbiprofen</td>
<td>Myriad Genetics</td>
</tr>
<tr>
<td></td>
<td>LY450139</td>
<td>Eli Lilly</td>
</tr>
<tr>
<td>Aβ Immunization / mAbs</td>
<td>Bapineuzumab</td>
<td>Elan/Wyeth</td>
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<tr>
<td></td>
<td>RN1219</td>
<td>Rinat/Pfizer</td>
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<tr>
<td></td>
<td>CAD-106</td>
<td>Cytos Biotechnology</td>
</tr>
<tr>
<td>Aβ Aggregation inhibitors</td>
<td>Tramiprosate</td>
<td>Neurochem</td>
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<tr>
<td></td>
<td>PBT2</td>
<td>Prana Biotechnology</td>
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<tr>
<td></td>
<td>AZD-103</td>
<td>Transition</td>
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<td></td>
<td></td>
<td>Therapeutics/Elan</td>
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</table>

- **Failed**: JANSSEN (TAU) – ApoE4 excluded
- **Ongoing**:

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*Expert Opin. Invest. Drugs 2007, 16(8), 1183-1196*
Targeting Tau and Microtubules

- Exciting potential for disease modification
- Fundamental mechanism important across a number of CNS diseases

<table>
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<th>Mechanism of action</th>
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<th>Company</th>
<th>Phase</th>
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<tr>
<td>Microtubule and tau</td>
<td>AL-108</td>
<td>Allon Therapeutics</td>
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<td>NP031112</td>
<td>Neuropharma</td>
<td>I</td>
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<td>SAR-502250</td>
<td>Sanofi Aventis</td>
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<td></td>
<td>SRN-003-556</td>
<td>Sirenaide</td>
<td>Preclinical</td>
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</table>

Allon (TAU) continues

Expert Opin. Invest. Drugs 2007, 16(8), 1183-1196
Neuroprotection in Parkinson’s disease
<table>
<thead>
<tr>
<th>PD Research Portfolio</th>
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<tbody>
<tr>
<td><strong>Human genetics</strong> - Identifying new genes involved in familial PD</td>
</tr>
<tr>
<td><strong>Genomics</strong> - Using information from the DNA sequence of the human genome to aid in genetic studies, and to search for the expression of genes associated with the disease</td>
</tr>
<tr>
<td><strong>Animal Models</strong>: Transgenic mice, transgenic rats and Drosophila (fruit flies)</td>
</tr>
<tr>
<td><strong>Assay development and high throughput drug screening</strong></td>
</tr>
<tr>
<td><strong>Cell replacement/Stem cell</strong> research as potential for replacing dying neurons</td>
</tr>
<tr>
<td><strong>Providing important trophic factors</strong> to dying cells and <strong>Gene Therapy and Diagnostic Biomarkers</strong></td>
</tr>
</tbody>
</table>
# Current treatments for PD

- Levodopa drugs
- Dopamine agonists
- Catechol-O-methyl transferase (COMT) inhibitors
- Anticholinergics
- MAO-B inhibitors
- Amantidine

# New PD Treatments on the Horizon

- **Symptomatic drugs**
  - Opioid antagonists
  - NMDA antagonists
  - Adenosine A2a receptor antagonists. Interact with the specific dopamine receptor subtype D2 in the basal ganglia, making it more sensitive to dopamine. **SYN-115 Phase IIa ended**
- **Neuroprotective agents**
- **Neural tissue transplants**
- **Cell implants e.g. genetically engineered dopamine producing cells**
## Drugs Selected for Investigation In the Neuroprotection Clinical Trial

<table>
<thead>
<tr>
<th>Drug</th>
<th>Primary Mechanism</th>
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<tbody>
<tr>
<td>Coenzyme Q10</td>
<td>Antioxidant/Mitochondrial Stabilizer</td>
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<tr>
<td>Creatine</td>
<td>Mitochondrial Stabilizer</td>
</tr>
<tr>
<td>GPI 1485</td>
<td>Trophic Factor</td>
</tr>
<tr>
<td>Minocycline</td>
<td>Anti-inflammatory/Anti-apoptotic</td>
</tr>
<tr>
<td>Rasagiline</td>
<td>Anti-oxidant/Anti-apoptotic</td>
</tr>
</tbody>
</table>

## Drugs Under Consideration for Future Study

<table>
<thead>
<tr>
<th>Drug</th>
<th>Primary Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amantadine</td>
<td>Glutamate Antagonant</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Azulenyl Nitrone</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Adenosine Antagonant</td>
</tr>
<tr>
<td>COX I-II Inhibitors</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Undetermined/Multiple</td>
</tr>
<tr>
<td>Estrogen</td>
<td>Undetermined/Multiple</td>
</tr>
<tr>
<td>Folate</td>
<td>Undetermined/Multiple</td>
</tr>
<tr>
<td>GM-1 ganglioside</td>
<td>Trophic Factor</td>
</tr>
<tr>
<td>Modafanil</td>
<td>Unknown</td>
</tr>
<tr>
<td>N-acetyl Cysteine</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Unknown</td>
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<tr>
<td>Pramipexole/Ropinirole</td>
<td>Antioxidant/Vesicular Trafficking</td>
</tr>
<tr>
<td>Remacemide</td>
<td>Glutamate Antagonant</td>
</tr>
<tr>
<td>Selegiline</td>
<td>Antioxidant/Anti-apoptotic</td>
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</tbody>
</table>
Providing important **trophic factors** to dying cells

Glial-derived neurotrophic factor (GDNF), a protein thought to affect dopamine synthesis, stored and uptake. (Amgen Inc.)

Subjects having pumps inserted in their abdomen and holes drilled in their skull.

**Phase II trial.** A six-month placebo phase during which time half of the research participants would receive no treatment whatsoever, while the other half received GDNF.

In 2004 Amgen received results from certain primate studies on GDNF in which four out of seventy monkeys that were given GDNF suffered **cerebellar toxicity** at doses 10 times higher than used in humans. Could result of sudden withdrawal of the drug rather than the drug itself??

Some patients developed **antibodies to GDNF**

**Trial was stopped**

Recent lawsuits involving the safety of drugs like Vioxx were a huge factor.

**Vaccination (preclinical)**

Triggering the immune system to prevent neuronal death and its manifestation into PD is promising. (Benner et al., PNAS, 2004, 101: 9435-9440)
Ceregene _ A biotechnology company, Gene therapy with GDNF family ligand, Neurturin.

In April 2010, the company announced further phase II trials of Neurturin, even though previous attempts found no evidence of benefit for Parkinson's disease symptoms in patients: two autopsies of patients from the first trial suggested that neurturin had failed to stimulate new dopaminergic connections from the substantia nigra. *Delivery problem?*

**TRANSEURO** Europe’s premiere clinical study on the treatment of Parkinson’s Disease (PD) patients using a cell therapy approach is a 5-year Collaborative Project supported through the FP7 European Commission Health programme, contract number 242003.
Sources for implantable cells

• Fetal tissue and cultured stem cells from embryonic sources
• Cells from the adrenal medulla and retinal pigment epithelium (RPE) as source of DA.

• Recent advances: induced pluripotent stem cells, which are produced by genetic treatment of adult cells from skin or other tissues, may provide cells suitable for therapeutic transplantation, as well as for in vitro drug screening.
DA neurons implanted into people with PD survive without pathology for 14 years

- Postmortem analysis of 5 subjects with PD 9–14 years after transplantation of fetal midbrain cell suspensions revealed surviving grafts that included dopamine and serotonin neurons without pathology despite ongoing degeneration of DA neurons in the host brain.

Brief Communication
Published online: 6 April 2008 | doi:10.1038/nm1747

Lewy body–like pathology in long–term embryonic nigral transplants in Parkinson's disease

Jeffrey H Kordower¹, Yaping Chu¹, Robert A Hauser², Thomas B Freeman³ & C Warren Olanow⁴

A case report from 2008 described pathological changes within the grafted neurons of a patient with PD who died 14 years posttransplantation (as evidenced by α–synuclein and ubiquitin staining).
Main results

- Some of the grafted neurons were identical in staining pattern and morphology to neurons of the host striatum.


Brief Communication


Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation

Jia-Yi Li¹, Elisabet Englund², Janice L Holton³, Denis Soulet¹, Peter Hagell⁴, Andrew J Lees³, Tammaryn Lashley³, Niall P Quinn⁵, Stig Rehncrona⁶, Anders Björklund⁷, Håkan Widner⁴, Tamas Revesz³,⁹, Olle Lindvall⁴,⁸,⁹ & Patrik Brundin¹,⁹

The disease seems to have spread from the host to the graft.

Curing Parkinson disease with grafted tissue?
Present perception of neurodegeneration: multi-etiological progressive nature involving various pathological and molecular events occurring in parallel or sequentially.
A Cocktail of Drugs as a Better Therapy for Neuroprotection, Which ones??

- Anti-Inflammatory Drugs
- Polyphenols
- Iron Chelators
- NEUROPROTECTION
- MAO-B Inhibitors
- iNOS Inhibitors
- DA Agonists
- Antioxidants
- Glutamate Antagonists
Novel Pharmacological Strategies for Neuroprotection

Polypharmacology- Cocktail of drugs

Multifunctional Compounds- Drugs acting on various brain targets
Neuroprotection-Neurorescue In Neurodegenerative Diseases

- Healthy food
  - Green tea
  - Ginko Biloba
  - Red wine
  - Pomegranate
  - Blueberries
  - Turmeric

- Exercise

- Intellectual activities
Green tea polyphenols

Green and black teas come from the same plant, Camellia sinensis. The differences are in the way they are grown, harvested, and processed.

- Potent oxygen and nitric radical scavenging
- Indirect antioxidant effects through activation of transcription factors and antioxidant enzymes
- Iron chelating
- Anti-inflammatory activities
- Neuroprotective in vitro and in vivo against several neuroroxins

Polyphenol content in green tea extract:
- EGCG > EGC > EC > ECG
Multifunctional Activities of Green Tea Catechins

Reactive oxygen and nitric oxide species radical scavengers

Indirect antioxidants through
• activation of transcription factors
• antioxidant enzymes (SOD, catalase)
• Phase II detoxifying enzymes (GST)

Divalent metal (iron, copper) chelators

Anti-inflammatory
Anti-bacterial
Anti-viral
Anti-fungal

Anti-proliferative
Anti-carcinogenic
Anti-angiogenic

Anti-obesity
Decrease:
• Glucose (activation of glycolitic enzymes)
• triglycerides
• Lipogenesis (inhibition of fatty acid synthase)
• Lipid intestinal absorption

Increase
Fat oxidation thermogenesis

Neuroprotective/neurorescue
• Mitochondrial membrane stabilization
• Inhibition of DA presynaptic transporters and catechol-O-methyltransferase (COMT) activity
• Activation of PKC
• Decrease of bad, bax
• Decrease of APP, Aβ and increase of soluble APPα

Cardiovascular system
Oxidized LDL
Atherosclerosis
cholesterol

Decrease of APP, Aβ and increase of soluble APPα

Fat oxidation thermogenesis
Prevention of MPTP induced Dopaminergic Neurotoxicity by Green Tea Polyphenol, EGCG. Tyrosine Hydroxylase Immunoreactivity

- Rasagiline, deprenyl (MAO-B inhibitors)
- Apomorphine (DA agonist)
- Clioquinol (Iron chelator)
- Melatonin (Antioxidants)
- Mynocyclin

![Image of brain sections with different treatments: Saline, MPTP, EGCG, EGCG+MPTP]
Effect of EGCG After Long-Term (72h, Neurorescue) Serum Starvation Period of PC12 Cells

A. Control  
Serum free  

- NGF (50 ng/ml)  
- EGCG (0.1 μM)  
- EGCG (1 μM)  
- EGCG (10 μM)  

B.  

<table>
<thead>
<tr>
<th>Condition</th>
<th>Folds of control</th>
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<tr>
<td>Full serum</td>
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<tr>
<td>EGCG (μM) 0.1</td>
<td>4</td>
</tr>
<tr>
<td>EGCG (μM) 1</td>
<td>8</td>
</tr>
<tr>
<td>EGCG (μM) 10</td>
<td>12</td>
</tr>
<tr>
<td>NGF (50ng/ml)</td>
<td>15</td>
</tr>
</tbody>
</table>

* p < 0.05 vs control
EGCG Promotes Differentiation of PC12 Cells; Expression of GAP43

**GAP-43 expression**

**Control**

**2 days**

**4 days**

**EGCG (0.1 µM)**

**EGCG (1 µM)**

**Neurite outgrowth**

* p<0.05 vs Full Serum 2d
** p<0.01 vs Full Serum 4d

Full Serum

2-4 days EGCG treatment

* p<0.05 vs Full Serum 2d
** p<0.01 vs Full Serum 4d
**EGCG Activates PKC Isoforms in Neuroblastoma SH-SY5Y**

A.  

<table>
<thead>
<tr>
<th>Condition</th>
<th>p-PKC (pan)</th>
<th>β-actin</th>
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<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
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<tr>
<td>EGCG (15min)</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
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B.  

- **Control**  
  - ![Image](image3)
  - ![Image](image4)

- **Serum free**  
  - ![Image](image5)
  - ![Image](image6)

**EGCG 1μM**  
- ![Image](image7)
- ![Image](image8)

**EGCG 10μM**  
- ![Image](image9)
- ![Image](image10)

**p-PKC (pan)**  
- ![Image](image11)
- ![Image](image12)

**PKCα**  
- ![Image](image13)
- ![Image](image14)
APP processing

Plasma membrane → Cytoplasm

1. EGCG effect on holo-APP expression

Amyloidogenic derivatives:

- sAPPβ
- Aβ
- β− CTF

2. EGCG ability to promote non-amyloidogenic pathway

Non-amyloidogenic derivatives:

- sAPPα
- α−CTF

3. EGCG ability to inhibit Aβ secretion
### Involvement of α-Secretase in EGCG-Stimulated sAPPα Release

<table>
<thead>
<tr>
<th>Condition</th>
<th>EGCG (μM)</th>
<th>PMA (1 μM)</th>
<th>Ro31-9790 (100 μM)</th>
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</tr>
<tr>
<td>20</td>
<td>+</td>
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#### Neurofibrillary tangles

- 10
- 20
- 20

### Involvement of PKC Activity in EGCG-Stimulated sAPPα Release

<table>
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<tr>
<th>Condition</th>
<th>EGCG (μM)</th>
<th>PMA (1 μM)</th>
<th>GF 109203X (2.5 μM)</th>
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<tr>
<td>20</td>
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<td>+</td>
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#### Amyloid plaques

- Neuron
- Amyloid plaques

#### Non-amyloidogenic derivatives

- sAPPα
- α-CTF
Comparative analysis of the Fe$^{2+}$ chelating potency of EGCG and other iron chelators.

**Figure:**

- **Graph:**
  - **Y-axis:** Metal chelating effect (%)
  - **X-axis:** Concentration (log M)
  - **Data Points:**
    - DFO: $4.8 \pm 1.0 \times 10^{-6}$ M
    - EGCG: $4.9 \pm 1.1 \times 10^{-6}$ M

- **Equation:**
  - Chelating effect (%) = \[1 - \frac{\text{absorbance of sample at 562 nm}}{\text{absorbance of control, without drugs, at 562 nm}}\] \times 100

**Chemical Structures:**

- EGCG

**Note:**

- HLA20, VK28, M30: novel iron chelators.
Translational regulation of iron-responsive proteins

Iron chelation
Iron deficiency

IRP1  IRP2
IRE-BS  IRE-BS

Transferrin Receptor
IREx5
mRNA
3' 5'

Ferritin
IRE
mRNA
3' 5'

α-synuclein
APP

Fe metab
Fe(II) abolishes EGCG-induced differential regulation of APP and TfR

**APP**

| EGCG (µM) | - | 10 | 10 | 10 | - | - | - | - | - | - | - |
| DFO (µM)  | - | - | - | - | - | 10 | 10 | 10 | 50 | 50 | 50 |
| Fe$_2$SO$_4$ (µM) | - | 10 | 50 | - | - | 10 | 50 | - | 10 | 50 |

**TfR**

| EGCG (µM) | - | 10 | 10 | 10 | - | - | - | - | - | - | - |
| DFO (µM)  | - | - | - | - | - | 10 | 10 | 10 | 50 | 50 | 50 |
| Fe$_2$SO$_4$ (µM) | - | 10 | 50 | - | - | 10 | 50 | - | 10 | 50 |
EGCG modulates the translation of a luciferase reporter gene driven by the APP 5'-UTR sequences.

Iron deficiency
Iron chelation

**IRE**

**SV40 promotors**

**Luciferase**

**APP 3'-UTR**

Stop translation

Luciferase activity
Effect of EGCG on APP 5'-UTR conferred translation of a luciferase reporter mRNA in the human U-87-MG glioma cells

These results suggest that the reduction of APP by EGCG is modulated in part through chelation of iron.

* * *

\[ * p < 0.01, \text{ vs control} \]
EGCG elevates sAPP\(\alpha\) secretion and p-PKC isoforms

1. These results are consistent with previously shown PKC activation by EGCG.
2. EGCG may modulate APP processing via elevation of sAPP\(\alpha\) secretion
Proposed Mechanism of EGCG Action for the reduction of Aβ production

EGCG

Labile Fe(II) → p-PKC-α, -ε

α-secretase

sAPPα

Destabilization of Aβ fibrils

Aβ

Irreversible

APP translation

APP mRNA

RPs

IRE

5’

3’

NEUROPROTECTION/NEURORESCUE

NEUROPLASTICITY
GRAND CANYON EFFECT
Neuroprotection-Neurorescue In Neurodegenerative Diseases

- Healthy food
  - Green tea
  - Red wine
  - Blueberries

- Exercise

- Intellectual activities