Beta-amyloid in the Gastrointestinal Tract may Cause Cognitive Deficits: Protective Action of Soy Flavonoids

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Does Peripherally Aβ Contribute to AD?

- In AD patients, Aβ plaques are found in many other organs including the skin, subcutaneous tissues and the intestine. (Joachim, C.L., *Nature*, 1989)

- Amyloid deposit are seen in the antrum and duodenum of the GI tract ahead of the brain in transgenic AD mice. (Hui C.W., unpublished)

DAB staining on transgenic AD mice and its WT
The GI tract has an enteric nervous system with an extensive cholinergic component.

Could pathology first occur in the GI tract ahead of the brain?

Could pathology in the GI tract migrate to the brain?

Could treating GI tract early delay or prevent CNS degenerative changes?
Evidence for Prion-Like Activity of Aβ

• Intraperitoneal injection of Aβ seeds robustly induced amyloidogenesis in the brain of mice at 7 months. (Eisele, et al., Science, 2010)


In Parkinson’s Disease Rat Model:
Animal Models for Early Stage AD

Current commonly used AD animal models may be too aggressive and acute:

• Direct hippocampal/ intracerebroventricular injection of Aβ seems to have a low translational value.

• Transgenic models that overproduce Aβ, do not represent the majority of AD cases where environmental cases may contribute to an estimated 30% of AD cases (Dorszewska, et al., 2016).

There is an urgent need for new animal models...
Hypothesis: Aβ translocates from gut to brain
Administration of Aβ into GI Tract

Discrete injection of Alexa Fluor 647-tagged Aβ into the GI wall close to myenteric plexus

M: Mucosal layer
SP: Submucosal plexus
CM: Circular muscle
MP: Myenteric plexus
LM: Longitudinal muscle
E: Epithelial layer
Tracking Tagged-Aβ: Bruker Imaging System

Location of Aβ one month after administration into stomach

Excitation wavelength 600nm, emission wavelength 700nm and exposure of 5 seconds. Background image with the same laser settings was subtracted in all images.
Brainstem

ChAT (choline acetyltransferase) staining

Keys:
CC – Central canal
DMX – dorsal motor nucleus of the vagus nerve
XII – Hypoglossal nucleus
AP – Area Postrema
Brainstem

Blue – DAPI – Nucleus
Yellow – labelled βA
Red – ChAT – cholinergic neurons of the dorsal motor nucleus of vagus nerve
Aβ is Found in Vagus Nerves One Month After Injection into Stomach

Vagus nerve taken along esophagus. 1 month after surgical injection of 8μg in 4μL of Alexa Fluor 647-tagged Aβ into stomach serosa, fluorescent signal of Alexa Fluor 647 in green is seen along fibers of vagi in 3 out of 4 mice. Tissues were co-stained with DAPI in blue for nuclei and PGP9.5 in red for neuronal fibers. N = 4
Daily Intake of Soybean Flavonoids May Prevent Neurodegeneration and Cognitive Loss

- Soy flavonoids are phytoestrogens found in soy beans.

- Act via estrogen receptors, and considered safer option to estrogen replacement therapy.

- Soy flavonoids, daidzein, genistein have been shown to have neuroprotective actions, and can enhance memory performance in human studies. (Kajta, 2013) (Pan, et al., 2012) (Bagheri, 2011) (Pisani, 2012) (Zhao, 2000) (Thorpe, 2009) (File, 2001) (Gleason CE, 2015)
Can Chronic Administration of Soy Flavonoids Reduce the Spread of Aβ? – *in vitro* studies

**Aims:**

- Locate estrogen receptors
- Investigate if soy flavonoids protect the enteric nervous system against Aβ

1. Estrogen receptors (ERs) expression on enteric neurons

2. Aβ toxicity towards enteric neurons

3. Soy flavonoids to reverse neuronal loss induced by Aβ toxicity
Can Chronic Administration of Soy Flavonoids Reduce the Spread of Aβ? – *in vitro* studies

1. Estrogen receptor (ER) expression on enteric neurons

2. Aβ toxicity towards enteric neurons

3. Soy flavonoids to reverse neuronal loss induced by Aβ toxicity

Estrogen receptor alpha (ERα)
Estrogen receptor beta (ERβ)
G-protein coupled estrogen receptor 30 (GPR30)
Estrogen Receptors Are On Myenteric Neurons in Female ICR Mice

Estrogen receptor α

Estrogen receptor β

GPR30

Tissues were co-stained with DAPI in blue for nuclei and GFAP in red for glial fibers. N = 3
Estrogen Receptors Are On Myenteric Neurons in Male ICR Mice

Tissues were co-stained with DAPI in blue for nuclei and GFAP in red for glial fibers. N = 3
Aβ Internalized into Enteric Neurons Resulting in Neuronal Fiber Loss

Alexa Fluor 555 tagged oligomeric Aβ
24 h tissue culture colon longitudinal muscle myenteric plexus
Tissues were co-stained with DAPI in blue for nuclei and PGP9.5 in green for neuronal fibers or choline acetyltransferase (ChAT) in green for cholinergic neurons. N = 5
Can Chronic Administration of Soy Flavonoids Reduce the Spread of Aβ? – *in vitro* studies

- Estrogen receptor (ER) expression on enteric neurons
- Aβ toxicity towards enteric neurons
- Soy flavonoids to reverse neuronal loss induced by Aβ toxicity

- Vehicle (-ve control)
- β-estradiol (+ve control) (100nM)
- Daidzein (1μM)
- Genistein (1μM)
- Glycitein (1μM)
- Luteolin (1μM)
Estrogen and Soy Flavonoids Prevent the Neurotoxic Action of Aβ in Myenteric Neuronal Cell Cultures From the Colon

24 h tissue culture of colon longitudinal muscle myenteric plexus. Tissues were stained with PGP9.5 in green for neurons, and GFAP in red for glial cells.
Quantification of the Neuroprotective Action of Flavonoids on the Colon

PGP9.5 as general neuronal marker
ChAT (choline acetyltransferase) for cholinergic neurons
nNOS (neuronal nitric oxide synthase) for nitrergic neurons
GFAP as glial cell marker
Soy flavonoids as long term treatment against Aβ migration – *in vitro* work

- Estrogen receptor (ER) expression on enteric neurons
- Aβ toxicity towards enteric neurons
- Soy flavonoids to reverse neuronal loss induced by Aβ toxicity
Long Term *in-vivo* Studies

**Table 6.2. Surgical and drug treatment group arrangement.** [1] NH$_4$OH was used to dissolved βA(1-42) power in saline [2] Mixed formula was designed with the same proportion of soy flavonoids presence in soy bean milk, where daidzein: glycitein: genistein was 5: 1: 9. [3] 10 animals for 6 month and 10 animals for 12 month time point

<table>
<thead>
<tr>
<th>Label</th>
<th>Surgeries</th>
<th>Drug treatment</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>Injection with 0.4% NH$_4$OH in saline [1]</td>
<td>Vehicle (0.05% DMSO)</td>
<td>10/10$^{[3]}$</td>
</tr>
<tr>
<td>β</td>
<td>βA(1-42) injection</td>
<td>Vehicle (0.05% DMSO)</td>
<td>10/10</td>
</tr>
<tr>
<td>X</td>
<td>βA(1-42) injection</td>
<td>Daidzein</td>
<td>10/10</td>
</tr>
<tr>
<td>Y</td>
<td>βA(1-42) injection</td>
<td>Genistein</td>
<td>10/10</td>
</tr>
<tr>
<td>Z</td>
<td>βA(1-42) injection</td>
<td>Glycitein</td>
<td>10/10</td>
</tr>
<tr>
<td>M</td>
<td>βA(1-42) injection</td>
<td>Mixed formula$^{[2]}$</td>
<td>10/10</td>
</tr>
</tbody>
</table>
Long Term *in-vivo* Studies

**Aims:**
To investigate if Aβ injected into the GI tract can induce GI dysfunction and cognitive deficits in studies lasting up to 12 months.

1. Surgical injection of Aβ along GI tract & initiation of soy flavonoid treatment
2. Memory task 6/12 month after surgery
3. GI tract functional studies
4. Molecular and histochemical studies
At 6 months, Aβ had made learning more difficult

Hunger-driven T-maze memory task

- Aβ group spent more time to decide which arm to enter.
- Deficits were compensated on longer training times.
Memory deficits were found at 12 months

- Aβ group showed significant memory deficits in the NOR task.
- Deficits were reversed by soy flavonoids.
Long Term *in-vivo* Studies

1. Surgical injection of Aβ along GI tract & initiation of soy flavonoid treatment
2. Memory task 6/12 month after surgery
3. GI tract functional studies
4. Molecular and histochemical studies

Organ bath set-up
At 12 months, Aβ reduced the frequency of spontaneous contractions of the ileum.
Long Term *in-vivo* Studies

- Surgical injection of Aβ along G tract I & initiation of soy flavonoid treatment
- Memory task 6/12 month after surgery
- GI tract functional studies
- Molecular and histochemical studies
At 12 months, Aβ deposits were found in the hippocampus

DAB staining of immunohistochemistry of Aβ

Data adopted from Michelle Y.Y. SUN with permission
Glycitein is the best soy flavonoids at creating a neuroprotective environment

**Induction of neurotrophic factor**

- Nerve growth factor (NGF), Brain-derived neurotrophic factor (BDNF), Glial cell derived neurotrophic factor (GDNF)

**Increased estrogen receptor expression**

- Estrogen receptor alpha (ERα)
- G-protein coupled estrogen receptor (GPR30)

**Increased synaptic protein – synaptophysin (SNT)**

**Reduced expression of beta-secretase (BACE) for cleaving amyloid precursor protein**
Conclusion

• GPR30 are on enteric neurons of male and female mice.

• Aβ injected into the serosal layer of the GI tract internalized into neurons and translocated to the brain via the vagus nerves.
  • There was an associated change in spontaneous contractile frequency of the ileum.
  • Memory was impaired at 6 and 12 months.

• Early treatment with soy flavonoids had protective effects against Aβ-induced toxicity in the GI tract and could prevent cognitive deficits.
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