21st US-Japan Cellular and Gene Therapy Conference
Neurodegenerative Diseases: Biology, Cellular and Gene Therapy

FDA White Oak Campus, Building 31, Great Room, 1503 A
Silver Spring, Maryland 20993

Thursday, March 1, 2018
9:00 am – 5:00 pm

Aging and Anti-Aging of Cognitive Functions

Tatsuhiro Hisatsune
Graduate School of Frontier Sciences
The University of Tokyo, Japan
Rise in the Number of Dementia Patients

Japan

2014 4.6 million

Seven Years

2025 7.0 million

(Estimation)

World

2015 46 million

Thirty Years

2050 132 million

(Estimation)

https://www.asahi.com/articles/ASK4R7J67K4RUBQU00S.html

http://www.worldalzreport2015.org/

http://www.who.int/mental_health/neurology/dementia/dementia_thematicbrief_epidemiology.pdf
The Course of Healthy Aging, Mild Cognitive Impairment (MCI), and Alzheimer's Disease (AD)
Cognitive function of elderly people (≥ 65 years-old)

CDR: 0*  CDR: 0.5  CDR: 1

CN: Cognitive Normal  MCI: Mild Cognitive Impairment  Dementia

10%/y

Reversion

Cognitive function (DSM-5)*

Learning & memory
Language
Executive function
Social cognition
Complex attention
Perceptual-motor

MCI due to AD
Slight Decline
Normal
Normal
Normal
Normal
Normal

AD
Definite Decline

CDR: 0*
CDR: 0.5
CDR: 1

http://www.alz.org/jp

- CDR: Clinical Dementia Rating
- DSM-5: Diagnostic and Statistical Manual of Mental Disorders-5th version
Neuroinflammation: as a target of regenerative medication for MCI

Neurovascular damage → Aging → Senile Plaques

→ Toxic Aβ peptide → Neuroinflammation

Defect in Neurovascular drainage of Aβ peptide → Glial Activation (Astrocytes, Microglia)

Control of Neuroinflammation

→ Normal Neurovascular function

→ Normal Neurogenesis

Inflammatory Cytokines

→ Alteration in neural network

→ Tau Phosphorylation

→ Decline in Cognitive Functions

Reversion

→ Cognitive normal

→ Memory decline

Dementia

Regenerative medication for the prevention of the onset of Dementia
Strategies to control neuroinflammation

Many trials with:

• Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)
  NSAIDs, such as ibuprofen and indomethacin
  COX-2 inhibitors, such as rofecoxib and celecoxib

• To control inflammatory cytokines
  Antagonists, Specific blockers
  Antibodies toward inflammatory cytokines

• Anti-inflammatory small molecules
  → Anti-inflammatory drugs, such as ChE inhibitors
  → Anti-inflammatory natural products

Ref “Can Alzheimer’s Disease Be Prevented?” NIH Publication No. 09-5503, April 2009
Age-related declines in Cognitive Functions in Animal and Human

Mouse
- Adult Hippocampal Neurogenesis
- Alzheimer’s Disease Model Mouse
- NIH NINDS
- Stroke model

Monkey
- Adult Neurogenesis
- Stroke monkey

Human
- Neuroinflammation
- <Glial Activation>
- Mild Cognitive Impairment
- Alzheimer’s Disease

Sato et al. (2009)
Alzheimer’s Disease (AD) Model Mouse

【B6C3-Tg(APPswe,PSEN1dE9)85Dbo/J】

Wild Type  AD Transgenic

Senile Plaques (18 month olds: Red, Anti-Aβ staining)
Control of memory decline and neuroinflammation by Choline-esterase inhibitor (ChEI)

Water Maze (Probe test)

ChEI has:
- no effect on plaque formation
- suppressive effect on neuroinflammation

ChEI Recovers Adult Neurogenesis in AD model mouse

Imidazole-di-peptides

Anti-inflammatory natural products from vertebrate muscle

Anserine

Carnosine

Chicken (100g):
Anserine (0.6g)
Carnosine (0.2g)

Salmon (100g):
Anserine (0.5g)

Carnosine protects neurovascular damage and memory decline in High Fat Diet-AD model mouse

Imidazoledipeptide Carnosine (β-alanyl-histidine)

Sigma C9625

Memory performance
test
Contextual Fear Conditioning

Memory freezing (%)

Control AD model mouse

Carnosine

Carnosine protects neurovascular damage and memory decline in High Fat Diet-AD model mouse

Anserine protects neuroinflammation and memory decline in 18 months old AD model mouse

**Imidazolidepeptide**
**Anserine (Ans)**
(β-alanyl-methyl histidine)

Purification (>93%) from Salmon

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**Kaneko et al. Scientific Reports (2017)**
Anserine recovers degeneration of brain capillary pericytes

Aging and Anti-Aging of Cognitive Functions in Mouse

1) Alzheimer’s Disease model mouse showed declines in cognitive function and glial neuroinflammation.

2) The control of neuroinflammation in AD model mouse leaded to the improvement of cognitive functions.

3) Cognitive declines in Stroke model mouse were also prevented in (less glial neuroinflammation) P2Y1R knock-out mouse.

## Aging and Anti-Aging of Cognitive Functions

- Translation from Animal Models to Human clinical trials -

<table>
<thead>
<tr>
<th>Species</th>
<th>Mouse</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Behavior Tests</strong></td>
<td>Morris Water Maze</td>
<td>Wechsler Memory Scale</td>
</tr>
<tr>
<td></td>
<td>Contextual Fear Conditioning</td>
<td>Clinical Dementia Rating (CDR)</td>
</tr>
<tr>
<td><strong>Brain Examinations</strong></td>
<td>Brain Tissue Staining</td>
<td>Brain MRIs VBM, fMRI ASL, DTI</td>
</tr>
</tbody>
</table>
Imidazololedipeptide ameliorates memory decline in elderly people

<table>
<thead>
<tr>
<th>Elderly People (60-80 years old)</th>
<th>Random Grouping (Active:Placebo=1:1)</th>
<th>Test Schedule (pre and post tests)</th>
<th>Statistics Two-way repeated ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active Imidazololedipeptide</td>
<td>6 month</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carnosine 0.25 g/d Anserine 0.75 g/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Test food from Nipponham)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Test Schedule**

- **6 month**
  - **Active Group**
    - **BL**: Base Line
    - **FU**: Follow Up
  - **Placebo Group**
    - **BL**: Base Line
    - **FU**: Follow Up

**Preservation of prefrontal cortex**

- **Active vs Placebo**
  - VBM analysis ($p < 0.005$)

**Preservation of episodic verbal memory**

- **Wechsler Memory Scale - Logical Memory (delayed recall)**
  - Change in Score of WMS-LM2
  - **P < 0.05**

- **Active vs Placebo**
  - **Active (n=42)**
  - **Placebo (n=42)**

**Decrease in Inflammatory cytokine, IL-8, in plasma**

- **P < 0.01**


17/30
Alteration in network activity (fMRI study)

Resting-state fMRI analysis

PCC: Posterior Cingulate Cortex

Hippocampus

Spearman’s $r = 0.446$, $P = 0.043$


In collaboration with NCNP, IBIC (Dr. Matsuda)
Arterial Spin Labeling (ASL): to evaluate brain blood flow
Effect of ACS on the preservation of brain blood flow at the frontal areas

Ding et al. *Aging and Disease* (2017)

Brain Areas of Blood flow preservation
Table 3
ApoE4 as a risk gene for AD

Japanese (2313)

<table>
<thead>
<tr>
<th>APOE Genotype</th>
<th>No.</th>
<th>Odds Ratio (95% Confidence Interval)</th>
<th>Breslow-Day P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3/E3</td>
<td>1661</td>
<td>1.0 (Referent)</td>
<td>...</td>
</tr>
<tr>
<td>E2/E2</td>
<td>9</td>
<td>1.1 (0.1-17.2)</td>
<td>.52</td>
</tr>
<tr>
<td>E2/E3</td>
<td>149</td>
<td>0.9 (0.4-2.5)</td>
<td>.84</td>
</tr>
<tr>
<td>E2/E4</td>
<td>19</td>
<td>2.4 (0.4-15.4)</td>
<td>.80</td>
</tr>
<tr>
<td>E3/E4</td>
<td>430</td>
<td>5.6 (3.9-8.0)</td>
<td>.11</td>
</tr>
<tr>
<td>E4/E4</td>
<td>45</td>
<td>33.1 (13.6-80.5)</td>
<td>.62</td>
</tr>
</tbody>
</table>

*Odds ratios for APOE genotypes derived assuming a reference odds ratio of 1 for APOE ε3/ε3 genotype.
†These P values are a test for heterogeneity of odds ratios for genotype among data sets.

Lindsay et al., JAMA 278(16):1349-1356 (1997)
Acceleration AD in ApoE4 carrier

Corder et al. Science (1993)
Original Article

Anserine/Carnosine Supplementation Preserves Blood Flow in the Prefrontal Brain of Elderly People Carrying APOE e4

Qiong Ding¹, Kitora Tanigawa¹, Jun Kaneko¹, Mamoru Totsuka², Yoshinori Katakura³, Etsuko Imabayashi⁴, Hiroshi Matsuda⁴, Tatsuhiro Hisatsune¹*

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³Graduate School of Systems Life Sciences, Kyushu University, Higashi-ku, Fukuoka, Japan
⁴Integrative Brain Imaging Center (IBIC), National Center of Neurology and Psychiatry, Tokyo, Japan

<table>
<thead>
<tr>
<th>APOE4⁺ group</th>
<th>APOE4⁻ group</th>
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<tbody>
<tr>
<td>E4/E4</td>
<td>E3/E3</td>
</tr>
<tr>
<td>E4/E3</td>
<td>E3/E2</td>
</tr>
<tr>
<td>E4/E2</td>
<td>E2/E2</td>
</tr>
</tbody>
</table>

Active 1 7 0 23 0 0
Placebo 2 2 0 33 0 0
Effect of ACS on the preservation of the prefrontal tract in an ApoE4 double carrier

ApoE4/E4 Pre-DTI

Post-DTI 1 year
Randomized Controlled Trial to assess the effect of Anserine/Carnosine on the prevention of Alzheimer’s Disease

Participants: Mild Cognitive Impairment (MCI)

<table>
<thead>
<tr>
<th>Visit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recruit</td>
<td>0 week Baseline</td>
<td>4 week</td>
<td>8 week</td>
<td>12 week Follow-up</td>
<td>Post observation</td>
</tr>
<tr>
<td>Informed Consent</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intervention Period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical Examination</td>
<td>○</td>
<td></td>
<td>Anserine/Carnosine (1 g) / day</td>
<td>○</td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>CDR (Clinical Dementia Rating)</td>
<td></td>
<td>○</td>
<td></td>
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<tr>
<td>MMSE, WMS-LM2</td>
<td>○</td>
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<tr>
<td>ADAS-cog, GDS-S-J</td>
<td>○</td>
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<td></td>
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<tr>
<td>Blood Test</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
<td>○</td>
<td></td>
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<tr>
<td>Genotyping of ApoE</td>
<td>○</td>
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</tbody>
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Under UMIN# R000023455
**Interventional Clinical Study (individuals with MCI)**

- **CN**: Cognitive Normal
  - CDR: 0
  - 20~30%/y
- **MCI**: Mild Cognitive Impairment
  - CDR: 0.5
  - 10%/y
- **Dementia**: CDR: 1, 2, 3

**Recruitment and Study Design**
- **54** individuals
- **Pre 0w (Baseline)**: 27 (Active) + 27 (Placebo)
  - 2 Withdrawn
- **Post 12w (Follow-up)**: 25 (Active) + 25 (Placebo)
  - 2 Withdrawn

**Intervention**
- Imidazoledipeptide (Anserine/Carnosine)
  - 0.75g/0.25g per day

**Outcome**
- **Active (Total)**: 6 Reversion, 19 Stable
- **Active (ApoE4+)**: 4 Stable, 4 Reversion

Masuoka et al. Presentation at Neuroscience 2017, Masuoka et al. (in revision)
## CLINICAL DEMENTIA RATING (CDR):

<table>
<thead>
<tr>
<th>Impairment</th>
<th>None 0</th>
<th>Questionable 0.5</th>
<th>Mild 1</th>
<th>Moderate 2</th>
<th>Severe 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory</td>
<td>No memory loss or Slight inconsistent forgetfulness</td>
<td>Consistent slight forgetfulness; Partial recollection of events; &quot;benign&quot; forgetfulness</td>
<td>Moderate memory loss; more marked for recent events; defect interferes with everyday activities</td>
<td>Severe memory loss; Only highly learned material retained; new material rapidly lost</td>
<td>Severe memory loss; only fragments remain</td>
</tr>
</tbody>
</table>

### Memory Questions for Informant (Excerpt):

1. Does he/she have a problem with his/her memory or thinking?
   - Yes [ ] No [ ]

1a. If yes, is this a consistent problem (as opposed to inconsistent)?
   - Yes [ ] No [ ]

2. Can he/she recall recent events?
   - Usually [ ] Sometimes [ ] Rarely [ ]

3. Can he/she remember a short list of items (shopping)?
   - Usually [ ] Sometimes [ ] Rarely [ ]

4. Has there been some decline in memory during the past year?
   - Yes [ ] No [ ]

5. Is his/her memory impaired to such a degree that it would have interfered with his/her activities of daily life a few years ago (or pre-retirement activities)? (collateral sources opinion)
   - Yes [ ] No [ ]

6. Does he/she completely forget a major event (e.g., trip, party, family wedding) within a few weeks of the event?
   - Usually [ ] Sometimes [ ] Rarely [ ]

7. Does he/she forget pertinent details of the major event?
   - Usually [ ] Sometimes [ ] Rarely [ ]

8. Does he/she completely forget important information of the distant past (e.g., birthdate, wedding date, place of employment)?
   - Usually [ ] Sometimes [ ] Rarely [ ]
Reports regarding reversion from MCI

Effect of reversion from mild cognitive impairment (MCI) on cumulative incidence of dementia


Summary (Human Clinical Studies)

1) **Regenerative Medicine** to reduce the risk of Dementia can be feasible in Individuals with Mild Cognitive Impairment (MCI). MCI is a reversible transition stage between cognitive normal (CN) and Alzheimer’s Disease (AD). Reversion, from MCI to CN, reduces the risk of the onset of AD, to about 50%.

2) **Neuroinflammation** as a downstream cellular responses is a target to develop regenerative medicine for Dementia.

3) **Anserine/Carnosine**, natural products from animal meat (imidazoledipeptides), suppressed neuroinflammation-related neurovascular dysfunction and cognitive declines in elders.

4) **In MCI Individuals carrying ApoE4, a major AD risk gene**, Anserine/Carnosine supplementation induced reversion and may reduce the risk of AD onset. Large scale clinical study to verify its effect is definitely awaited.
Collaborators and Acknowledgements

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