HA as a pathogen for Alzheimer’s disease

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Abstract

It has been considered that Alzheimer’s disease may be induced by multi-pathogenic factors. However recent Nature Genetics has reported that sporadic Alzheimer’s disease is also genetically determined by such as ApoE4, Clusterin, so on. We already understand familiar Alzheimer’s disease is determined by such APP, Presenilin genes. These reports should allow us to consider that these genes stimulate the biological process and consequently Alzheimer’s disease would be determined by certain pathogen. Then what is the pathogen?

First this pathogen should be stimulated by certain biological process by these genetic activation, which I mean that these genes separately work to induce this pathogen.

I really propose that homocysteic acid (HA) is this pathogen.

Now I am clarifying why HA is the pathogen from these genes’ stimulation.

Main point of our issue is that our vaccine decreased strongly the urinary HA level with time, which consisted with the good memory performance of 3xTg-AD mice. (Fig. 1 and Fig.2 and Table 1,2,3)

Anti-HA vaccine showed the strong cure effect on 3xTg-AD mice memory impairment with time, which is consisted with the strong inhibition of HA vaccine for urinary excretion of HA with time.

Relation between HA and APP, Presenilin, and ApoE4

Recently we have discussed about the HA production by preseniline activation. Alzheimer’s model 3xTg-AD mice have the activated 3 genes, APP, Preseniline, and Tau. 3xTg-AD mice shows the higher level of HA in brain than control. (Table 4)

It is well known that the activated Preseniline activates the calcium signalling (1), which activated the calcium dependent cystathionine beta-synthase (CBS) activity (2). This enzyme gene is located in chromosome 21(3). And this enzyme is also vitamin B6 dependent. We observed that CBS produced HA from homocysteine in the presence of
calcium, or activated superoxide production or B6 deficiency (unpublished observation). Then B6 deficiency induces the production of HA. Down syndrome is well known to have triple chromosome 21 and shows the strong production of HA, which was confirmed in strong higher urinary excretion of HA by us (Table 5).

CBS catalyzes the HA production from homocysteine. CBS is calcium dependent, of which activity is stimulated by Preseniline. This hypothesis is supported by our observation.

Preseniline 1 transgenic mice L286V developed by Tabira et al showed the beta-amyloid accumulation into neuron at 12 months old (4). Beta-amyloid accumulation into neuron was induced by HA and our preliminary observation showed L286V produced higher level of HA in urine than control. (Table 6)

ApoE4 is a risk factor for Alzheimer’s disease. ApoE4 is also known to correlate with anxiety reaction (5), which induces the strong mental stress and consequently increases HA level. (6) The anxiety is known the earlier sign of Alzheimer’s disease (5). Then ApoE4 increases HA level in brain.

APP transgene is also known as Alzheimer’s gene. Overexpression of APP induces activation of the adrenergic neuron (7), which increases HA production (8).

Clusterin has a role in dampening down inflammation in the brain, of which activated inflammation induces glutamate signal activation (9), and consequently induces HA activation (10).

Mechanism:
In the presence of beta-amyloid, HA accumulates beta-amyloid 42 into neuronal cell, which induces alpha-synuclein and consequently induces tau aggregation. Then neuronal degeneration will be induced by lower level of HA.(11)

Higher level of HA also induces the neuronal degeneration in the absence of amyloid by oxidative stress and mitochondrial inhibition.

Materials and Methods

3xTg-AD mice*
The mouse germline used in this study was kindly gifted by Professor F. M. Laferla (University of California, Irvine). Housing environment (12h/12h light/dark cycle) was germ-free clean room. 3xTg-AD hemizygous male mice (5 and 7 month-old) were 7 mice used. Also Non-Tg mice were 4 mice used. The 3xTg-AD mice develop both plaque and tangle pathology in AD-relevant brain regions. The 3xTg-AD mice develop extracellular As deposits prior to tangle formation, consistent with the amyloid cascade hypothesis. Despite equivalent overexpression of the human sAPP and
human tau transgenes, As deposition develops prior to the tangle pathology, consistent with the amyloid cascade hypothesis. In addition, these mice exhibit deficits in synaptic plasticity, including long-term potentiation (LTP) that occurs prior to extracellular As deposition and tau pathology, but is associated with intracellular As immunoreactivity. These studies support the view that synaptic dysfunction is a proximal defect in the pathobiology of AD, preceding extracellular plaque formation and neurofibrillary pathology. As these 3xTg-AD mice phenocopy critical aspects of AD neuropathology, this model will be useful in pre-clinical intervention trials, particularly because the efficacy of anti-AD compounds in mitigating the neurodegenerative effects mediated by both signature lesions can be evaluated.

**Vitamin B6-deficient food**
Vitamin B6-deficient food was purchased from Kyudo Ltd. Nutrient composition has been described further in the study.

**Anti-HA vaccine**
Anti-HA antibodies were purchased from MoBiTec Co. (Germany). Polyclonal antigen was raised in mice after immunization with a glutaraldehyde-containing HA conjugate KLH, following which antibody specificity was determined by performing ELISA with competition experiments involving HA-G-BSA (compound cross-reactivity ratio 1:1), cysteine-G-BSA (1:85), and homocysteine-G-BSA (1:231). This vaccine was administrated with IP.

**Morris water maze test**
The apparatus used for all Morris water maze tasks comprised a circular aluminum tank (1.5 m in diameter) painted white and filled with water maintained at 26–29°C. The maze was located in a room containing several simple, visual extramaze cues. To reduce stress, mice were placed on a platform in both the hidden and cued versions of the task for 10 s prior to the first training trial.

**Spatial reference Morris water maze training**
Mice were trained to swim to a 14-cm circular clear Plexiglas platform placed 1.5 cm beneath the water surface that was invisible to the mice while swimming. The platform location was randomly selected for each mouse, but was kept constant for that mouse throughout the training period. In each trial, the mouse was placed in the tank at one of the four designated start points in a pseudorandom order. Mice were allowed to search for and escape to the submerged platform. If a mouse failed to find the platform within 60 s, it was manually guided to it and allowed to remain there for 10 s. Then, each mouse was placed in a holding cage under a warming lamp for 25 s until the start of the next trial. To ensure that memory differences were not due to the lack of task learning, the mice were trained for four trials a day for as many days as required to meet the criterion, which was defined as <20-s mean escape latency before
the first probe trial was run. To prevent overtraining, probe trials were run for each group as soon as they met the group criterion and stopped after all the groups met the criterion. Retention of spatial training was assessed 1.5 and 24 h after the last training trial. Both probe trials consisted of a 60-s free swim in the pool without the platform. Mice were monitored by a camera mounted on the ceiling directly above the pool, and all trials were stored on videotape (burnt onto a DVD) or subsequent analysis. The parameters measured during the probe trial comprised (1) initial latency time to reach the platform,

**HA level measurement**

HA was extracted from mouse brain (hippocampus and cortex) with an acid of trichloroacetic acid. Brain samples were prepared by a modification of the method of Reed and Bellerche. Brains (1.50–2.00 g) were isolated from 4-month-old 3xTg-AD homozygous male mice. The mice were killed by rapid decapitation and their brains were quickly excised and placed on an ice-cold Petri dish. For the gradient highperformance liquid chromatography (HPLC) method, tissue samples were weighed and homogenized using a sonicator for 10 s in ice in 4 ml of ice-cold 10% (w/v) trichloroacetic acid per 100 mg tissue (wet weight). HA (4 g) was added as the internal standard. For isocratic HPLC, tissue samples were divided into six aliquots. The samples were homogenized as described above. The homogenates for isocratic or gradient HPLC were left on ice for 1 h and centrifuged at 20,000 xg for 25 min. The supernatant was washed five times with an equal volume of diethyl ether and the aqueous phase was maintained. Residual ether was evaporated under nitrogen at room temperature for 5 min. Immediately thereafter, 20 L was injected into the HPLC system.

**Urinary HA level**

Urine of 3xTg-AD male mice (14 month-old) were collected for 24 hrs. 24 hr of Human urine were also collected in down syndrome male patients (n=7, 36.1± 5.3 years old). These urinary HA level was measured according to the method of HPLC system.

References

(1) Johnston JM, Burnett P, Thomas AP, Tezapsidis N, Calcium oscillations in type-1 astrocytes, the effect of a presenilin 1 (PS1) mutation. Neurosci Lett. 2006 ;395(2):159-64

(2) K. Qu, S.W. Lee, J.S. Bian, C.-M. Low and P.T.-H. Wong, Hydrogen sulfide: Neurochemistry and neurobiology, Neurochemistry International

(4) Chui DH, Tanahashi H, Ozawa, K. et al, Transgenic mice with Alzheimer’s presenilin 1 mutations show accelerated neurodegeneration without amyloid plaque formation, Nat. Med. 5, 560-564, 1999


(6) Tohru Hasegawa, Medical Hypotheses (2007) 69, 1135–1139

(7) Debby Van Dam, Bart Marescau, Sebastiaan Engelborghs, Thomas Cremers, Jan Mulder, Matthias Staufenbiel and Peter Paul De Deyn: Analysis of cholinergic markers, biogenic amines, and amino acids in the CNS of two APP overexpression mouse models, Neurochemistry International Volume 46, Issue 5, April 2005, Pages 409-422


Table 1  
HA level in 3xTg-AD brain at 12 months old

| Control | 71.5 ± 20.3 | pmoles/mg brain |
Table 1  HA level of 3xTg-AD mice after vaccine treatment.
After measurement of Morris water maze test, these mice were sacrificed and their
brain were immediately freezeed with liquid nitrogen. After that, HA level was
measured with the the method described in reference 1.

<table>
<thead>
<tr>
<th>Vaccine treatment</th>
<th>30.6 ± 18.4 pmoles/mg brain</th>
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<tbody>
<tr>
<td>n=10</td>
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Table 2  HA level in urine of 3xTg-AD (15 months old) (n=5)

<table>
<thead>
<tr>
<th>Control</th>
<th>22.5 ± 8.5 M</th>
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<tbody>
<tr>
<td>vaccine treatment</td>
<td>7.8 ± 9.1 M (35% of control)</td>
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</tbody>
</table>

Table 3  HA level in urine of 3xTg-AD (18 months old) (n=5)

<table>
<thead>
<tr>
<th>Control</th>
<th>27 ± 5.6 M (20% increase of 15 month )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine treatment</td>
<td>6.8 ± 4.1 M (25% of control)</td>
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Table 4

Homocysteic acid level in 3xTg-AD -H mice brain at 4 month old compared to Non Tg control mice

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<thead>
<tr>
<th></th>
<th>Hippocampus</th>
<th>Cortex</th>
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<tbody>
<tr>
<td>Control</td>
<td>23.63 ± 9.2</td>
<td>20.41 ± 6.6</td>
</tr>
<tr>
<td></td>
<td>n=5</td>
<td>n=5</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>3xTg-AD</td>
<td>41.25 ± 5.4</td>
<td>32.16 ± 4.8</td>
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<tr>
<td></td>
<td>n=4</td>
<td>n=4</td>
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Table 5

Comparison of urinary HA level between down syndrome and normal

<table>
<thead>
<tr>
<th></th>
<th>Down syndrome</th>
<th>Normal control</th>
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<tr>
<td></td>
<td>34.8 ± 8.1 m moles</td>
<td>non detect</td>
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</table>
Male Down syndrome (36.1 ± 5.3 years old) n=5

Male normal control (35 ± 2 years old) n=5

Spot urine was adjusted with specific gravity 1.020

Table 6

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<thead>
<tr>
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<th>Urinary HA level of Presenilin 1 L286V mice (6 months old) n=5</th>
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</thead>
<tbody>
<tr>
<td>L286V mice (male)</td>
<td>15.3 ± 8.1 moles/day</td>
</tr>
<tr>
<td>Control (C57BL, male)</td>
<td>non detect (6 month old)</td>
</tr>
</tbody>
</table>