Urinary Homocysteic Acid Levels Correlate with Mini-Mental State Examination Scores in Alzheimer’s Disease Patients

Tohru Hasegawa, Masayoshi Ichiba, Shin-ei Matsumoto, Koji Kasanuki, Taku Hatano, Hiroshige Fujishiro, Eizo Isekki, Nobutaka Hattori, Tatsuo Yamada and Takeshi Tabira

Saga Woman Junior College, Saga, Japan
Social Medicine, Faculty of Medicine, Saga University, Saga, Japan
Department of Neurology, Graduate School of Medicine, Juntendo University, Bunkyo-ku, Tokyo, Japan
PET/CT Dementia Research Center, Juntendo Tokyo Koto Geriatric Medical Center, Juntendo University School of Medicine, Koto-ku, Tokyo, Japan
Department of Neurology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

Accepted: 22 March 2012

Abstract. Homocysteic acid (HA) has been suggested as a pathogen in a mouse model of Alzheimer’s disease (AD), 3xTg-AD. However, it is not established whether HA is involved in humans. We investigated the relationship between urinary HA levels and Mini-Mental State Examination (MMSE) scores in AD patients (n = 70) and non-AD controls (n = 34). We found a positive, statistically significant relationship between the two variables (the urinary HA level and MMSE score) (r = 0.31, p = 0.0008, n = 70). This relationship was stronger in females than males (r = 0.43, p = 0.005, n = 44 in females; r = 0.48, p = 0.02, n = 22 in males). The urinary HA levels were significantly different in AD patients than controls (AD: 8.7 ± 7.5, n = 70; non-dementia control: 13.3 ± 9.4, n = 34, p < 0.01). In addition, aging and smoking were found as lowering factors for urinary HA levels.

Our preliminary study showed a negative, statistically significant relationship between blood HA (micromole) and urine HA levels (r = −0.6, p = 0.007, n = 19), and between blood HA levels and MMSE scores (r = −0.79, p = 0.000518, n = 19). On the basis of these results, we speculate that reduced urinary excretion induces elevated HA levels in blood, resulting in cognitive dysfunctions. This study also suggests that HA may be a candidate of neurotoxins for uremic encephalopathy. Since amyloid-β increases HA toxicity and HA is an agonist of N-methyl-D-aspartic acid (NMDA) receptor, we speculate that elevated blood HA affects the brain cognitive function through NMDA receptor-mediated toxicity in AD.

Keywords: Aging, Alzheimer disease, homocysteic acid, MMSE, smoking, uremia

Supplementary data available online: http://dx.doi.org/10.3233/JAD-2012-120022

INTRODUCTION

We have recently shown that vitamin B6 deficiency induces elevated levels of homocysteic acid (HA) in the brain in association with accumulation of amyloid-β (Aβ) and cognitive dysfunctions in C57BL/6 mice, which were reversed by immunotherapy targeting HA [1]. This was confirmed in an Alzheimer’s disease (AD) mouse model, 3xTg-AD mice carrying human amyloid-β protein precursor, presenilin 1, and tau gene mutations, which were successfully treated with anti-HA antibodies [2]. These results suggest that HA might be involved in the pathological mechanism of AD. In

ISSN 1387-2677/14/$27.50 © 2014 – IOS Press and the authors. All rights reserved
the present study, we measured urine HA levels in humans and found that urine HA levels correlate well with Mini-Mental State Examination (MMSE) scores.

MATERIALS AND METHODS

The study protocol was approved by the Juntendo University Ethics Committee, and all individuals agreed with the procedure by providing informed written consent. In the case where demented patients did not understand the procedure, the main family care giver provided informed written consent. The individual profiles are shown in Table 1. Control individuals consisted of normal healthy individuals and individuals with other diseases such as hypertension \((n=5)\), cerebral infarction \((n=4)\), Parkinson’s disease \((n=3)\), amyotrophic lateral sclerosis \((n=1)\), spastic paraplegia \((n=1)\), epilepsy \((n=1)\), cervical spondylosis \((n=1)\), myositis \((n=1)\), polyneuropathy \((n=1)\), hyperlipidemia \((n=1)\), diabetes mellitus \((n=1)\), ovarian cyst \((n=1)\), prostatic hypertrophy \((n=1)\), and frontotemporal dementia \((n=1)\). All AD cases including 2 cases with mild cognitive impairment (MCI) met the NINCDS-ADRDA criteria, and the diagnosis was assisted by CT, MRI, and SPECT findings. Urine samples were collected at Juntendo Hospital and Juntendo Tokyo Koto Geriatric Medical Center, and kept frozen without preservatives at \(-20^\circ\text{C}\) until use.

In addition, as a preliminary study for blood HA, patients were recruited at the Memory Clinic of Fukuoka University. Those patients were composed of normal \((n=2,\text{ male})\), MCI \((n=6,\text{ male})\), and AD \((n=11)\) (female, \(n=1\); male, \(n=8,73\pm6\) years). They all agreed with this observation.

Measurement of HA

The specific gravity of each urine sample was measured, and the urinary HA level was adjusted to that of 1.020 specific gravity. High performance liquid chromatography (HPLC) with an ECD detector was performed using a previous method with modifications \([3]\). Urine was diluted 10 times with water, 4 mg of HA was added to 1 ml of each urine sample as an internal standard, and 20 \(\mu\)l of the urine sample was added to the measurement solution, composed of 150 \(\mu\)l o-phthaldialdehyde reagent and 150 \(\mu\)l mercaptoethanol. Fifteen minutes after mixing the diluted urine sample with the measurement solution, the sample was injected into the HPLC system. The urine HA measurement was done blind. Blood HA levels were measured as previously described \([3]\). Typical chromatograms of authentic HA, urine, and blood are shown (supplementary Figure 1A–C; available online: http://www.j-alz.com/issues/31/vol31-1.html#supplementarydata02), and a titration curve is shown in Fig. 1. The retention time of HA was 3.9–4.1 min in urine and 3.6–3.8 min in blood. When the urine samples were measured, HPLC-ECD detector sensitivity was modified 10 times lower than that of blood.

Statistical significance was estimated by Student’s \(t\) test, and we analyzed the correlation by Spearman analysis.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (years)</td>
<td>Body weight (Kg)</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>72.9 ± 7.5</td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>75.4 ± 8.7</td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
<td>79.7 ± 6.2</td>
</tr>
<tr>
<td>GND*</td>
<td>5</td>
<td>74.2 ± 7.2</td>
</tr>
</tbody>
</table>

Table 1

Profiles of AD patients and controls

*GND, gender not described.

![Fig. 1. The titration curve of authentic homocysteic acid (HA). This is to show the titration curve of authentic HA by ECD-HPLC chromatograms.](http://www.j-alz.com/issues/31/vol31-1.html#supplementarydata02)
Fig. 2. Relationship between urinary homocysteic acid (HA) levels and Mini-Mental State Examination (MMSE) scores. All urine samples were collected at Juntendo University Hospital and Juntendo Tokyo Koto-Geriatric Medical Center. Illustrated are relationships between urinary HA levels and MMSE scores in AD patients (A) (n = 70; females, n = 44; males, n = 22; age, 75 ± 8 years) and non-AD controls (B) (n = 34, 72.4 ± 7.9 years).

Fig. 3. Relationship between urinary homocysteic acid (HA) levels and Mini-Mental State Examination (MMSE) scores in females (n = 44; age, 79.7 ± 6.2 years) (A) and in males (n = 22; age, 75.4 ± 8.7 years) (B). As shown, the correlation is stronger in females than males.

RESULTS

Urinary HA levels were positively correlated with MMSE scores in AD patients. In other words, the lower the urinary HA levels, the lower the MMSE scores (Fig. 2). In controls, we could not observe a relationship between urinary HA levels and MMSE scores. This correlation was stronger in females than in males.
Table 2

<table>
<thead>
<tr>
<th></th>
<th>Urinary HA (mM)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AD patients</td>
<td>Controls</td>
<td>Significance</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>14</td>
<td>AD versus control ( p &lt; 0.01 )</td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>GND*</td>
<td>5</td>
<td>10.16</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>13.34</td>
<td>AD versus control ( p &lt; 0.01 )</td>
</tr>
</tbody>
</table>

*GND, gender not described.

Table 3

<table>
<thead>
<tr>
<th>Effect of smoking on urinary HA levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary HA (mM)</td>
</tr>
<tr>
<td>Without smoking</td>
</tr>
<tr>
<td>With smoking</td>
</tr>
</tbody>
</table>

\( *p < 0.05 \)

When urinary HA levels were compared between AD and non-AD controls, the difference was statistically significant \( (p < 0.01) \) (Table 2).

We looked for factors that may correlate with the reduced HA in urine. In addition to cognitive dysfunction, aging was found to be a significant factor for reducing urine HA levels (Fig. 4A, B). In controls, there was no relationship between age and urinary HA levels. Further, smoking was also found to be a suppressing factor for the urinary HA excretion (Table 3).

We are interested in the relationship between urinary HA levels and blood HA levels. Our preliminary study shows an inverse relationship between urinary HA levels and blood HA levels \( (r = -0.6, p = 0.007, n = 19) \) (Fig. 5). As shown in Fig. 6, there was a strong negative relationship between blood HA levels and MMSE scores \( (r = -0.79, p = 0.0005, n = 19) \). The 19 cases include 2 normal individuals and 6 MCI patients.

DISCUSSION

Our results clearly indicate that urinary HA levels were positively correlated with MMSE scores. When comparing AD patients and non-AD controls, urinary HA levels were significantly lower in AD patients (Table 2). However, there was a strong discrepancy in the relationship between urinary HA levels and MMSE scores. As shown in Fig. 2B, some patients with other diseases and normal controls showed lower urinary HA levels, but their MMSE scores were within normal range, except for a case with frontotemporal dementia. These discrepancies between AD and non-AD controls are based on the fact that Aβ increases HA toxicity [20].

Since HA is excreted into urine, the results suggest that those patients with lower urinary HA levels may have higher HA levels in blood. Indeed, our preliminary study showed an inverse correlation between urinary HA and blood HA levels \( (r = -0.6, p = 0.007, n = 19) \) (Fig. 5). Further, there was a strong negative relationship between blood HA levels and MMSE scores, which indicated that blood HA might induce cognitive impairment in AD. Recently many papers reported that Aβ oligomers induced synaptic damage through stimulation of N-methyl-D-aspartic acid.
Fig. 5. The relationship between urinary homocysteic acid (HA) levels and blood HA levels. 19 AD patients submitted their urine and blood at Fukuoka University Hospital. They all agreed with the collection of urine and blood (female, \( n = 11 \) 75 ± 5 years; male, \( n = 8 \), 73 ± 6 years). As shown, levels of urinary HA and blood HA are inversely correlated \((r = -0.6, p = 0.007)\).

(NMDA) receptor \([4, 5]\). Since HA is known as an agonist of NMDA receptor, blood HA might induce cognitive impairment through activation of NMDA receptor and augmentation of NMDA toxicity.

It is well known that homocysteine (HC) is a risk factor for AD and vascular dementia \([6]\). However, it has not been clearly shown that HC is neurotoxic. It is known that HA is formed from HC by cystathionine-\(\beta\)-synthase at renal tubules, which is secreted into urine and reuptake of HA does not occur in a normal condition, because HA was detected in the blood of normal controls by 0.2 \(\mu\)M \([11]\), while the urinary HA level was at mM. Thus, urinary HA reabsorption does not seem to occur. In AD patients, excretion of HA is reduced or reuptake of HA is increased for some reason. Some studies have recently reported that kidney functions of patients with AD are impaired \([7, 8]\). Other papers showed that exogenous NMDA receptor agonists including HA disrupted blood brain barrier permeability \([9, 10]\), so that HA could enter the brain and work as a neurotoxin. In uremic patients, several factors are suggested to induce brain dysfunction. In the pup brain, metabolism was changed to strong glycolysis \([19]\). Moreover, HA induces intraneuronal accumulation of \(A_\beta\) \([20]\), and antibodies to HA attenuate AD pathology in 3X-Tg mice \([2]\). Second, it has been reported that lowering of HC by B vitamins slows down the rate of accelerated brain atrophy in MCI \([21]\). This report suggests that HC induces brain atrophy, but we think HA produced from HC acted as a neurotoxin. Indeed, HA induces neurodegeneration \(in vitro\) \([22]\).

In conclusion, our findings indicate that when urinary HA excretion is decreased, HA rises to a toxic level in the body, particularly in the brain. This retention of HA may induce the AD pathogenic process and cognitive impairment. In the future, we should investigate a relationship with ApoE genotypes and examine HA levels in cerebrospinal fluid.
ACKNOWLEDGMENTS

Authors are grateful to Dr. Toshiki Nakahura, Dr. Hei Arai, and Ms. Yuka Hasegawa for their help in this study. This study was partially supported by a grant of Strategic Research Foundation Grant-aided Project for Private Universities from Ministry of Education, Culture, Sport, Science, and Technology, Japan (MEXT), 2011–2015.

Authors’ disclosures available online (http://www.j-alz.com/disclosures/view.php?id=1234).

REFERENCES


