INTRODUCTION

Alzheimer’s disease (AD) is the most common form of dementia in the elderly, accounting for 60-80% of dementia cases worldwide (Alzheimer’s Association, 2013). Although AD was first identified more than 100 years ago, the mechanism of AD pathogenesis remains unconfirmed. Hallmarks of brain abnormalities are extracellular accumulation of plaques and evidence of nerve cell damage and death in the brain (Mattson, 2003; Mayeux, 2003; ADI, 2013). Plaques are accumulated by aggregates of amyloid beta protein (Aβ) (Mattson, 2004). These plaques result in the production of reactive oxygen species (ROS) and increased oxidative damage, leading to neuronal death (Babusikova et al., 2011). The deposits of Aβ are believed to interfere with neuron-to-neuron communication at synapses and contribute to cell death. AD patients’ brains show dramatic shrinkage due to cell loss and widespread debris as a result of dead and dying neurons (ADI, 2013).

Aβ is a small peptide (~4.5 kDa) made up of 40-42 amino acids (Yan et al., 1999). It is derived from the amyloid precursor protein (APP) by beta-site APP-cleaving enzyme 1 (BACE1), an authentic β-secretase (Yan et al., 1999). Aβs have many different isoforms (Selkoe and Schenk, 2003). In particular, Aβ 1-42 has a high propensity for aggregation and fibrillogenesis and is a common component of plaques (Prelli et al., 1988; Seubert et al., 1992; Donnelly et al., 2007). Decreased Aβ 1-42 in the cerebrospinal fluid (CSF) is considered a major hallmark of AD patients (Galasko et al., 1998; Sunderland et al., 2003; Martorana et al., 2012; Rosen et al., 2012).

To reduce the toxicity and damage associated with Aβ, it has to be degraded and/or removed efficiently from the brain. Aβ produced in the brain is degraded by proteases, such as neprilysin (NEP) (Shirotani et al., 2001; Mercury-induced amyloid-beta (Aβ) accumulation in the brain is mediated by disruption of Aβ transport

Dong-Kyeong Kim, Jung-Duck Park and Byung-Sun Choi

Department of Preventive Medicine, College of Medicine, Chung-Ang University, 84 Heukseok-Ro, Seoul, Korea

(Received March 5, 2014; Accepted June 6, 2014)

ABSTRACT — According to a recent study, mercury (Hg) exposure contributes to Alzheimer’s disease (AD). However, the underlying mechanisms are not understood. This study investigated the effect of methylmercury (MeHg) treatment on the generation, degradation, and transport of amyloid β-protein (Aβ) in the brain. Wistar rats were administered MeHg by gavage (0, 20, 200, and 2,000 μg Hg/kg/day) for 4 weeks. The total Hg in the blood and brain regions was measured, and the levels of Aβ12 in plasma, cerebrospinal fluid (CSF), and brain regions were estimated. The expression of amyloid precursor protein (APP), beta-site APP-cleaving enzyme 1 (BACE1), and neprilysin (NEP) in the brain regions was determined, in addition to the expression of low-density lipoprotein receptor-related protein 1 (LRP1) and the receptor for advanced glycation end products (RAGE) in the brain capillary endothelium (BCE). Finally, the amount of soluble low-density lipoprotein receptor-related protein (sLRP) in the plasma was determined. Aβ12 levels were decreased in the CSF of the 2,000 μg Hg/kg/day group compared with controls, and Aβ42 levels increased in the hippocampus (HC) in a dose-dependent manner. MeHg decreased LRP1 expression but increased RAGE levels in BCE. sLRP levels were decreased in the plasma of the MeHg-treated rats. They were positively correlated with CSF Aβ12 and negatively correlated with Aβ42 and Hg levels in HC. These results imply that MeHg reduces the transportation of Aβ, thereby resulting in the accumulation of the protein in the HC. Plasma sLRP levels may be an early biomarker of Hg-induced Aβ accumulation in the brain.

Key words: Alzheimer’s disease, Mercury, Amyloid-beta protein, Low-density lipoprotein receptor-related protein 1, Receptor for advanced glycation end products

Correspondence: Byung-Sun Choi (E-mail: bschoi@cau.ac.kr)
Mercury (Hg) is a toxic metal associated with AD, with several studies showing that Hg levels were elevated in postmortem brain tissue, plasma, and blood of AD patients (Hock et al., 1998; ATSDR, 1999). The role of methylmercury (MeHg) in the pathogenesis of AD is not completely understood. According to an in vitro study, Hg increased the secretion of Aβ (Olivieri et al., 2000). However, this result was not confirmed in an in vivo study. Moreover, that study investigated only the effect of mercury on Aβ levels and provided no mechanistic explanation. At present, there are no efficient biomarkers of AD in plasma. Although the CSF is a very good source of biomarkers, such as Aβ1-42, its utility in the clinic is low, as sampling procedures may have various side effects. An efficient indicator is needed to estimate brain Aβ levels in plasma.

Therefore, this study was designed to investigate whether Hg induces Aβ accumulation in the brain and decreases Aβ in the CSF, which is shown in AD patients. It also examined mechanisms underlying the generation, degradation, and transport of Aβ in the brain in response to Hg treatment. In addition, we evaluated plasma sLRP levels may be an early biomarker of Hg-induced Aβ accumulation in the brain.

MATERIALS AND METHODS

Animals and treatment

Male Wistar rats (7 weeks old) were purchased from Samtaco Inc. (Ohsan, Korea). The animals were housed under controlled temperature (23 ± 2°C) and humidity (50 ± 2%) conditions under a 12 hr light cycle and were allowed free access to water and food. The animal studies were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee (CAU-IACUC-11-0057). Twenty-four male adult rats were assigned to the following groups: distilled water (control) or 20, 200, and 2,000 μg Hg/kg/day administered by oral gavage for 4 weeks. After 4 weeks of the treatment, the rats were anesthetized with tribromoethanol (Aldrich, St. Louis, MO, USA; 300 mg/kg, i.p.), and a sample of CSF was collected from the cisterna magna with a 25-gauge butterfly needle (Becton Dickinson Korea, Guri, Korea). The blood was sampled from abdominal vena cava. The frontal cortex (FC), hippocampus (HC), and cerebellum (CB) were dissected from the brain, and the remaining brain regions were harvested. All samples were stored at -80°C until analysis.

Separation of brain capillary endothelium (BCE)

The capillary endothelia were separated from remaining brain tissue using dextran gradient centrifugation (Preston et al., 1995; Choi and Zheng, 2009). Briefly, the brain was homogenized in 3 volumes of cold buffer (10 mmol/l HEPES; 141 mmol/l NaCl; 4 mmol/l KCl; 1 mmol/l NaH₂PO₄; 2.5 mmol/l CaCl₂; and 10 mmol/l glucose, pH 7.4) with 7 strokes in a 7 ml tissue grind pestle (Pyrex, Corning, NY, USA). Dextran 70 solution was added to a final concentration of 15% (w/v), and the solution was homogenized with 3 additional strokes. The homogenate was centrifuged at 5,400 × g for 15 min at
4°C. The pellet (capillary-enriched fraction) was separated from the supernatant (capillary-depleted fraction). Light microscopic examination confirmed that the pellet consisted mainly of networks of brain vessels.

**Analysis of total Hg**

Total Hg in the whole blood and regional brain tissue was determined using a Direct Mercury Analyzer-80 (DMA-80, Milestone, Italy) with the gold-amalgam method (Berman *et al.*, 2008; Wang *et al.*, 2012). The Hg content was calculated from the standard calibration curve with standard Hg (1,000 ppm, Aldrich, St. Louis, MO, USA). The limit of detection was 0.1 μg/l, and the recovery rates were 98.2-102.8% for the standard added to the samples. Standard reference material (SRM, Bio-Rad, Hercules, CA, USA) was used for validation in the analysis.

**Quantification of Aβ42**

Aβ42 levels in brain tissue, plasma, and CSF were analyzed with a human/rat β amyloid (42) enzyme-linked immunosorbent assay (ELISA) (High-Sensitive; Wako, Osaka, Japan) according to the manufacturer’s instructions. Briefly, regional brain tissues (FC, HC, and CB) were homogenized in 10 volumes of ice-cold guanidine buffer (5.0 mol/l in 50 mmol/l Tis-HCl, pH 8.0) and mixed for 4 hr at room temperature. The brain homogenates were further diluted 1:10 with cold casein buffer (0.25% casein; 0.05% sodium azide; 20 μg/ml aprotinin; 5 mmol/l EDTA, 10 μg/ml leupeptin in PBS, pH 8.0), and then centrifuged at 16,000 × g for 20 min at 4°C. The supernatant was used for Aβ analysis. Plasma samples were diluted 4-fold with the standard diluent in the kit, and CSF samples were diluted 50-fold with the same solution.

**Quantification of sLRP levels in plasma**

Plasma sLRP levels were determined with an ELISA, as previously described (Quinn *et al.*, 1997; Sagare *et al.*, 2011). Briefly, 96-well plates were coated with recombinant rat receptor-associated protein (1 μg/well) in carbonate buffer (50 mM carbonate, pH 9.6) for 2 hr at 37°C. Subsequently, blocking buffer (20 mM HEPES; 0.15 M NaCl; 2 mM CaCl2, pH 7.4) was added and incubated for 30 min at 37°C for blocking. Plasma samples or recombinant LRP1 antigen (Santa Cruz Biotechnology, Santa Cruz, CA, USA) solution were diluted in the blocking buffer, added to each well, and incubated for 1 hr at room temperature. Next, the 96-well plates were incubated with 100 μl/well of primary antibody (1:200, Santa Cruz Biotechnology) for 1 hr at room temperature. After removing the primary antibody solution, horseradish peroxidase (HRP)-conjugated secondary antibody (1:1,000, Millipore, Billerica, MA, USA) solution was added and incubated for 1 hr at room temperature. Finally, 100 μl of 3,3',5,5'-tetramethylbenzidine substrate (Sigma, St. Louis, MO, USA) was added and stopped with stopping solution (1 N HCl) after 15 min. Absorbance was determined at 450 nm using a spectrophotometer (Sunrise, Tecan, Grodig, Austria).

**Quantification of mRNA expression by real-time RT-PCR**

Total RNA was isolated and purified using an RNasy Plus Mini kit (Qiagen, Valencia, CA, USA), and cDNA was synthesized by a Prime Script RT reagent kit (Takara, Kyoto, Japan). Real-time RT-PCR using an Mx3005P (Agilent, CA, USA) was used to quantify the mRNA expression levels of APP, BACE1, and NEP in FC, HC, and CB and LRP1 and RAGE in BCE. The mRNA levels of beta-actin, glyceraldehydes 3-phosphate dehydrogenase (GAPDH), and 18s rRNA were determined, and the mRNA expression levels of target genes were normalized using BestKeeper software (Andersen *et al.*, 2004; Pfaffl *et al.*, 2004). Each PCR reaction contained 2 μl of cDNA, 10 μl of SYBR Premix Ex Taq (Takara), and 125-500 nM of forward and reverse primers (Table 1). After initial denaturation at 95°C for 30 sec, the amplification program consisted of 40 cycles of denaturation at 95°C for 5 sec, annealing at 55°C for 30 sec, and extension at

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Orientation</th>
<th>Primer Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP</td>
<td>F</td>
<td>AAC ATG TGC GCA TGG TGG A</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>CAC GGC AGG GAC GTT GTA GA</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>TGG TGG ACA CGG GCA GTA G</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TCG GAG GTC TCG GTA TGT ACT GG</td>
</tr>
<tr>
<td>BACE1</td>
<td>F</td>
<td>CCG GCC AGA GTA GTC AGT CA</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TGC CAT GGA TGC TCC ACT TC</td>
</tr>
<tr>
<td>NEP</td>
<td>F</td>
<td>TGG GTG TCC CGA AAT CTG TT</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>ACC ACC GCA TTC TTA AAG GA</td>
</tr>
<tr>
<td>LRP1</td>
<td>F</td>
<td>TTC AGC TG TGG TTA AGC CTG AA</td>
</tr>
<tr>
<td>RAGE</td>
<td>F</td>
<td>CAC CGG TTT CTG TGA CCC TGA T</td>
</tr>
<tr>
<td>betta-actin</td>
<td>F</td>
<td>GGA GAT TAC TGC CCT GGC TCC TA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F</td>
<td>GCC ACA GTG AAG CCT GAT G</td>
</tr>
<tr>
<td>18s rRNA</td>
<td>F</td>
<td>ATG GTG GTG AAG ACG CCA GTA</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>AAG TTT CAG CAC ATC CTG CGA GTA</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TGG GTG AGC TCA ATG TCT GCT TTC</td>
</tr>
</tbody>
</table>
72°C for 30 sec. The relative differences in mRNA expression in the MeHg-treated samples were calculated and expressed as a relative increase, setting the control at 100%.

**Western blot analysis**

Regional brain tissue and BCE were homogenized with protein extraction solution (NP40, Elpis, Daejeon, Korea) in a 7 ml tissue grind pestle (Pyrex). The homogenates were additionally homogenized on ice by passing 10 times through a 23-gauge needle and incubated for 20 min at 4°C and then centrifuged at 13,000 × g for 5 min at 4°C. Equal amounts of protein (25 μg) were loaded and separated on 10% sodium dodecyl sulfate polyacrylamide gel and transferred to a PVDF membrane (GE Healthcare, Pittsburgh, PA, USA). After blocking with 5% nonfat milk in Tris-buffered saline for 1 hr at room temperature, blots were incubated overnight at 4°C with one of the following primary antibodies: rabbit anti-APP (1:200, Santa Cruz Biotechnology), mouse anti-BACE1 (1:200, Santa Cruz Biotechnology), goat anti-LRP1 (1:200, Santa Cruz Biotechnology), rabbit anti-NEP (1:200, Santa Cruz Biotechnology), and rabbit anti-RAGE (1:100, Santa Cruz Biotechnology). HRP-conjugated goat anti-rabbit IgG (1:2,500, Invitrogen, Carlsbad, CA, USA), goat anti-mouse IgG (1:2,500, KOMA Biotech, Seoul, Korea), and rabbit anti-mouse IgG (1:2,500, Millipore) were used as the secondary antibodies. Signals were detected using the standard chemical luminescence method (Power Opti-ECL, Animal Genetics, Suwon, Korea) and LAS-4000 (GE Healthcare). The density of each band was quantified using ImagQuant TL (GE Healthcare).

**Statistics**

All data were expressed as the mean ± S.E.M. Statistical analyses of the differences between groups were carried out by one-way analysis of variance (ANOVA), followed by a posthoc multiple comparison with Duncan’s test (Figs. 1-7) and the Student’s t-test (Fig. 6) using the PASW statistics package for Windows program (version 18.0). Pearson’s correlation analysis was used for determining correlations between sLRP in plasma and other factors associated with AD (Table 3). The differences between groups and the correlation coefficient were considered significant at p ≤ 0.05.

**RESULTS**

**Total Hg levels in whole blood (WB) and brain regions**

Total Hg increased in a dose-dependent manner in WB and brain regions after 4 weeks of treatment with MeHg by oral gavage (Table 2). Total Hg levels in WB were about 10-fold higher than those in the brain regions. There were no significant differences between the Hg levels of each brain regions (FC, HC, CB) in the same group. However, the Hg levels were higher in the HC than in the FC and CB in the 20 μg Hg/kg/day group (p < 0.05).

**Aβ42 in CSF, plasma, and brain regions**

Aβ42 in the CSF of the 2,000 μg Hg/kg/day group was decreased by 56.7% compared with the control. Aβ42 levels in the HC increased in a dose-dependent manner (p < 0.01). However, Aβ42 levels in other brain regions and plasma were not different from those of the controls (Fig. 1).

**Protein expression related to Aβ production**

Analysis of the effect of MeHg treatment on mRNA and protein expression of APP and BACE1 in the FC, HC, and CB revealed no changes in any of the studied brain regions (Figs. 2 and 3).

**Protein expression related to Aβ degradation**

Analysis of mRNA and protein expression levels of NEP, which is an Aβ-degrading enzyme, following MeHg treatment revealed no changes in any of the studied brain regions (Fig. 4).

**Table 2.** Total mercury (Hg) levels in whole blood (WB) and brain sections (frontal cortex [FC], hippocampus [HC], and cerebellum [CB]) after treatment with methylmercury (MeHg) at 0, 20, 200 and 2,000 μg Hg/kg/day for 4 weeks (μg/kg wet weight)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Control</th>
<th>20 μg Hg/kg/day</th>
<th>200 μg Hg/kg/day</th>
<th>2000 μg Hg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>5.39 ± 0.40</td>
<td>1924.59 ± 89.73</td>
<td>28748.75 ± 1479.97</td>
<td>303286.85 ± 22397.38</td>
</tr>
<tr>
<td>FC</td>
<td>2.29 ± 0.47</td>
<td>284.34 ± 38.37</td>
<td>2383.20 ± 305.09</td>
<td>21914.91 ± 1767.58</td>
</tr>
<tr>
<td>HC</td>
<td>3.02 ± 0.65</td>
<td>389.02 ± 31.63</td>
<td>2963.25 ± 282.47</td>
<td>22872.44 ± 1390.54</td>
</tr>
<tr>
<td>CB</td>
<td>2.21 ± 0.38</td>
<td>273.70 ± 27.49</td>
<td>2609.52 ± 183.63</td>
<td>24744.21 ± 1707.20</td>
</tr>
</tbody>
</table>
Expression of proteins associated with Aβ transport across the BBB

Methylmercury disrupts amyloid-beta transport in the brain

LRP1 mRNA expression in BCE decreased in the 200 and 2,000 μg Hg/kg/day (64.8 and 74.0% respectively) groups. The protein levels of LRP1 also decreased (48.5, 77.1, and 59.2%, respectively) in 20, 200, and 2,000 μg Hg/kg/day groups, in comparison to the control. mRNA expression of RAGE increased by 133, 131, and 135% in the 20, 200, and 2,000 μg Hg/kg/day treated groups compared with the control (Figs. 5 and 6).

Table 3. The relationship between levels of soluble low-density lipoprotein receptor protein (sLRP) in plasma and other factors of associated with Alzheimer’s disease (AD) after treatment with methylmercury (MeHg) at 0, 20, 200 and 2,000 μg Hg/kg/day for 4 weeks

<table>
<thead>
<tr>
<th>Variables</th>
<th>r value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ42 in plasma</td>
<td>-0.272</td>
<td>0.209</td>
</tr>
<tr>
<td>Aβ42 in CSF</td>
<td>0.589</td>
<td>0.004</td>
</tr>
<tr>
<td>Total Hg in WB</td>
<td>-0.569</td>
<td>0.005</td>
</tr>
<tr>
<td>Total Hg in FC</td>
<td>-0.553</td>
<td>0.006</td>
</tr>
<tr>
<td>Total Hg in HC</td>
<td>-0.552</td>
<td>0.009</td>
</tr>
<tr>
<td>Total Hg in CB</td>
<td>-0.562</td>
<td>0.005</td>
</tr>
<tr>
<td>Aβ42 in FC adjusted by total protein</td>
<td>0.024</td>
<td>0.914</td>
</tr>
<tr>
<td>Aβ42 in HC adjusted by total protein</td>
<td>-0.556</td>
<td>0.006</td>
</tr>
<tr>
<td>Aβ42 in CB adjusted by total protein</td>
<td>-0.245</td>
<td>0.261</td>
</tr>
</tbody>
</table>

WB: whole blood, FC: frontal cortex, HC: hippocampus, CB: cerebellum
sLRP as an indicator of MeHg-mediated Aβ accumulation in the brain

After treatment with MeHg at 20, 200, and 2,000 μg Hg/kg/day, sLRP levels in plasma declined to 60.3%, 60.3%, and 24.9%, respectively, in comparison with the control (Fig. 7). In addition, the plasma sLRP concentration was positively correlated with Aβ42 in CSF and negatively correlated with total Hg in blood and brain tissue and the Aβ42 concentration in the HC (Table 3).

DISCUSSION

Hg exposure, which is common in the environment, exerts severe neurotoxic effects on humans (ATSDR, 1999). In the general population, the major source of Hg is diet, especially seafood and freshwater fish containing MeHg (Clarkson et al., 2003; Kim et al., 2013b). MeHg is generated by the methylation of inorganic mercury, which is catalyzed by sulfate-reducing bacteria in the aquatic environment (Hintelmann, 2010). MeHg is readi-
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Ly absorbed by the gastrointestinal tract (90% of amount ingested), and it is rapidly distributed in tissues throughout the body (Harada, 1995). MeHg can easily enter the brain through the BBB. It is then readily converted to an inorganic form and remains “trapped” in the brain for a long time (Yoshida et al., 1980; Harada, 1995; ATSDR, 1999). The CNS is one of the main targets of MeHg toxicity, which causes irreversible damage to the nervous system (NRC, 2000). In recent studies performed to estimate the toxic effect of MeHg on rats, those treated with 1 and 2 mg of Hg/kg/day for 20 d (Fossato da silva et al., 2011) or 0.5, 1, and 3 mg Hg/kg/day for 14 d showed no toxic effects (Abd El-Aziz et al., 2012). Therefore, in the present study, the rats were treated with 0, 20, 200, and 2,000 μg Hg/kg/day of MeHg. Signs of acute or sub-acute toxicity of MeHg, such as losing weight, hair loss,
and behavioral abnormalities during the treatment period, were not observed in the rats. There were also no significant differences in the body weight or organ weight of the Hg-treated and control groups.

Treatment of the rats with MeHg resulted in a dose-dependent increase of Hg in WB and brain sections (FC, HC, and CB). This finding is in agreement with previous studies reported that blood levels of Hg were 12-20 fold higher than brain levels (Iverson et al., 1973; Jin et al., 2007).

The levels of Aβ42 in the CSF decreased in a dose-dependent manner, and they were negatively correlated with Hg levels in WB and brain sections. In the HC, the levels of Aβ42 increased in a dose-dependent manner following MeHg treatment. However, we did not observe any difference in Aβ42 levels in the FC and CB. Several studies showed that the HC is one of the main target organs for AD pathogenesis (Jack et al., 2002; Bekris et al., 2010; Ringman et al., 2012). In the present study, Aβ42 levels in the HC were positively correlated with Hg levels in WB and the HC. These results suggest that oral exposure to MeHg might be a risk factor for AD.

Regulation of levels of Aβ in the brain depends on balancing its generation, degradation, and transport in the brain. According to the amyloid cascade hypothesis, disordered APP metabolism results in the generation of Aβ. The accumulation of Aβ is associated with overproduction of the protein rather than decreased Aβ degradation and/or transport, which is the main pathomechanism in late-onset AD, the most common form of the AD (Hardy and Selkoe, 2002; Bekris et al., 2010; Ringman et al., 2012; Rosen et al., 2013). In the present study, MeHg led to accumulation of Aβ in HC by adversely affecting the transport of Aβ across the BBB. This type of Aβ accumulation is typically seen in late-onset AD models.

In a previous AD animal model study, the authors observed an increase in RAGE but a decrease in LRP1 at the BBB (Herring et al., 2008). Other studies reported that decreased LRP1 and increased RAGE were involved in Aβ uptake in a BBB cell model using a rat brain endothelial cell line (Yamada et al., 2008; Kim et al., 2013a). In the present study, MeHg had no effect on levels of APP, BACE1, or NEP in the brain. However, it downregulated the expression of LRP1 and upregulated that of RAGE. These results indicate that MeHg does not affect Aβ generation and degradation in brain but that it inhibits the transport of Aβ from the brain into the systemic circulation. Thus, MeHg increases the accumulation of Aβ in the HC by reducing the transport of Aβ from the brain. The findings suggest that LRP1 and RAGE may be new therapeutic targets in AD and biological markers of the disease.

The early diagnosis of AD is important for treatment because disease-modifying drugs are more effective in delaying memory loss in the earlier stages of the disease before plaques have accumulated in the brain (Olah et al., 2012). The CSF is an important source of biomarkers of AD because it is in direct contact with the extracellular space of the brain (Blennow et al., 2012). Levels of Aβ1-42 in the CSF were shown to be negatively correlated with the postmortem plaque load (Strozyk et al., 2003; Tapiola et al., 2009). Although the CSF is a very good source of biomarkers of AD, such as Aβ1-42, sampling procedures have various side effects (de Souza et al., 2012). Levels of Aβ1-42 levels in plasma and serum are inconsistent because the circulating Aβ is not derived exclusively from the brain (Salin et al., 2008). Thus, levels may increase due to liver catabolism or renal excretion (Salin et al., 2008). In addition, platelets can also secrete Aβ (Chen et al., 1995). Circulating Aβ also binds to chaperones, such as sLRP. Thus, Aβ1-42 in plasma and serum does not serve as a reliable biomarker of AD (Maler et al., 2007).

sLRP has been studied as a biomarker for AD because levels of this protein in plasma are associated with continuous removal of Aβ in the brain (Sagare et al., 2007). sLRP is cleaved by β-secretase at the N-terminal extracellular domain (von Arnim et al., 2005) and circulates in plasma (Quinn et al., 1997; Sagare et al., 2007). In plasma, Aβ binds to sLRP, with 70-80% Aβ normally binding in neurologically healthy humans and mice (Sagare et al., 2007). In the present study, levels of sLRP in plasma declined in a dose-dependent manner. Levels of sLRP were most notably negatively associated with Hg levels in WB and brain sections and with Aβ42 levels in the HC.
However, they were positively associated with $\alpha_42$ levels in the CSF. Increases in levels of $\alpha_42$ in the brain and decreases in the CSF are well-known factors in AD pathogenesis (Mattson, 2003; Martorana et al., 2012; Rosen et al., 2012). According to recent studies using different animal models, increases in the levels of oxidized sLRP in plasma lead to elevated levels of free $\alpha_{40}$ and $\alpha_{42}$, which then re-enter the brain (Deane et al., 2003; Ujiie et al., 2003; Donahue et al., 2006; Sagare et al., 2007). The results of the present study suggest that levels of sLRP in plasma might be an early biomarker of MeHg-induced $\beta$ accumulation in the brain, which is a risk factor for AD pathogenesis. They also indicate that decreases in blood sLRP levels may lead to an influx of $\beta$ from the blood to the brain.

ACKNOWLEDGMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NO. 2012-0001666).

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