Increased Plasma Urotensin-II and Carotid Atherosclerosis are Associated with Vascular Dementia

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**Aim:** Human urotensin-II (U-II) is a cyclic neuropeptide with potent vasoconstrictive activity in the vasculature. The expression of U-II and its receptor (UT) mRNA is detected at high levels in the brain. We evaluated the relationship between plasma U-II levels and vascular dementia (VaD) caused by stroke or atherosclerotic small vessel disease.

**Methods:** Carotid artery intima-media thickness (IMT), plaques, plasma levels of immunoreactive U-II (IR-U-II), and atherosclerotic biomarkers were determined in 42 patients with VaD, 197 with Alzheimer’s disease (AD), and 47 non-demented elderly controls.

**Results:** Age, gender, body mass index, systolic blood pressure (SBP), fasting plasma glucose, insulin, triglycerides, high-density lipoprotein cholesterol, leptin, and plasminogen activator inhibitor-1 levels were not significantly different among these groups. IR-U-II, low-density lipoprotein (LDL) cholesterol, lipoprotein(a), lipid peroxides, interleukin-6, and high-sensitive C-reactive protein (hs-CRP) levels, and maximum IMT were significantly higher in VaD than in AD patients or controls. IR-U-II level showed a significantly positive correlation with SBP or maximum IMT. Multivariate logistic regression analysis revealed a significantly independent association between IR-U-II levels or increased maximum IMT (≥1.1 mm) and VaD as compared with SBP, LDL cholesterol, and interleukin-6 levels.

**Conclusion:** Increased plasma IR-U-II levels and carotid atherosclerosis may be involved in the pathogenesis and progression of VaD.


**Key words:** Human urotensin-II, Atherosclerotic biomarkers, Carotid atherosclerosis, Vascular dementia

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**Introduction**

The incidence of vascular dementia (VaD) is high in Japan; nearly 50% of that of senile dementia, and equivalent to that of Alzheimer’s disease (AD)¹. Most VaD cases in Japan are of the multiple lacunar type or Binswanger disease, often without any episode of stroke. As the number of patients with atherosclerosis in large cervical and cerebral arteries has increased in Japan, VaD may present with different clinical pictures. We have reported the close relationships of VaD with carotid atherosclerosis, atherogenic dyslipidemia (high concentrations of low-density lipoprotein [LDL] cholesterol, lipoprotein(a), and lipid peroxides, and the high prevalence of small dense LDL)², Chlamydia pneumoniae infection, increased high-sensitive C-react...
tive protein (hs-CRP)\textsuperscript{3}, and a unique pattern of neurotrophic anti-apoptosis factors, \textit{i.e.}, low levels of insulin-like growth factor-1 (IGF-1) and high levels of hepatocyte growth factor (HGF)\textsuperscript{4,5}.

Human urotensin-II (U\textsubscript{II}), a cyclic undecapeptide (Glu-Thr-Pro-Asp-c[Cys-Phe-Trp-Lys-Tyr-Cys]-Val), serves as an endogenous ligand for G protein-coupled receptor-14 or sensory epithilum neuropeptide-like receptor (SENR)\textsuperscript{6}, which was recently renamed UT. Both U\textsubscript{II} and UT are expressed at high levels in the central nervous system (CNS)\textsuperscript{7,8}. There are high-density \textsuperscript{[125I]}U\textsubscript{II} binding sites in the lateral septal, medial habenula, and interpeduncular nuclei in rats and the cerebral cortex in humans\textsuperscript{7,8}. Intracerebroventricular injection of U\textsubscript{II} induces anxiogenic- and depressant-like effects in mice, suggesting that U\textsubscript{II} may be involved in psychiatric disorders\textsuperscript{9,10}. Administration of U\textsubscript{II} into the lateral cerebral ventricle increased brain damage following cerebral ischemia in rats\textsuperscript{11}. U\textsubscript{II} induces the secretion of amyloid $\beta$40-protein (A$\beta$40) from the human neuroblastoma cell line IMR32\textsuperscript{12}.

This peptide is known as the most potent vasoconstrictor identified to date\textsuperscript{13}. U\textsubscript{II} in circulating blood is produced mainly by the cardiovascular system, liver, and kidneys\textsuperscript{14}. Plasma levels of U\textsubscript{II} are increased in vascular endothelial dysfunction-related diseases, such as hypertension, ischemic heart disease, congestive heart failure, diabetes mellitus, and renal failure, and are positively correlated with systolic blood pressure (BP) and severity of atherosclerosis\textsuperscript{14-16}. U\textsubscript{II} (molecular weight: 1388) is regarded as a biomarker for atherosclerosis and vascular disease\textsuperscript{14}; however, the association between U\textsubscript{II} and VaD is not clear.

We examined associations among plasma U\textsubscript{II} levels, carotid atherosclerosis, and VaD in comparison with AD patients and non-demented elderly subjects.

**Methods**

**Subjects**

The study population consisted of 286 elderly Japanese subjects (131 men, 155 women; aged 60–97): 197 patients with AD and 42 patients with VaD hospitalized at Showa University Karasuyama Hospital, and 47 age- and sex-matched healthy controls with no evidence of dementia. None of the subjects were taking drugs that influence cognitive performance and had inflammatory diseases. Patients with heart or renal failure and liver cirrhosis known to increase plasma U\textsubscript{II} levels were excluded by history and routine laboratory examination\textsuperscript{14}. All participants abstained from smoking for only the night before blood sampling, because smoking within 10 minutes increases plasma U\textsubscript{II} levels\textsuperscript{17}. The study was performed in accordance with the declaration of Helsinki and was approved by our Institutional Ethical Review Board. Informed consent was obtained from all subjects and their immediate family.

**Dementia Diagnosis**

All subjects underwent uniform structured clinical evaluation, which included their medical history, neurological examination, cognitive function assessment, information interview, and standard laboratory tests. Computed tomography (CT) and magnetic resonance imaging (MRI) of the brain were performed for all subjects, and all images were assessed by the same experienced neuroradiologist blinded to the clinical diagnosis or severity of dementia. Diagnosis of dementia was made by the consensus of physicians and neuropsychologists based on the criteria for probable dementia of the National Institute of Neurological and Communicative Disorders and Stroke, the Alzheimer’s Disease and Related Disorders Association\textsuperscript{18}, and the National Institute of Neurological Disorders and Stroke/Association Internationale pour la Recherche et l’Enseignement en Neurosciences\textsuperscript{19}. Diagnostic classification was made blinded to the results of laboratory examination and carotid ultrasonography. On brain CT and MRI, all VaD patients had significantly more vascular lesions, such as strokes, lacunae, and leukoariosis, than AD patients and controls.

Cognitive function was evaluated using the Mini-Mental State Examination (MMSE), a 20-item global cognitive function test that includes questions on orientation in time and place, registration, attention and calculation, recall, language, and visual construction that is widely used in clinical practice and scientific studies, with possible scores ranging from 0–30 points\textsuperscript{20}. MMSE investigates cortical functions, which are important for daily functioning and are severely affected in dementia. Cognitive impairment was defined as a score $< 24$ points\textsuperscript{20}.

**Past History of Smoking and Alcohol Intake**

Information on cigarette smoking and alcohol intake was obtained from each subject or a surrogate regarding the age at which they started smoking, total number of years spent smoking, cigarette consumption, and usual weekly intake of alcoholic beverages over the previous several months. Alcohol intake was converted to daily equivalent in terms of “go,” a traditional Japanese unit of sake volume (1 go = 180 mL, 22.7 g ethanol). One go corresponds to one bottle (633
mL) of beer, two shots (75 mL) of whisky, or two glasses (180 mL) of wine. Subjects who reported consuming >1 go per week were regarded as drinkers\(^2\), and smokers were defined by current smoking >10 cigarettes per day for >1 year\(^2\).

**Assays**

Blood samples were collected in the morning after overnight fasting and in a non-smoking state. Plasma glucose and serum triglycerides levels (Vitros Slides; Ortho-Clinical Diagnosis, Raritan, NJ, USA) were analyzed by enzymatic methods using automated techniques\(^5\). Serum levels of LDL cholesterol (BLF Eiken II; Eiken Chemical, Tokyo, Japan) and high-density lipoprotein (HDL) cholesterol (Vitros Slide) were determined by the precipitation method\(^5\). Serum levels of apolipoprotein (apo) A1, apoB, and apoE (ApoA1, B, E Auto N; Daiichi Chemicals, Tokyo) were determined by turbidimetric immunoassays\(^7\). Serum levels of insulin (Lumipulse Presto Insulin; Fujirebio Inc., Tokyo) and hs-CRP (N latex CRP; Dade Behring Inc., Deerfield, IL, USA) were determined by enzyme immunoassay and latex-enhanced immuno-nephelometric assay, respectively\(^9\). Serum levels of IGF-1 (IGF-1 IRMA; Daiichi Radioisotope Laboratories, Chiba, Japan) and leptin (Human Leptin RIA kit; Linco Research, Inc., St. Charles, MO, USA) were determined by radioimmunoassay\(^4\).

Serum levels of HGF (HGF Otsuka ELISA kit; Otsuka Pharmaceutical Co., Ltd., Tokyo) and interleukin (IL)-6 (Human IL-6; Fujirebio Inc., Tokyo) and leptin (Human Leptin RIA kit; Daiichi Radioisotope Laboratories, Chiba, Japan) were determined by radioimmunoassay and latex-enhanced immuno-nephelometric assay, respectively\(^9\). Serum levels of IGF-1 (IGF-1 IRMA; Daiichi Radioisotope Laboratories, Chiba, Japan) and leptin (Human Leptin RIA kit; Linco Research, Inc., St. Charles, MO, USA) were determined by radioimmunoassay\(^4\).

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Serum levels of HGF (HGF Otsuka ELISA kit; Otsuka Pharmaceutical Co., Ltd., Tokyo) and interleukin (IL)-6 (Human IL-6; Fujirebio Inc., Tokyo), and plasma levels of plasminogen activator inhibitor-1 (PAI-1, LPIA•tPAI test; Mitsubishi Chemical Medience Corp., Tokyo) were determined by enzyme-linked immunosorbent assay (ELISA)\(^22\). Serum adiponectin levels were measured by a monoclonal antibody against high molecular weight adiponectin purified from human serum\(^23\).

**Immunoreactive UII (IR-UII) Measurements**

Blood was sampled in EDTA tubes containing aprotinin and immediately spun for 15 minutes at 3,000 rpm. Supernatants were stored at −80°C until analysis. Plasma IR-UII measurements were carried out with commercial ELISA kits (Urotensin II [human]-EIA kit; Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) according to the manufacturer’s instructions. Investigations related to the present study were performed at Phoenix Laboratories to test the immunoreactivity of this antibody with UII. The kit showed 100% immunoreactivity with human UII, 15.7% with human UII-related peptide, 0% with human prepro-UII, and 15% and 22% with human UII 4–11 and 5–11 fragments, respectively. The lower detection limit was 0.05 ng/mL and the mean recovery was 96.6%. Our additional studies confirmed the accuracy of the ELISA kit by dilution linearity test using human plasma and human UII (Sigma, St Louis, MO, USA). All of the samples (50 μL) were processed in a duplicate assay. Intra- and interassay coefficients of variation (CV) were 4% and 9%, respectively.

**Carotid Ultrasonography**

Carotid atherosclerosis was evaluated by high-resolution B-mode ultrasonography using a 7.5-MHz linear-array transducer with axial resolution range from 0.2–0.4 mm (SSA-700A; Toshiba Corp., Tokyo). The CV for interobserver variability was found to be 8% and the CV for intraobserver variability was 6%. Maximal IMT and plaque number, as indices of carotid atherosclerosis, were determined by three trained operators blinded to the subjects’ clinical records. Ultrasound images were obtained with the subject in the supine position with the neck mildly extended and rotated to the contralateral side, and IMT and plaque number were determined on frozen frames, perpendicular to the vascular walls by scanning bilateral common and internal carotid arteries. IMT was measured in 8 sites of the far walls in the bilateral carotid arteries, excluding plaque, and the unilateral maximum IMT value, which was higher than the other side, was defined as maximum IMT\(^2\). The upper normal limit of IMT is 1.0 mm, and lesions with focal IMT ≥1.1 mm were defined as plaques\(^2\).

**Immunohistochemistry**

To identify the localization of IR-UII in human carotid atherosclerotic plaques, fluorescent double staining was carried out using human carotid arteries obtained at autopsy. Formalin-fixed sections were incubated with rabbit anti-UII polyclonal antibody (Alpha Diagnostic International, San Antonio, TX, USA), and mouse monoclonal antibodies (DakoCyotomation A/S, Glostrup, Denmark) of anti-α-smooth muscle actin (αSMA), a VSMC marker, anti-CD68, a macrophage marker, or anti-von Willebrand Factor (vWF), an EC marker. Alexa 488-labeled anti-mouse and Alexa 546-labeled anti-rabbit antibodies (Molecular Probes) were used as secondary antibodies. Sections were then examined using a Nikon E-600 fluorescence microscope (Tokyo).

**Statistical Analysis**

Results are expressed as the means±SEM for continuous variables and frequencies for categorical variables. One-way analysis of variance followed by
Bonferroni correction was used to test differences in continuous variables, and the chi-square test was used to analyze differences in categorical data. Pearson’s correlation coefficient was used to analyze relationships between the plasma IR-U levels and other parameters in 286 subjects to assess independent risk factors for VaD. Associations were calculated as odds ratios (OR) with 95% confidence intervals (CI). Statistics were performed with Statview Version 5.0 (Abacus Concepts Inc., Berkeley, CA). A p value of <0.05 was considered significant.

**Results**

**Clinical Characteristics and Laboratory Parameters of VaD and AD Patients and Non-Demented Controls**

The clinical characteristics of VaD and AD patients, and non-demented controls are summarized in **Table 1**. Mean age, male ratio, body mass index, and prevalence of alcohol consumption, smoking, diabetes mellitus, hyperlipidemia, hypertension, ischemic heart disease, and peripheral vascular disease, and systolic and diastolic BP did not differ significantly among the three groups. The prevalence of cerebrovascular disease (CVD), such as stroke and transient ischemic attack (**Table 1**) and maximum IMT (**Fig. 1A**) was significantly greater in VaD than AD patients or controls. AD patients had significantly greater maximum IMT than controls (**Fig. 1A**). MMSE scores were significantly lower in VaD and AD patients than controls but did not differ significantly between VaD and AD patients (**Table 1**).

The plasma IR-U levels were significantly higher in VaD than AD patients or controls, and the level was lowest in AD (**Fig. 1B**). As shown in **Table 2**, HDL cholesterol, triglycerides, apoA-1, apoB, apoE, glucose, insulin, HGF, leptin, and PAI-1 levels did not differ significantly among the three groups. Serum levels of LDL cholesterol, lipoprotein(a), lipid peroxides, and hs-CRP were significantly higher in VaD than AD patients or controls. Serum adiponectin and IL-6 levels were significantly higher in VaD and AD patients than controls. The serum IGF-1 level was significantly lower in VaD and AD patients than controls, and the level was lowest in AD.

**Correlations Between Plasma IR-U Level and Other Parameters in All Subjects**

We examined the correlations between the plasma IR-U level and other parameters in 286 subjects. Plasma IR-U levels were positively correlated

**Table 1. Clinical characteristics of VaD or AD patients and non-demented controls**

<table>
<thead>
<tr>
<th></th>
<th>VaD (n=42)</th>
<th>AD (n=197)</th>
<th>Control (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>77±1</td>
<td>80±1</td>
<td>75±1</td>
</tr>
<tr>
<td>Male (%)</td>
<td>55</td>
<td>40</td>
<td>62</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21±1</td>
<td>21±1</td>
<td>22±1</td>
</tr>
<tr>
<td>Alcohol consumption (%)</td>
<td>17</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Smoking habit (%)</td>
<td>12</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>17</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>26</td>
<td>27</td>
<td>38</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>57</td>
<td>44</td>
<td>57</td>
</tr>
<tr>
<td>Cerebrovascular disease (%)</td>
<td>100²</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Ischemic heart disease (%)</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Peripheral vascular disease (%)</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>135±3</td>
<td>127±1</td>
<td>131±1</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75±2</td>
<td>72±1</td>
<td>75±1</td>
</tr>
<tr>
<td>MMSE (points)</td>
<td>9±1 ²</td>
<td>8±1 ²</td>
<td>27±2</td>
</tr>
</tbody>
</table>

Data are expressed as the means±SEM. *p<0.001 vs. other groups; †p<0.0001 vs. control group.

**Fig. 1.** Comparison of maximum IMT (A) in the carotid artery and plasma IR-UII levels (B) among VaD patients, AD patients, and non-demented controls. Results are expressed as the means±SEM.
Table 2. Laboratory parameters of VaD or AD patients and non-demented controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>VaD (n=42)</th>
<th>AD (n=197)</th>
<th>Control (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>138±7*</td>
<td>123±2</td>
<td>121±4</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>55±3</td>
<td>58±1</td>
<td>56±3</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>85±7</td>
<td>82±3</td>
<td>86±4</td>
</tr>
<tr>
<td>ApoA-1 (mg/dL)</td>
<td>130±4</td>
<td>136±2</td>
<td>138±4</td>
</tr>
<tr>
<td>ApoB (mg/dL)</td>
<td>105±5</td>
<td>94±2</td>
<td>104±5</td>
</tr>
<tr>
<td>ApoE (mg/dL)</td>
<td>5.6±0.4</td>
<td>4.5±0.1</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>Lipoprotein(a) (mg/dL)</td>
<td>32±4</td>
<td>20±4</td>
<td>19±1</td>
</tr>
<tr>
<td>Lipid peroxides (nmol/mL)</td>
<td>4.7±1.7*</td>
<td>2.3±0.2</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>105±4</td>
<td>108±2</td>
<td>104±3</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>8.0±1.0</td>
<td>7.8±0.7</td>
<td>8.4±1.2</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>114±7*</td>
<td>104±4*</td>
<td>152±7</td>
</tr>
<tr>
<td>HGF (ng/mL)</td>
<td>0.3±0.02</td>
<td>0.3±0.01</td>
<td>0.2±0.10</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>4.4±0.7</td>
<td>4.3±0.4</td>
<td>5.4±0.7</td>
</tr>
<tr>
<td>Adiponectin (mg/mL)</td>
<td>7.5±1.3*</td>
<td>6.0±0.7*</td>
<td>3.6±0.9</td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>24±3</td>
<td>19±1</td>
<td>22±3</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>9.0±3.1*</td>
<td>6.1±0.7*</td>
<td>2.2±0.3</td>
</tr>
<tr>
<td>Hs-CRP (ng/mL)</td>
<td>9.250±3.114*</td>
<td>6.375±1.126</td>
<td>2.039±411</td>
</tr>
</tbody>
</table>

Data are expressed as the means±SEM. *p<0.05, †p<0.001 vs. other groups; ‡p<0.0001, §p<0.005, ¶p<0.05 vs. the control group.

Fig. 2. Correlation between plasma IR-U11 levels and maximum IMT in the carotid artery among VaD patients (closed circles), AD patients (open squares), and non-demented controls (open circles).

with systolic BP (r=0.14, p<0.02), diastolic BP (r=0.15, p<0.01), or maximum IMT (r=0.44, p<0.0001; Fig. 2). In comparison in plasma IR-U11 levels between VaD patients and healthy controls who had increased maximum IMT ≥1.1 mm, IR-U11 levels were significantly still higher in 39 VaD patients than in 19 healthy volunteers (5.8±0.5 vs. 3.5±0.5 ng/mL, p<0.01). There were no significant correlations between IR-U11 levels and other atherosclerotic parameters.

Table 3. Associations of parameters with vascular dementia in all subjects (n=286)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>1.006</td>
<td>0.999–1.014</td>
<td>0.1110</td>
</tr>
<tr>
<td>IR-U11 (ng/mL)</td>
<td>1.272</td>
<td>1.099–1.473</td>
<td>0.0013</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.999</td>
<td>0.977–1.022</td>
<td>0.9256</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>1.015</td>
<td>0.998–1.032</td>
<td>0.0775</td>
</tr>
<tr>
<td>Maximum IMT (≥1.1 mm)</td>
<td>6.085</td>
<td>2.816–13.14</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Logistic Regression Analysis of Risk Factors for VaD

Univariate logistic regression analysis of all parameters measured in 286 subjects revealed that LDL cholesterol (OR: 0.909, 95%CI: 0.850–0.971, p = 0.0048), IR-U11 (OR: 3.300, 95%CI: 1.735–6.429, p = 0.0003), IL-6 (OR: 0.811, 95%CI: 0.643–1.022, p = 0.0048), systolic BP (OR: 1.123, 95%CI: 1.035–1.219, p = 0.0055), and increased maximum IMT (≥1.1 mm) (OR: 10.55, 95%CI: 1.446–77.02, p = 0.02) were significantly associated with VaD. Further, multivariate logistic regression analysis was performed in 286 subjects to assess independent risk factors for VaD among these above parameters. This analysis indicated significantly independent associations of plasma IR-U11 levels and increased maximum IMT (≥1.1 mm) with VaD as compared with serum LDL.
cholesterol and IL-6 levels and systolic BP (Table 3).

**Expression of IR-U11 in Carotid Atherosclerotic Plaques**

Photomicrograph of staining with anti-U11 antibody shows that IR-U11 is expressed at high levels in atherosclerotic plaques of human carotid arteries from a patient with CVD (Fig. 3). Merge images of fluorescent double staining show that IR-U11 is expressed highly in vascular smooth muscle cells (VSMCs) and sporadically in macrophage foam cells and endothelial cells (ECs).

**Discussion**

The present study indicated that plasma IR-U11 and atherosclerotic biomarker (LDL cholesterol, lipoprotein(a), lipid peroxide, IL-6, and hs-CRP) levels and carotid atherosclerosis were significantly greater in VaD than in AD patients or controls. We and others previously showed that plasma levels of vasoactive agents and inflammatory cytokines such as serotonin, hs-CRP, IL-1β, and tumor necrosis factor-α, were significantly higher in VaD patients than in AD patients. Atherosclerotic indices, such as carotid IMT and the ankle-to-brachial index, were significantly greater in VaD than in AD. Increased carotid IMT is associated with the severity of intracranial arterial stenosis and VaD. Although no differences in serum apoE levels were found among our three groups, apoE polymorphism (ε4 allele) is well known to be associated with AD rather than VaD. However, recent studies reported that carotid atherosclerosis and apoE polymorphism are observed in...
both AD and VaD patients with almost similar incidence. In our present and previous studies, plasma IR-U II levels showed a closely positive correlation with carotid maximum IMT as well as BP, suggesting that U II may become a reliable biomarker for atherosclerosis. Importantly, it is the first report that both increased plasma IR-U II levels and carotid atherosclerosis showed a significantly independent association with VaD. This study provides insights into the combined use of both parameters to predict the risk for VaD.

The expression of U II is shown in VSMCs, ECs, and macrophage foam cells within human carotid atherosclerotic plaques. These vascular cells are also the major cell types expressing UT. U II acts in an autocrine-paracrine manner in the vessel wall. Levels of U II and UT expression are up-regulated by inflammatory cytokines, such as interferon-γ, IL-6, and IL-1β, and by hypoxia (ischemia) and mechanical stimuli. U II contributes to the development of atherosclerosis by inducing macrophage foam cell formation, EC and VSMC proliferation, and extracellular matrix production. U II accelerates macrophage foam cell formation by up-regulating acyl-CoA:cholesterol acyltransferase-1, and has synergistic interactions with mildly oxidized LDL in inducing VSMC proliferation at the highest rate among vasoactive agents. Our recent study showed that chronic infusion of U II into apoE-knockout mice enhances atherosclerotic lesions. These findings indicate that U II contributes to the progression of atherosclerosis in the large and small cerebral arteries, leading to the major etiology of CVD followed by VaD.

There are several potential limitations in the present study. First, there is some controversy as to the use of some immunoassays for measuring U II levels. Disparity was found between the results of different assays, such as radioimmunoassay and ELISA from different commercial or in-house sources. One reason for discrepant findings between studies that employ different immunoassay methodologies might be the differing selectivity of the antibody used for mature U II over prepro-U II, U II-related peptide, and U II fragments. Douglas stated that establishing differential assays for immunoreactive and bioactive U II may help explain the wide variation in ELISA assay, however, such sensitive assays are not yet available. Second, serum levels of adiponectin, known as an anti-atherosclerotic and anti-inflammatory adipocytokine, were significantly higher in patients with VaD and AD than non-demented controls. This finding is in sharp contrast to serum adiponectin levels being decreased in patients with intracranial atherosclerosis. Recent studies showed that serum adiponectin levels are compensatorily increased in chronic inflammatory vascular diseases, such as systemic lupus erythematosus or congestive heart failure to repair vascular endothelial damage. In the present study, adiponectin levels increased with the grade of hs-CRP and also were significantly negatively correlated with IGF-1 levels ($r = -0.28$, $p < 0.0001$). Future studies are required to examine whether the decreased IGF-1 levels in dementia observed in our present and previous studies may lead to increased levels of adiponectin. Finally, plasma IR-U II levels were rather lower in AD patients than in the age-matched controls. The causal relationship between the reduction of plasma U II levels and AD pathogenesis is unclear in the present study. Further studies are needed to clarify this point.

In conclusion, our results suggest that the plasma IR-U II level is a candidate biomarker for atherosclerosis, and thus increased IR-U II levels along with severe carotid atherosclerosis may act as risk factors for VaD; therefore, blockade of the U II/UT system may be a promising therapeutic strategy for VaD.

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