Endothelin-1 in Cerebrospinal Fluid in Elderly Patients With Hypertension and Dementia

Masayoshi Nakajima, Shigeto Morimoto, Shoshi Takamoto, Shoichi Kitano, Keisuke Fukudo, Toshio Onishi, Toshio Ogihara

Abstract Endothelin-1, a potent endothelium-derived vasoconstrictive peptide, is also known to exist in the central nervous system. We determined endothelin-1-like immunoreactivity in cerebrospinal fluid by a radioimmunoassay in 32 normotensive or hypertensive elderly subjects (79±8 years old) with or without multi-infarction dementia. The mean value of endothelin-1-like immunoreactivity in cerebrospinal fluid was significantly (P<.05) elevated in subjects with essential hypertension (>160/95 mm Hg, n=5, 79±9 years old) compared with those with borderline hypertension (140-159/90-94 mm Hg, n=4, 78±5 years old) and normotensive subjects (<140/90 mm Hg, n=23, 79±8 years old). The value of endothelin-1-like immunoreactivity in cerebrospinal fluid was significantly (P<.05) positively correlated with both systolic (r=.38) and diastolic (r=.42) blood pressures in all subjects. On the other hand, mean values of endothelin-1-like immunoreactivity in cerebrospinal fluid were also significantly (P<.05) elevated in the groups of patients with multi-infarction dementia that had profoundly decreased Mini-Mental State scores (≤10, n=6) and moderately decreased Mini-Mental State scores (11 to 20, n=14) compared with those values in subjects with normal cognitive function (score for Mini-Mental State ≥21, n=12). The value of endothelin-1-like immunoreactivity in cerebrospinal fluid was significantly negatively correlated (r=−.48, P<.05) with scores for the Mini-Mental State. Moreover, scores for the Mini-Mental State correlated negatively with both systolic (r=−.52, P<.01) and diastolic (r=−.36, P<.05) blood pressures. These results indicate that endothelin-1 in cerebrospinal fluid is associated with hypertension, cerebrovascular injury, or both in the elderly. (Hypertension. 1994;24:97-100.)

Key Words • endothelins • cerebrospinal fluid • aged • dementia, multi-infarct

Endothelin-1 (ET-1) is a potent vasoconstrictive peptide recently identified in the conditioned medium of cultured aortic endothelial cells.1 The extremely potent vasoconstrictor action of ET-1 and the wide distribution of its binding sites2 suggest that ET-1 may play a crucial role in the control of blood pressure (BP) and also in the pathogenesis of hypertension.3-6 ET-1 and big ET-1 have also been identified in human cerebrospinal fluid (CSF).7,8 Specific receptors for ET-1 were also identified in the brains of rats9,10 and humans.9 Moreover, intracisternal or intracerebroventricular administration of ET-1 provoked significant BP elevation in rats11,12 and cerebral vasospasm in dogs.13 These observations indicate possible roles of CSF ET-1 as a brain peptide in the regulation of both cardiovascular functions and cerebrovascular tone. In the present study we evaluated the possible participation of CSF ET-1-like immunoreactivity (ET-1-LI) in BP regulation and/or in multi-infarction dementia (MID) in elderly subjects.

Methods

Subjects

The subjects studied were 32 elderly individuals (12 men and 20 women; mean±SD age, 79±8 years) with or without essential hypertension and with or without MID undergoing myelography for the workup of back or neck pain. All the subjects studied were residents of a nursing home. These subjects were classified into 23 normotensive subjects with BP below 140/90 mm Hg (9 men and 14 women, 79±8 years), 4 borderline hypertensive subjects with systolic BP of 140 to 159 mm Hg and/or diastolic BP of 90 to 94 mm Hg (1 man and 3 women, 79±5 years), and 5 hypertensive subjects with systolic BP of 160 mm Hg or higher and/or diastolic BP of 95 or higher (2 men and 3 women, 79±9 years). BP was taken as the mean of at least two different measurements in the morning of 3 different days. None of the subjects suffered from acute cerebrovascular disease, ischemic heart disease, congestive heart failure, renal disease, diabetes mellitus, or other severe illness. The clinical diagnosis of MID was established by criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM III-R).14 The MID patients, having scores of 20 or less on the Mini-Mental State (MMS),15 were further divided into a dementia group (n=6, 3 men and 3 women, 78±5 years) with scores of 10 or less and a predementia group (n=14, 3 men and 11 women, 78±5 years) with scores of 11 to 20. All the nondementia elderly subjects (n=12, 6 men and 6 women, 79±8 years) had MMS scores of 21 to 30. The mean values of MMS scores were 1.8±2.9, 16.2±2.6, and 25.8±3.4 in the dementia, predementia, and nondementia groups, respectively. Patients in whom dementia was a part of another neurological disorder, such as senile dementia of Alzheimer's type, Parkinson's disease, or with evidence of dementia caused by endocrine or electrolyte disorder, were not included. None of the subjects were receiving any medication that may affect BP or cognitive function, including antihypertensive or nonsteroidal anti-inflammatory drugs, for at least 4 weeks before the study. Blood samples were collected by venipuncture, and CSF samples were withdrawn via lumbar puncture before the injection of water-soluble myelographic dye for the evaluation of back or neck pain, in the morning after an overnight fast.
Clinical and Laboratory Characteristics of Elderly Subjects Studied

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normotensive</th>
<th>Borderline Hypertensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (men/women)</td>
<td>23 (9/14)</td>
<td>4 (1/3)</td>
<td>5 (2/3)</td>
</tr>
<tr>
<td>Age, y</td>
<td>79±8</td>
<td>78±5</td>
<td>79±9</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.55±0.09</td>
<td>1.56±0.09</td>
<td>1.53±0.08</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>51±7</td>
<td>52±8</td>
<td>51±7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>20±2</td>
<td>21±2</td>
<td>22±2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>120±12</td>
<td>148±4†</td>
<td>171±5‡</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>72±7</td>
<td>92±4†</td>
<td>97±7†</td>
</tr>
<tr>
<td>Mini-Mental State score</td>
<td>19.3±8.3</td>
<td>13.2±9.8</td>
<td>10.0±9.4*</td>
</tr>
<tr>
<td>Serum albumin, μmol/L</td>
<td>520±40</td>
<td>520±40</td>
<td>540±60</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>70±27</td>
<td>80±18</td>
<td>80±18</td>
</tr>
<tr>
<td>Plasma renin activity, (ng/L)/s</td>
<td>0.44±0.36</td>
<td>0.11±0.08</td>
<td>0.11±0.06</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>147±64</td>
<td>139±36</td>
<td>108±42</td>
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<tr>
<td>Plasma epinephrine, pmol/L</td>
<td>224±77</td>
<td>224±98</td>
<td>213±71</td>
</tr>
<tr>
<td>Plasma norepinephrine, pmol/L</td>
<td>1627±1728</td>
<td>1737±580</td>
<td>1260±420</td>
</tr>
<tr>
<td>Plasma endothelin-1, pmol/L</td>
<td>0.75±0.19</td>
<td>0.81±0.08</td>
<td>0.97±0.16*</td>
</tr>
</tbody>
</table>

*P<.05, †P<.01, normotensive vs borderline hypertensive or hypertensive.
‡P<.01, borderline hypertensive vs hypertensive.

Subjects and/or their relatives were informed about the nature of the study and gave written consent. The study was approved by an institutional review committee, and the procedures followed were in accordance with institutional guidelines. Plasma and CSF samples were kept at −20°C until assay for ET-1-LI.

Measurement of ET-1-LI

CSF and plasma samples, collected into tubes containing EDTA and aprotinin, were acidified by addition of 2 mol/L HCl (1:0.25 mL), centrifuged at 10,000 g for 5 minutes at room temperature, and loaded on an ethyl silica minicolumn (Amprep C2, 500 mg; Amersham) that had been equilibrated by washing with methanol (2 mL) followed by deionized water (2 mL) before the loading. Each column was washed with 5 mL of 0.1% (vol/vol) trifluoroacetic acid and eluted with 2 mL of 0.1% (vol/vol) trifluoroacetic acid in 80% (vol/vol) methanol. The eluate was dried under vacuum centrifugation into polypropylene tubes. The extracts were dissolved in 0.25 mL of 20 mmol/L phosphate buffer, pH 7.0, containing 10% Block Ace, HCl (1:0.25 mL), centrifuged at 10,000 g for 5 minutes at room temperature, and loaded on an ethyl silica minicolumn (Amprep C2, 500 mg; Amersham) that had been equilibrated by washing with methanol (2 mL) followed by deionized water (2 mL) before the loading. Each column was washed with 5 mL of 0.1% (vol/vol) trifluoroacetic acid and eluted with 2 mL of 0.1% (vol/vol) trifluoroacetic acid in 80% (vol/vol) methanol. The eluate was dried under vacuum centrifugation into polypropylene tubes. The extracts were dissolved in 0.25 mL of 20 mmol/L phosphate buffer, pH 7.0, containing 10% Block Ace, 400 mmol/L NaCl, and 2 mmol/L EDTA. ET-1-LI was measured by a radioimmunoassay using a commercial kit (ET-1 high sensitivity assay system, Amersham). The sensitivity of the assay was 0.2 fmol per tube. The cross-reactivities for the assay of big ET-1 and ET-3 were 38% and 0.3%, respectively.

Measurement of Other Circulating Factors

Serum levels of creatinine and albumin were measured with a Technicon autoanalyzer. Plasma renin activity and plasma aldosterone concentration were measured by radioimmunoassay and plasma epinephrine and norepinephrine by a high-performance liquid chromatographic method, as previously reported.16

Statistical Analysis

Two-way ANOVA was used for comparison of any two groups. Spearman's analysis was used for correlation between any two parameters. Probability values less than .05 were considered significant.

Results

Characteristics of the Subjects

The Table summarizes the characteristics of the subjects studied. The mean age was similar in the normotensive, borderline hypertensive, and hypertensive groups. There were no significant differences among the three groups in the mean values of height, weight, body mass index, or serum levels of creatinine and albumin. The BP values were significantly higher in the hypertensive group compared with the other two groups and in the borderline hypertensive group compared with the normotensive group. The mean values of MMS score in the hypertensive group were significantly (P<.05) lower than those in the normotensive group. Values of both systolic (r = −.36, P<.05) and diastolic (r = −.52, P<.01) BP were significantly negatively correlated with MMS score.

CSF ET-1-LI

The mean CSF level of ET-1-LI was significantly (P<.05) elevated in the hypertensive subjects (11.1±2.5 pmol/L) compared with that in the borderline hypertensive and normotensive subjects (7.4±2.0 and 7.5±2.4 pmol/L, respectively) (Figure, a). The CSF ET-1-LI level was significantly (P<.05) positively correlated with values of both systolic (r = .38) and diastolic (r = .42) BP in all subjects. On the other hand, CSF ET-1-LI levels in both the dementia and predementia groups (9.6±2.6 and 8.9±2.9 pmol/L) were significantly (P<.05) higher than those in the nondementia group (6.4±1.2 pmol/L) (Figure, b). The CSF ET-1-LI level was significantly negatively (r = −.48, P<.01) correlated with MMS score in all subjects. The CSF ET-1-LI level in men (n=12, 10.3±3.5 pmol/L) did not differ significantly from that in women (n=20, 10.2±2.1 pmol/L), and there was no significant correlation of CSF ET-1-LI level with age of the subjects.
The mean plasma level of ET-1-LI was also significantly higher in the hypertensive group than in the normotensive groups (Table), although the ET-1-LI levels in plasma were obviously lower than those in CSF. The ET-1-LI level in plasma was significantly positively correlated with that in CSF (r = 0.42, P < 0.05) and with the values of both systolic (r = 0.32, P < 0.05) and diastolic (r = 0.35, P < 0.05) BP in all subjects. However, plasma ET-1-LI was similar in the dementia, predementia, and non-dementia groups and did not correlate significantly with MMS score (r = -0.06).

Circulating BP-Related Factors

The Table also summarizes the plasma levels of BP-related factors. The mean levels of plasma renin activity and plasma aldosterone concentration were slightly but nonsignificantly decreased in the hypertensive and borderline hypertensive groups compared with those in the normotensive group. Plasma levels of epinephrine and norepinephrine were similar in the three groups. Neither ET-1-LI level in CSF nor that in plasma significantly correlated with levels of any of these circulating BP-related factors in all subjects.

Discussion

In the present study we demonstrated for the first time significant elevation of CSF ET-1-LI level in elderly patients with essential hypertension and/or MID. It has been well known that hypertension is one of the most potent risk factors for cerebrovascular injury and resulting MID. Actually, our hypertensive subjects showed a significantly higher tendency to suffer from MID and significantly lower cognitive function compared with the normotensive subjects. Besides the elevation of CSF level of ET-1-LI, our elderly hypertensive subjects showed a significant elevation of plasma level of ET-1-LI compared with the normotensive subjects. This observation is compatible with most studies that measured circulating ET-1-LI, showing increases in the circulating levels of ET-1-LI in subjects with essential hypertension compared with normotensive subjects. In contrast to CSF ET-1-LI, plasma ET-1-LI levels were similar among the three groups with different degrees of cognitive function. These observations suggest an association of ET-1-LI level in both CSF and the circulation with hypertension but an association of only CSF ET-1-LI with MID.

The ET-1 levels in CSF were approximately 10 times higher than those in the plasma in the three groups. This observation is compatible with those of previous reports, although controversy persists. Furthermore, 121-ET-1 administered systemically in rats does not cross the blood-brain barrier. However, immunocytochemical studies have revealed the presence of ET-1-LI in neurons and astrocytes in the brain. Taken together, our results strongly suggest that ET-1-LI present in CSF may represent ET-1 or big ET-1 synthesized and secreted by neural tissues.

There are several possible explanations for the elevated level of CSF ET-1-LI in elderly subjects with essential hypertension and/or MID. The first is that CSF volume in elderly subjects with hypertension and/or MID is decreased, resulting in the increase in CSF ET-1-LI levels in these individuals. However, this possibility is not likely, because the volume of brain ventricles and cisterns is increased especially in elderly subjects with MID.

The second possibility is that increased synthesis of ET-1 in the brain causes the hypertension. In animal experiments, intracisternally or intracerebroventricularly administered ET-1 caused significant increases in BP and heart rate, probably through stimulation of sympathetic vasomotor activity, accompanied by elevation of plasma levels of epinephrine and norepinephrine. However, there was no significant correlation between CSF ET-1-LI and circulating levels of epinephrine and norepinephrine in our elderly subjects. Moreover, there was no difference in the level of CSF ET-1-LI between borderline hypertensive and normotensive subjects. These findings suggest that an elevated level of CSF ET-1-LI is more strongly associated with the progress than with the onset of hypertension in the elderly.

The third possibility is that synthesis of ET-1 may be increased in injured brain secondary to the development of hypertension and/or multi-infarction. Astrocytes not only are target cells for ET-1 but also produce ET-1. These cells are activated in the repair process of brain lesions. CSF ET-1-LI level is also known to be elevated and involved in vasospasm in subarachnoid hemorrhage. It also might be possible that elevated CSF ET-1-LI may cause local vasospasm of the cerebral arteries with resulting MID, since intracisternally administered ET-1 induces cerebral vasospasm. However, the magnitude of the increase in CSF ET-1-LI was modest in elderly subjects with hypertension and/or MID in the present study compared with that in patients with cerebral vasospasm after subarachnoid hemorrhage who demonstrated a twofold to severalfold increase. Although comparable levels of ET-1 (10^-11 mol/L and more) evoked cerebral vasospasms in animals and could displace the binding of labeled...
endothelin to its specific receptors on cultured neuronal cells,28,29 studies of the effects of endothelin on human cerebral arteries indicate that endothelin levels of approximately $10^{-7}$ mol/L or greater are needed to produce significant vasoconstriction.30

As the fourth explanation, it is also possible that the increased levels of CSF ET-1-LI in our study may reflect decreased clearance of CSF ET-1 in elderly subjects with hypertension and/or MID, since brain atrophy in elderly MID patients23 may also induce a decrease in the binding sites of brain cells and a subsequent decrease in clearance of ET-1-LI.

Further investigation is required to clarify whether ET-1 or big ET-1 participates as a CSF factor in the development of hypertension and/or MID in the elderly. A possible compositional change in ET-1 and big ET-1 in CSF in these situations should also be investigated. ET-3 is also reported to be present in the human brain.7,31,32 The possible association of CSF ET-3 with hypertension and/or MID in the elderly MID patients may also induce a decrease in clearance of CSF ET-3 in elderly subjects.

References


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