Blood-Brain Barrier Permeability Correlates with Medial Temporal Lobe Atrophy but Not with Amyloid-β Protein Transport across the Blood-Brain Barrier in Alzheimer’s Disease

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Key Words
Alzheimer’s disease · Blood-brain barrier · Medial temporal lobe atrophy · Amyloid-β protein

Abstract
Background/Aims: Alterations in the blood-brain barrier (BBB) may play an important role in the pathogenesis and treatment of Alzheimer’s disease (AD). We investigated BBB disturbance and its influence on the equilibrium of amyloid-β protein (Aβ) between plasma and cerebrospinal fluid (CSF) in AD patients. Methods: We analyzed albumin ratio as a marker of the BBB permeability and correlated it with the severity of dementia, brain atrophy on MRI, apolipoprotein E isoform, CSF levels of total tau, CSF and plasma levels of Aβ1–40 (Aβ40) and 1–42 (Aβ42), and CSF/plasma ratios of Aβ40 and Aβ42 in 42 AD patients. Results: The albumin ratio was positively correlated with the severity of medial temporal lobe atrophy but not with the other parameters including CSF/plasma ratios of Aβ40 or Aβ42. Conclusion: Our results suggest that progression of medial temporal lobe atrophy is associated with increased BBB permeability and that the transport of Aβ across the BBB is not influenced by the BBB alteration in AD.

Introduction
Alzheimer’s disease (AD) is the most common cause of dementia in the elderly. The pathogenesis of this disease has not yet been fully elucidated. Several studies have been performed to investigate whether blood-brain barrier (BBB) alterations play a significant role in AD [1–11]. Despite morphological evidence suggesting abnormalities of the brain microvasculature that constitutes the BBB in AD [2], there is still little clinical evidence supporting BBB disruption in AD patients [1, 3–5, 7–11]. Understanding of the BBB alteration is important because it may influence the regulation of entry of plasma-derived amyloid-β protein (Aβ) into the brain as well as the clearance of brain-derived Aβ. Changes in BBB permeability may also affect the delivery of anti-AD drugs into the brain.

In the present study, we investigated the cerebrospinal fluid (CSF)/serum albumin ratio, a marker of BBB permeability, and correlated it with dementia scales, brain atrophy on magnetic resonance imaging (MRI), apolipoprotein E (ApoE) isoform, CSF levels of total tau, CSF and plasma levels of Aβ and CSF/plasma ratios of Aβ in AD patients.
lobe atrophy (MTA) proposed by Scheltens et al. [13, 14].

We studied 42 consecutive patients (21 men/21 women) aged 53–81 years, who had consented to our study including lumbar tapping, and fulfilled the clinical criteria of the NINCDS-ADRDA Work Group for probable AD [12]. To exclude other diseases, we performed laboratory examinations including routine blood tests, CSF analyses, MRI of the brain and single photon emission computed tomography for cerebral blood flow with 99m Tc-etihyl cysteinate dimer.

As concomitant vascular lesions would influence the BBB permeability as well as dementia, we evaluated the vascular lesions on MRI using the Age-Related White Matter Change Rating Scale [13] and excluded patients with a score of 30 or more, which indicated significant signal hyperintensity on MRI in the periventricular areas, lobar white matter, basal ganglia and infratentorial areas. The severity of dementia was assessed by Clinical Dementia Rating (CDR) and Mini-Mental State Examination (MMSE). Patients with CDR 0.5 at their first visit were included in the AD group if they later progressed to fulfill the AD criteria [12]. Informed consent was obtained from each patient under the approval of the ethics committee of the institution. The clinical features of the 42 patients are summarized in table 1.

**Table 1.** Clinical features, brain atrophy, and albumin, total tau, Aβ42 and Aβ40 in CSF and serum/plasma in 42 patients with AD

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>53–81</td>
<td>69.9 ± 7.9</td>
</tr>
<tr>
<td>CDR</td>
<td>0.5–3</td>
<td>1.0 ± 0.7</td>
</tr>
<tr>
<td>MMSE</td>
<td>9–27</td>
<td>20.8 ± 4.7</td>
</tr>
<tr>
<td>MTA1</td>
<td>0–4</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>Serum albumin, mg/dl</td>
<td>12.4–39.3</td>
<td>25.0 ± 7.8</td>
</tr>
<tr>
<td>Albumin ratio</td>
<td>3.0–9.6</td>
<td>6.0 ± 1.8</td>
</tr>
<tr>
<td>CSF total tau</td>
<td>9.0–1,200.0</td>
<td>466.2 ± 357.3</td>
</tr>
<tr>
<td>Plasma Aβ40, pg/ml</td>
<td>21–6,848</td>
<td>539.0 ± 1,254.0</td>
</tr>
<tr>
<td>Plasma Aβ42, pg/ml</td>
<td>5.8–221.0</td>
<td>47.7 ± 39.3</td>
</tr>
<tr>
<td>CSF Aβ40, pg/ml</td>
<td>1,534–13,676</td>
<td>6,833 ± 3,263</td>
</tr>
<tr>
<td>Aβ40 Ratio1</td>
<td>0.64–365.6</td>
<td>83.2 ± 95.0</td>
</tr>
<tr>
<td>Aβ42 Ratio1</td>
<td>0.78–16.5</td>
<td>7.7 ± 4.1</td>
</tr>
</tbody>
</table>

MTA = Medial temporal lobe atrophy.

1 MTA on MRI: the rating scale of Scheltens et al [13].
2 Albumin ratio = CSF albumin (milligrams/deciliter)/serum albumin (grams/deciliter).
3 Aβ40 (42) ratio = CSF Aβ40 (42) (picograms/milliliter)/plasma Aβ40 (42) (picograms/milliliter).

**Method**

**Patients**

We studied 42 consecutive patients (21 men/21 women) aged 53–81 years, who had consented to our study including lumbar tapping, and fulfilled the clinical criteria of the NINCDS-ADRDA Work Group for probable AD [12]. To exclude other diseases, we performed laboratory examinations including routine blood tests, CSF analyses, MRI of the brain and single photon emission computed tomography for cerebral blood flow with 99mTc-ethyl cysteinate dimer.

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**Evaluation of Brain Atrophy on MRI**

MRI studies were performed using a 1.5-tesla unit (Signa, General Electric, N.Y., USA). For evaluation of progressive brain atrophy in AD, we used the visual rating scale of medial temporal lobe atrophy (MTA) proposed by Scheltens et al. [13, 14]. Briefly, the 5-point rating scale (0–4) is based on assessment of the size of the hippocampal formation relative to the surrounding CSF space on coronal T1-weighted images that best depict the hippocampal formation and surrounding structures. A score of 0 was assigned when no CSF was seen surrounding the hippocampus. A score of 4 was given if there was severe atrophy of the medial temporal lobe and the normal anatomy of the hippocampus was no longer visible, with enlargement of the temporal horn and the choroid fissure. This rating scale of MTA was reported to show good interobserver reliability [15]. The patients were rated by 2 neurologists (D.Y. and K.O.) blinded to any clinical information and laboratory findings.

**Analysis of CSF, Serum and Plasma**

Lumbar puncture was performed to obtain CSF samples; the CSF samples were analyzed for albumin, total tau, Aβ 1–42 (Aβ42) and 1–40 (Aβ40), which are major species of Aβ deposited in the AD brain [16, 17], in addition to routine CSF examination. At the same time as lumbar puncture, serum and plasma samples were obtained and examined for serum albumin, ApoE isoform, and plasma Aβ42 and Aβ40.

The CSF/plasma ratio for albumin [albumin ratio = CSF albumin (milligrams/deciliter)/serum albumin (grams/deciliter)] was calculated as a marker of BBB permeability [18]. For measurement of total tau, Aβ42 and Aβ40, CSF and plasma samples were kept frozen until the analysis. CSF levels of total tau and Aβ42 were measured by sandwich-type enzyme-linked immunosorbent assay (ELISA) using the standard Innotest assay format (Innogenetics, Ghent, Belgium); plasma Aβ42 was determined with the high sensitivity test format of the Innotest. CSF and plasma Aβ40 levels were measured by sandwich-type ELISA according to the manufacturer’s instructions (IBL, Gunma, Japan). Thereafter, the CSF/plasma ratios for Aβ42 and Aβ40 were calculated.

The ApoE isoform was determined by isoelectric focusing/immunoblotting using serum samples [19].

**Statistical Analysis**

The correlations of the albumin ratio with age, CDR, MMSE, MTA, CSF total tau, CSF and plasma levels of Aβ42 and Aβ40 and CSF/plasma, Aβ42 and Aβ40 ratios were analyzed statistically using Spearman’s rank correlation test. Associations of the presence of ApoE E4 with the albumin ratio were analyzed by Mann-Whitney U test. Correlations of ApoE E4 with albumin ratio were analyzed by Spearman’s rank correlation test. The level of significance was defined as p < 0.05. Statistical analyses were performed with StatView 5.0.

**Results**

Albumin, total tau, Aβ42 and Aβ40 in CSF and serum/plasma as well as MTA on MRI from the 42 AD patients are shown along with their clinical features in table 1.

The albumin ratio is significantly and positively correlated with age, CDR, MMSE (fig. 1a) but not with age, CDR, MMSE (fig. 1b), ApoE E4 isoform, CSF total tau or CSF and plasma levels of Aβ42 and Aβ40.
The CSF/plasma ratios for Aβ_{42} or Aβ_{40} were not correlated with the albumin ratio (fig. 2). In addition, there were no correlations between CSF Aβ_{42} or Aβ_{40} levels and those in plasma (data not shown), as reported previously [20].

**Discussion**

Our results indicated that BBB permeability, evaluated as the albumin ratio, is correlated with the severity of MTA, which would represent severity of AD pathology,
peripherally acting effects [26–28]. These observations reduce the Aβ/H9252 over of Aβ antibodies to Aβ studies have indicated that peripheral administration of and that CSF/plasma ratios for Aβ production and Aβ may arise from a chronic imbalance between Aβ and AD may be due to BBB permeability changes in AD [2, 6, 24]. However, there is little clinical evidence for breakdown of the BBB in AD patients, and the results are still controversial [1, 3–5, 7–11], in contrast with cerebral ischemia and vascular dementia, in which clinical abnormalities of Aβ are evident [21–23]. The albumin ratio did not differ significantly between AD and control cases in some reports [4, 7, 9], while it was higher in AD in other reports [3, 10, 11]. Our results indicated, for the first time, that BBB permeability is increased in association with severity of MTA in AD.

There are several possible explanations for the conflicting results of earlier studies, including (1) no exclusion of asymptomatic cerebrovascular disease by MRI evaluation and (2) evaluation of AD severity only by dementia scales, such as MMSE. In contrast to the previous studies [3, 4, 7, 9–11], our study included only AD patients who fulfilled AD criteria [12] and had no significant vascular lesions on MRI. Further, we used not only CDR and MMSE as dementia scales but also MTA on MRI as an indicator of the severity of AD. MTA and CDR/MMSE represent different aspects of the severity of AD. In the present study, only the MTA was correlated with the albumin ratio. The lack of correlation between CDR or MMSE and the albumin ratio in our study was consistent with a previous report [4]. Our results indicate that the BBB permeability changes in AD would be more closely associated with the severity of MTA, a pathological substrate characteristic of AD, than clinical severity of dementia. Our data provide clinical evidence supporting the pathological observations of microvascular abnormalities in the AD brain. The pathomechanisms underlying the alteration of BBB permeability may include microvascular degeneration, amyloid angiopathy and intrathecal inflammation that would damage BBB integrity, as demonstrated in multiple sclerosis [6, 24].

The deposition of Aβ, mainly Aβ42 and Aβ40, in the brain is an invariant neuropathological feature of AD, and AD may arise from a chronic imbalance between Aβ production and Aβ clearance. Aβ is detectable in soluble form in the CSF and plasma [20, 25]. Although the turnover of Aβ in CSF and plasma remains unclear, recent studies have indicated that peripheral administration of antibodies to Aβ or agents with an affinity to Aβ can reduce the Aβ levels in the brain, most likely because of peripherally acting effects [26–28]. These observations [26–28] suggest that Aβ dynamics between the periphery and brain play an important role in the cerebral Aβ deposition and provide the possibility of peripheral therapeutic approaches using such agents. Several studies have indicated that Aβ transport is mediated by Aβ receptors at the BBB, such as LDL receptor-related protein-1 and receptor for advanced glycation end products [29, 30]. Our results suggest that Aβ transport across the BBB is not influenced by BBB alterations in the AD brain because the BBB permeability represented by the albumin ratio was not related to the CSF/plasma ratio for Aβ42 or Aβ40 in this study.

This study has some limitations; first, there were no data of control cases because of the difficulty of obtaining CSF from normal subjects; second, MTA used as a morphological marker of AD in this study is not specific to the disease; third, the number of patients was relatively small. Since there was no correlation of the age with MTA or albumin ratio in our 42 patients, the factor of aging does not seem to influence our results. We performed the laboratory investigations, as described in the ‘Method’ section, to exclude other diseases than AD. However, further study with a larger number of AD patients and control subjects is necessary to confirm our results.

In conclusion, our results demonstrated, for the first time, that the BBB permeability is increased with the severity of MTA, a pathological substrate of AD, and that Aβ transport across the BBB would not be influenced by the increased BBB permeability.

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