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ORIGINAL PAPER

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Serum BDNF, TNF-α and IL-1β levels in dementia patients

Comparison between Alzheimer's disease and vascular dementia

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Abstract Neurotrophins such as the brain-derived neurotrophic factor (BDNF) are reportedly related to the pathogenesis of Alzheimer's disease (AD). Several studies have revealed an alteration in BDNF expression in the postmortem brains of AD patients. BDNF has great potential as a therapeutic agent because of its ability to cross the blood–brain barrier and due to its wide in vivo distribution. However, little is known about in vivo BDNF in dementia patients. Moreover, the immunological function of neurotrophins such as BDNF has received great interest. Therefore, we investigated the serum levels of BDNF and cytokines such as TNF-α and IL-1β in dementia patients by the enzyme-linked immunosorbent assay (ELISA). The following subjects were included in this study: 60 AD patients, 60 vascular dementia (VaD) patients and 33 healthy controls. AD and VaD patients were matched for age, gender and severity of dementia. Serum BDNF levels in AD patients were significantly lower than those in VaD patients and controls. TNF-α and IL-1β levels showed no significant difference among the three groups. In the dementia groups, neither the TNF-α nor the IL-1β levels correlated with the BDNF levels. Our results suggest that BDNF may play a pathological role in some cases of AD.

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Introduction

Alzheimer's disease (AD) is characterized by severe neuronal and synaptic degeneration of the cholinergic, hippocampal and cortical neurons in the basal forebrain [1]. The damage and the loss of basal forebrain cholinergic neurons and their projections, which play a crucial role in the functions of memory and cognition, correlates with the degree of dementia [2].

Neurotrophins such as the brain-derived neurotrophic factor (BDNF) support the function and survival of the basal forebrain cholinergic neurons [3, 4], suggesting that these factors might play a role in the aetiology of AD. As a therapeutic agent, BDNF has a great potential because it can cross the blood–brain barrier in both directions [5] and is widely distributed in vivo.

Limited information is available regarding in vivo BDNF in dementia patients. Several studies have revealed that the levels of the BDNF protein and mRNA in the postmortem brains of AD patients were decreased [6–8]. However, the serum BDNF levels in dementia patients have not yet been reported. Moreover, the immunological function of neurotrophins such as the BDNF has generally received great interest. Therefore, in the present study, we examined the serum levels of BDNF and cytokines such as TNF-α and IL-1β in the dementia patients and compared these values with in those from AD and vascular dementia (VaD) patients. In addition, we also investigated whether a possible correlation between BDNF and the cytokines would be valid to clarify the direction of a proposed functional relationship.

Methods

Subjects

The subjects included in this study were as follows: 60 AD patients, comprising 20 males and 40 females, 61–93-years-old (mean ± SD, 77.9 ± 7.0 years); 60 VaD patients, comprising 20 males and 40 females, 55–94-years-old (mean ± SD, 78.9 ± 8.2 years); and 33 healthy normal control subjects (NC), comprising 8 males and 25 females, 61–84-years-old (mean ± SD, 71.1 ± 5.8 years).

The healthy normal control subjects had no psychiatric or neurological antecedents, and individuals with cognitive impairments were excluded from this group.

The patients were subjected to a structural interview and physical examination, and those with malignant diseases or severe infections were excluded from all the study groups. AD and VaD were diagnosed based on the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria and the Neuroepidemiology Branch of the National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l' Enseignement en Neuroscience (NINDS-AIREN) criteria, respectively. A computed tomography (CT) scan was performed to aid the diagnosis.

Hachinski's ischaemic score (HIS) was used to differentiate between AD and VaD. The patients with HIS scores less than 4 were assigned to the AD group. The Mini-Mental State Examination (MMSE) was used to assess cognitive function. AD severity was rated according to Functional Assessment Staging (FAST).

All the subjects provided a written informed consent for their participation in the study. The ethics committee of Osaka Medical College approved the present study.

Table 1 lists the relevant characteristics of all the subjects. AD and VaD patients were concluded to be successfully matched for age, gender and the severity of dementia because no significant differences in these characteristics were observed between these two groups. One-way analysis of variance (ANOVA) revealed significant differences among the three groups [$F(2,150) = 13.5$, $P < 0.0001$], and the age of the control group (71.06 ± 5.7 years) was significantly lower than that of the AD (77.93 ± 7.04 years, $P < 0.0001$) and the VaD groups (78.88 ± 8.17 years, $P < 0.0001$).

Statistical analysis

The data were presented as the mean ± SD. The age differences among the three groups were assessed using ANOVA followed by the Bonferroni/Dunn test for multiple comparisons. The age differences in the two groups, the differences of MMSE scores and the differences in the serum BDNF levels with regard to gender were assessed by using Student's *t*-test. The relationship between the two variables was ascertained using Pearson's correlation coefficient. In order to exclude the effect of age, the differences of the serum BDNF, TNF-α and IL-1β levels in the three groups or in the two groups were assessed by using the multiple regression analysis with diagnosis and age as independent predictor variables. The results were regarded to be significant when $P < 0.05$. All statistical analyses were performed using the statistical analysis software package SPSS 11.0J for Windows.

Procedures

Blood samples were collected before breakfast (6:30–7:30 AM) within a few days after psychological testing. Serum was immediately separated, removed by centrifugation and frozen until analysis.

The serum levels of BDNF, TNF-α and IL-1β were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine R&D System, Minneapolis, MN, USA) according to the manufacturer's instructions.

All samples and standards were run in duplicates, and the mean values of the duplicate samples were used for statistical analyses. The detection limits were 20 pg/ml for BDNF, 1.6 pg/ml for TNF-α and 1 pg/ml for IL-1β. The intra- and inter-assay analyses were performed using multiple measurements of the control sample. The intra-assay coefficients of variation were 5.0% for BDNF, 5.2% for TNF-α and 8.5% for IL-1β. The inter-assay coefficients of variation were 11.3% for BDNF, 7.4% for TNF-α and 8.4% for IL-1β.

Results

Differences in the serum BDNF levels with regard to gender and age

In the NC group, the serum BDNF levels did not show any significant differences with respect to gender (males: 18.5 ± 7.9 ng/ml vs. females: 20.1 ± 7.5 ng/ml). Moreover, the serum BDNF levels did not correlate with age ($r = -0.144$, $P = 0.4255$).

In the dementia groups, the serum BDNF levels did not differ with gender (males: 17.4 ± 7.2 ng/ml vs. females: 16.2 ± 6.2 ng/ml) and did not correlate with age ($r = 0.057$, $P = 0.5353$).

Table 1 Participant characteristics

	AD ($n = 60$)	VaD ($n = 60$)	NC ($n = 33$)	P		
Gender (M/F)	20/40	20/40	8/25			
Age (years)	77.93 ± 7.04	78.88 ± 8.17	71.06 ± 5.77	0.4753[a]	<0.0001[b]	<0.0001[c]
MMSE	6.88 ± 6.78	6.75 ± 6.89		0.9058[a]		
HIS	1.20 ± 0.84	10.17 ± 3.06				
FAST	6 d (mean)					
BDNF (ng/ml)	14.73 ± 5.88	18.45 ± 6.71	19.72 ± 7.53	0.002[a]	0.002[b]	0.569[c]
TNF-α (ng/ml)	17.67 ± 6.15	17.63 ± 8.39	17.57 ± 4.10	0.930[a]	0.935[b]	0.663[c]
IL-1β (ng/ml)	3.88 ± 2.23	3.26 ± 2.04	4.17 ± 2.44	0.101[a]	0.507[b]	0.116[c]

Values are shown as mean ± SD. NC, normal controls; AD, Alzheimer's disease; VaD, vascular dementia; BDNF, brain-derived neurotrophic factor; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin one beta; MMSE, Mini Mental State Examination; HIS, Hachinski's ischaemic score; FAST, Functional Assessment Staging

[a]Comparison between AD and VaD

[b]Comparison between AD and NC

[c]Comparison between VaD and NC

Serum levels of BDNF, TNF-α and IL-1β and diagnostic classification

Figure 1 shows the serum BDNF levels in the NC subjects and AD and VaD patients. The multiple regression analysis revealed significant differences among the three groups ($P < 0.001$). The serum BDNF levels in AD patients (14.73 ± 5.88 ng/ml) were significantly lower than those in the VD patients (18.45 ± 6.71 ng/ml, $P = 0.002$) or NC subjects (19.72 ± 7.53 ng/ml, $P = 0.002$). There was no significant difference in the BDNF levels between the VaD and NC groups ($P = 0.569$).

The TNF-α and IL-1β levels showed no significant difference among the three groups ($P = 0.845$ for TNF-α, $P = 0.854$ for IL-1β).

Comparisons between the serum BDNF, TNF-α and IL-1β levels and clinical rating scales (MMSE, HIS and FAST)

In AD patients, the serum BDNF levels did not correlate with the MMSE ($r = 0.188$, $P = 0.1504$), HIS ($r = 0.179$, $P = 0.1772$) or FAST results ($r = -0.168$, $P = 0.2001$). Additionally, the serum TNF-α levels did not correlate with the MMSE ($r = 0.037$, $P = 0.7782$), HIS ($r = -0.139$, $P = 0.2897$) or FAST results ($r = -0.022$, $P = 0.8703$). The serum IL-1β levels also did not correlate with the MMSE ($r = -0.137$, $P = 0.2996$), HIS ($r = 0.081$, $P = 0.5420$) or FAST results ($r = 0.171$, $P = 0.1916$).

In VaD patients, the serum BDNF levels correlated positively with the MMSE ($r = 0.287$, $P = 0.0259$) but not with the HIS results ($r = -0.241$, $P = 0.0635$). The serum TNF-α levels did not correlate with either the MMSE ($r = -0.021$, $P = 0.8718$) or the HIS results ($r = -0.052$, $P = 0.6952$). The serum IL-1β levels correlated negatively with the MMSE ($r = -0.297$, $P = 0.0210$) but not with the HIS results ($r = -0.035$, $P = 0.7903$).

In dementia patients (AD + VaD), the serum BDNF levels correlated positively with the MMSE results ($r = 0.228$, $P = 0.0121$). Moreover, the serum BDNF levels correlated positively with the HIS results ($r = 0.194$, $P = 0.0335$). The serum TNF-α levels did not correlate with either the MMSE ($r = 0.003$, $P = 0.9723$) or with HIS the results ($r = -0.030$, $P = 0.7482$). The serum IL-1β levels correlated negatively with the MMSE ($r = -0.210$, $P = 0.0214$) but not with HIS the results ($r = -0.133$, $P = 0.1482$).

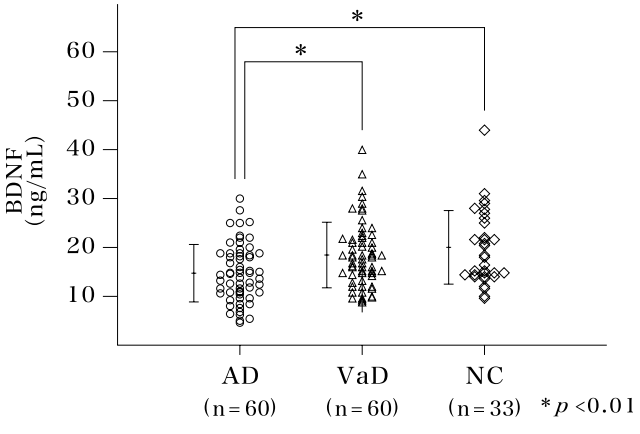

Fig. 1 Graph showing a comparison of the mean levels of the serum brain-derived neurotrophic factor (BDNF) in patients with Alzheimer's disease (AD), vascular dementia (VaD) and the normal control subjects (NC) Serum BDNF levels in AD patients [$n = 60$; 14.73 ± 5.88 ng/ml (mean ± SD)] were significantly lower ($P < 0.01$) than those in VaD patients ($n = 60$; 18.45 ± 6.71) or in the NC subjects ($n = 33$; 19.72 ± 7.53 ng/ml)

Comparison between the serum levels of BDNF and serum TNF-α or serum IL-1β

In AD patients, the serum BDNF levels did not correlate with either the TNF-α ($r = -0.144$, $P = 0.2729$) or the IL-1β levels ($r = -0.209$, $P = 0.1096$).

In VaD patients, the serum BDNF levels correlated negatively with the TNF-α ($r = 0.258$, $P = 0.0466$), but not with the IL-1β levels ($r = -0.052$, $P = 0.6955$).

In dementia patients (AD + VaD), the BDNF levels correlated with neither the TNF-α levels ($r = 0.095$, $P = 0.3013$) nor with the IL-1β levels ($r = -0.113$, $P = 0.2207$).

In all subjects (n = 153), neither the TNF-α ($r = 0.073$, $P = 0.3694$) nor the IL-1β ($r = -0.101$, $P = 0.2148$) levels correlated with the BDNF levels.

Discussion

In the present study, we demonstrated that the serum BDNF levels in AD patients were significantly lower than those in the VaD patients and NC subjects. This is the first report on the serum BDNF levels in dementia patients.

BDNF is highly concentrated in human plasma and is considerably more concentrated in the serum. This is due to the degranulation of platelets during the clotting process. Human platelets contain large amounts of the BDNF protein. Therefore, the difference between the serum and plasma BDNF levels appears to reflect the amount of BDNF stored in the circulating platelets. Notably, it has been postulated that platelet BDNF does not originate from megakaryocyte precursor cells. It seems to be acquired from the plasma and other compartments by internalization through binding sites that are unidentified so far [9]. The cellular sources of BDNF found in human plasma are not yet clearly defined; potential sources are the vascular endothelial and smooth muscle cells [10, 11]. Since BDNF crosses the blood–brain barrier in both directions, a substantial part of the circulating BDNF might originate from the neurons and glial cells of the central nervous system [5, 12].

Many studies have reported the levels of BDNF mRNA and its protein products in the postmortem brains of the AD patients. Connor et al. [7] reported decreased levels of the BDNF protein in the temporal cortex and hippocampus of AD patients. Ferrer et al. [13] reported decreased levels of the BDNF protein in the frontal cortex of AD patients.

Since the distribution of BDNF in the brain is greater than that of many other neurotrophic factors [14] and it supports the survival and function of a wide range of neurons, decreased BDNF levels could have extensive consequences. The decrease in the levels of BDNF mRNA and protein may possibly affect all basal forebrain cholinergic nuclei that are involved in AD.

Our results support previous reports that showed decreased levels of the BDNF protein and mRNA in the postmortem brains of AD patients.

In contrast, several reports from animal experiments have shown an increase in the BDNF mRNA and protein levels in the cerebrocortical and hippocampal neurons after brain injuries such as cerebral ischaemia [15–17]. This suggests that the BDNF exerts protective effects on the ischaemic brain. In addition, Lindvall et al. [17] showed that after brain ischaemia, increased BDNF mRNA levels gradually tapered off as time progressed. Therefore, it is possible that the levels of BDNF mRNA and protein may increase transiently after human cerebral ischaemia; subsequently, the increased BDNF levels may taper off gradually. In our study, there was no significant difference in the BDNF levels between the VaD and NC groups.

Numerous reports suggest that BDNF gene polymorphisms influence AD risk, hippocampal function and memory; however, the investigated BDNF polymorphisms appear to be neither robust genetic factors nor determinants of BDNF protein levels in AD [18].

Some controversy persists around the data available on TNF-α and IL-1β in AD patients and patients with other forms of dementia [19, 20]. In this study, the TNF-α and IL-1β levels did not differ significantly among the three groups. A number of studies have analyzed peripheral inflammatory indices such as blood cytokines in dementia patients. However, the data are sparse and inconclusive.

We also investigated whether a possible correlation between BDNF and cytokines would be valid to clarify the direction of a proposed functional relationship. Although a possible correlation was observed between TNF-α and BDNF in VaD patients, overall, we did not find any correlation between TNF-α and BDNF in all the subjects. Therefore, we concluded that we did not observe any correlation between BDNF and cytokines such as TNF-α and IL-1β in this study.

Our study showed no significant gender differences regarding serum BDNF levels and no correlation between the age and serum BDNF level in the NC subjects or in the dementia groups (AD + VaD). These results are in agreement with those reported previously [21].

Our results did not include information on drug effects on the serum BDNF level. Since nonsteroidal anti-inflammatory drugs (NSAIDs) and psychotropic drugs such as antidepressants are known to modulate the release of BDNF [22, 23], the findings presented in this study may differ under the influence of drugs. Further studies are required to examine the effect of the drugs.

Unfortunately, due to ethical constraints, we could not examine the BDNF levels in the cerebrospinal fluid (CSF). The presence of BDNF in the CSF needs to be investigated.

The clinical-based differential diagnosis of AD is not definitive, and it is characterized by an error range of ~10–15%. Moreover, it is well known that the pathological events of AD and VaD overlap in a certain percentage of patients. Since the clinical and CT-based diagnosis in this study has been supported neither by CSF biomarkers nor by neuropathological examination, the possibility of misdiagnosis should be considered.

In conclusion, our results suggest that BDNF may play a pathological role in some cases of AD; however, further investigation is required to verify this.

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