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ORIGINAL RESEARCH ARTICLE

Cerebral changes and cerebrospinal fluid β-amyloid in Alzheimer's disease: a study with quantitative magnetic resonance imaging

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Pathological and biochemical studies indicate that β-amyloid (βA4) deposition is a hallmark in the pathogenesis of Alzheimer's disease (AD).[1–4] Neuroimaging studies demonstrate that the respective cerebral changes primarily strike the temporal lobe and the amygdala-hippocampus complex and may be reliably assessed using quantitative magnetic resonance imaging (MRI).[5,6] Therefore one may expect that reduced βA4-levels are significantly correlated with measures of the temporal lobe rather than global cerebral atrophy in AD patients. To test this hypothesis in a clinical study, cerebrospinal fluid concentrations of total βA4 and its major C-terminal variations βA4 1–40 and βA4 1–42 were compared with cerebral changes as assessed by quantitative magnetic resonance imaging (MRI). Significantly ($P < 0.05$) reduced βA4 1–40 and βA4 1–42 levels were found in the AD patients (17 female; six male; AD/NINCDS-ADRDA-criteria)[7] in comparison to the patients with major depression (seven female; two male; DSM-III-R).[8] Within the AD group, βA4 and βA4 1–42 levels were significantly correlated with the volume of the temporal lobes ($r = 0.46$ and $r = 0.48$, respectively) but none of the other volumetric measures. These findings indicate that changes in cerebral βA4 levels contribute to temporal lobe atrophy in AD and support the possibility that βA4 is central to the etiology of AD.

Total βA4, βA4 1–40, and βA4 1–42 levels measured for nine patients with endogenous psychoses and those obtained for nine of the AD patients carefully matched for age and gender, are given in Table 1. Patients with AD presented with reduced values; this difference was significant for βA4 1–40 (d.f. = 1; $F = 7.7$; $P<0.05$) and βA4 1–42 (d.f. = 1; $F = 9.8$; $P<0.01$). Similar results were found when values from all 23 AD patients were entered into the analysis. Comparison of patients receiving antihypertensive medication or neuroleptics with those who were medication free did not reveal any significant differences in total βA4, βA4 1–40, and βA4 1–42 levels.

Table 1 βA4, βA4 1–40, and βA4 1–42 levels (ng ml^{-1}) of patients with Alzheimer's disease and major depression with the results of an univariate analysis of variance

	βA4	*βA4 1–40*	*βA4 1–42*
Alzheimer's disease	5.12 ± 1.8	2.0 ± 0.9	0.62 ± 0.33
Major depression	6.43 ± 2.18	3.44 ± 1.32	1.57 ± 0.85
F	1.8	7.7*	9.8**

*$P<0.05$; **$P<0.01$.

Within the AD group, total βA4 ($r = 0.46$, $P<0.05$) and βA4 1–42 ($r = 0.48$, $P<0.05$) levels were significantly correlated with the volume of the temporal lobes (Figure 1). None of the CSF amyloid measures were significantly correlated with the other volumetric (intracranial, whole brain, frontal lobe, and amygdala-hippocampus complex volume), nor clinical (age and severity of illness) measures. Similar results were obtained when severity of illness was partialled out or only data from the patients with probable AD were entered into the analysis. No significant correlations

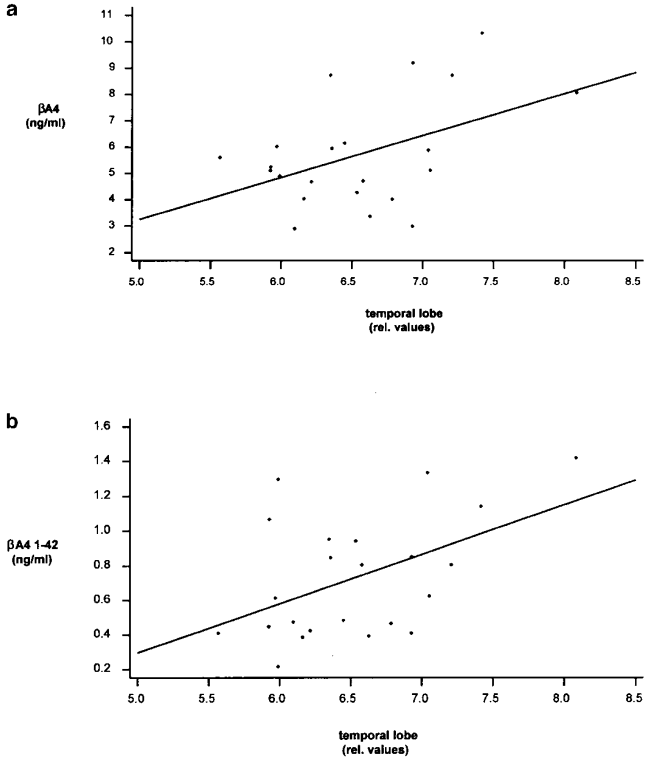

Figure 1 Correlation coefficients between (a) βA4 and (b) βA4 1–42-levels and temporal lobe volumes in AD patients ($r = 0.46$ and $r = 0.48$, $P<0.05$, respectively).

were found between CSF total protein concentrations and total βA4, βA4 1–40, and βA4 1–42 levels.

The present study provides two major findings: (1) further evidence that CSF levels of βA4 1–40 and βA4 1–42 are reduced in AD; and (2) an indication that βA4 and βA4 1–42 may contribute to temporal lobe atrophy in the disease.

Significantly reduced βA4 1–40 and βA4 1–42 levels were found in AD patients when compared with patients presenting with major depression. Previous studies reported decreased levels of βA4 1–42,[1,9,10] while others found no significant differences between AD patients and controls[11,12] or even increased levels of total βA4[13] in AD. In the former studies βA4 levels were mostly measured by sandwich ELISA methods, while we used a sensitive Western blot assay with immunoprecipitation to specifically measure βA4 variants. Hence, the divergent results are likely to be accounted for by methodological differences since an ELISA can be affected by the presence and concentration of other proteins which may cross-react or mask the epitope of antibodies used in this assay.[1] The small number of depressed patients included in the present study does not allow definite conclusions. However, our finding of significantly lower βA4 1–40 and βA4 1–42 levels in AD compared to depressed patients corresponds to the results of a recent MRI-based volumetric study[6] demonstrating significantly greater atrophic changes of the temporal lobe and amygdala-hippocampus complex in AD than in depressed geriatric patients.

Total βA4 and βA4 1–42 levels were significantly correlated with the volume of the temporal lobes but not with any of the other MRI volumetric measures. Since βA4 1–42 is a major component of amyloid plaques, this finding may correspond to a specific vulnerability of temporal structures. According to a recent MRI study,[5] changes of the medial temporal lobe are present even in the mildly demented. Moreover, a recent post-mortem study[3] found amyloid deposition in the entorhinal cortex to be significantly correlated with cognitive impairment. Therefore one may hypothesize that amyloid deposition in the temporal lobes may induce the atrophy process leading to a reduced βA4 and βA4 1–42 synthesis with decreased CSF levels of these peptides. Alternatively, the amount of βA4 and βA4 1–42 segregated in senile plaques may increase as the disease progresses. These hypotheses may be tested by longitudinal examinations of cerebral atrophy and βA4-levels in AD patients. Significant correlations between cerebral atrophy and βA4 1–40 were not found. Actual recent studies indicate that βA4 1–42 is a major component of both classical and diffuse plaques while the more soluble βA4 1–40 is mainly found in vascular amyloid.[14] Dilution effects due to global cerebral atrophy with consecutive enlargement of the CSF-spaces and medication effects have to be considered as potentially additive effects. However, significant correlations between βA4 measures and the volume of the CSF-spaces were not found. Little is known about the potential impact of medication on total βA4, βA4 1–40, and βA4 1–42 levels. Significant differences between patients receiving various drugs or patients who were drug-free at the time of the examination did not arise. Agents with pronounced anticholinergic side-effects may be of particular importance since recent studies indicate that cholinergic agents may modulate amyloid precursor protein processing. However, none of the drugs prescribed to the AD patients fall into this category. The existence of a third factor corresponding to both cerebral atrophy and decreased amyloid levels can neither be rejected nor confirmed. However, evidence from genetic studies supports the assumption that changes of the βA4 metabolism are central in the pathogenesis of AD.[14]

These results support the hypothesis that changes of the βA4 metabolism may contribute to cerebral atrophy in AD. Furthermore, combining βA4 measurements and neuroimaging methods may assist not only in clinical diagnosis but also in examining progression of AD.

Patients and methods

We examined 23 patients (17 females and six males; mean age 71.1 ± 8.5) with possible ($n = 5$) or probable AD (NINCDS-ADRDA criteria)[7] and nine patients (seven female and two males; mean age 66 ± 11.2 years) with major depression (DSM-III-R)[8] who were recruited consecutively. Computed tomographies taken in all patients revealed no pathological findings except cerebral atrophy of various degrees in the AD patients. Particular care was taken to exclude any signs of cognitive impairment in the depressed patients. This was done by repeated clinical examinations, and wherever necessary, by thorough neuropsychological examination. At the time of the examination, eight patients received antihypertensive agents and/or digitoxin. Six patients received neuroleptics to control agitation and/or sleeplessness, while nine patients were drug-free. All patients with major depression received an adequate antidepressive therapy. Severity of dementia was rated on the mini mental state examination (MMSE).[15] Mean MMSE scores were 16.7 ± 6.4 in the AD patients. In all patients, lumbar punctures were performed as part of the routine diagnostic procedure to exclude inflammatory diseases. CSF samples were obtained at the same time of the day (between 11 and 12 am). A fixed volume of 12 ml was withdrawn by using non-adsorbing tubes and immediately frozen (–80°C) until use. Total βA4 and βA4 1–40 levels were determined using a direct Western blot.[1] To quantify βA1–42 levels more specifically, a Western blot with immunoprecipitation was used: 150 nl of CSF were mixed with an equal volume of 0.1 M Tris-HCl buffer (pH 7.4) containing 0.25% bovine serum albumin and 0.05% Tween 20. G2-11 antibody (8 μg) and protein G-agarose (20 ml) were added and incubated overnight at 4°C with continuous rocking of the tubes. Gels were collected by centrifugation, washed, and the bound proteins were analyzed by Tris-Tricine SDS-PAGE followed by Western blot detection using WO-2 antibody

as described elsewhere.[1] In the AD patients quantitative MRI was performed on a 1.5T MRI system (Magnetom SP 4000, Siemens, Erlangen, Germany) using 3D MPRAGE and PSIF imaging sequences for the T1 and T2-weighted image data volumes.[16] Volumetric data were obtained from the intracranial space, the whole brain, the frontal and temporal lobes, and the amygdala-hippocampus complexes using a specially developed software NMRWin as described elsewhere.[5] To address potential interindividual differences in premorbid brain size, all volumetric measures were divided by the subject's intracranial volume. The study was approved by the local ethical committee. All patients gave informed consent. Following the regulations of the committee, the ability of patients to understand the diagnostic procedures and their potential side-effects was confirmed by an independent physician. For data analysis, Pearson correlation coefficients and analyses of variance were calculated.

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