SHORT REPORT

Intra vitam lumbar cerebrospinal fluid and serum and postmortem ventricular immunoreactive apolipoprotein E in patients with Alzheimer's disease

Norbert Rösler, Ildiko Wichart, Kurt A Jellinger

Abstract

Using a specific enzyme linked immunosorbent assay (ELISA) method, total apolipoprotein E immunoreactivity (tApoE-IR) was measured in premortem lumbar CSF and serum of patients with "probable" Alzheimer's disease and in postmortem ventricular CSF of patients with Alzheimer's disease confirmed by necropsy. Concentrations were compared with those from patients with other neurological diseases and controls. The mean serum:lumbar CSF ratio of ApoE-IR was 15.9 suggesting that the main portion of lumbar ApoE-IR is synthesised intrathecally. No significant differences in ApoE-IR between patients with Alzheimer's disease and the other groups were detected in either CSF compartment. In lumbar CSF, there was no correlation between ApoE-IR of patients with Alzheimer's disease and their mini mental state scores. These results suggest that the diagnostic value of ApoE-IR measurements in CSF of patients with Alzheimer's disease as a single determination is less than that of other markers, in particular tau protein. On the other hand, ApoE determinations could be useful as part of a neurochemical profile of Alzheimer's disease.

(*J Neurol Neurosurg Psychiatry* 1996;60:452-454)

Keywords: cerebrospinal fluid; apolipoprotein E; Alzheimer's disease

Alzheimer's disease is the most common dementing disorder in elderly people, with a relative incidence of 70-80%.¹ It is clinically characterised by progressive intellectual deterioration. The accuracy of the ex vivo diagnosis using established clinical criteria reaches about 80-90%,² but the definite diagnosis remains a neuropathological one. At present, valid biological markers for the intra vitam diagnosis of Alzheimer's disease as a prerequisite of early diagnosis and treatment are still lacking, although increased concentrations of tau protein in CSF may strengthen the clinical diagnosis of probable Alzheimer's disease.^{3,4}

Human apolipoprotein E (ApoE) is a glycoprotein containing 299 amino acids (molecular weight 34 kDa) present in the brain, where it is synthesised by astrocytes and oligodendrocytes. As a constituent of several plasma lipoproteins, it plays a part in cholesterol and phospholipid metabolism, mainly by mediating the cellular uptake of lipid complexes through interaction with the ApoB/ApoE (low density lipoprotein (LDL)) receptor.⁵ Recent studies have provided evidence for an association between ApoE and the pathobiochemistry of Alzheimer's disease. ApoE is present within plaques, neurofibrillary tangles, and dystrophic neurites of the brains of patients with Alzheimer's disease⁶; it binds soluble and insoluble forms of β amyloid with high avidity,⁷ and it has been proposed that ApoE, acting as a molecular chaperone, influences conformational changes of amyloid fibrils during plaque formation.⁸ Genotyping studies have shown an increased frequency of the ApoE- ϵ 4 allele in Alzheimer's disease^{9,10} suggesting a role for ApoE- ϵ 4 as a susceptibility marker of the disease.

Patients and methods

Total apolipoprotein E immunoreactivity (tApoE-IR) was measured in 62 ex vivo lumbar CSF and serum specimens. Informed consent was obtained before lumbar puncture. The whole group was subdivided as follows (table 1): (1) 16 patients with "probable" Alzheimer's disease according to the NINCDS-ADRDA criteria; in addition, the mini mental state examination (MMSE)¹¹ was performed; (2) 20 patients with other neuro-

Ludwig Boltzmann
Institute of Clinical
Neurobiology, Lainz
Hospital, Vienna,
Austria
N Rösler
I Wichart
K A Jellinger

Correspondence to:
Dr Norbert Rösler,
Department of Psychiatry,
University Medical School,
Hauptstrasse 5, D79104,
Freiburg, Germany.

Received 5 June 1995
and in revised form
13 October 1995
Accepted 27 November 1995

Table 1 Clinical and neurochemical data of patients with "probable" Alzheimer's disease (AD), other neurological disorders (OND), any young (COY) and elderly (COE) controls

	Intra vitam lumbar CSF			
	AD	OND	COY	COE
n (M/F)	16 (6/10)	20 (9/11)	16 (4/12)	10 (5/5)
Age (y)	73.2 (3.6)	58.7 (3.4)	37.8 (2.5)	62.3 (3.9)
CSF cell count (μ l)	1.2 (0.5)	2.3 (0.8)	1.1 (0.3)	1.3 (0.2)
$Q_{A\beta}$ (CSF/serum) ($\times 10^{-3}$)	6.9 (0.6)	6.9 (1.0)	5.9 (0.4)	6.7 (0.8)
$Q_{tA\beta}$ (CSF/serum) ($\times 10^{-3}$)	3.2 (0.3)	3.1 (0.6)	2.4 (0.3)	2.9 (0.5)
Serum tApoE-IR (mg/l)	28.1 (3.1)	22.1 (1.8)	21.6 (1.8)*	28.7 (3.1)
Lumbar CSF-tApoE-IR (μ g/100 ml)	149 (7.0)	160 (12.0)	148 (11.0)	168 (14.0)
$Q_{tApoE-IR}$ (Serum/CSF)	18.9	14.1	14.6	17.1
MMSE (points)	16.1 (1.5)	—	—	—

Values are means (SEM).

*P < 0.05 v COE.

Table 2 Clinical and neurochemical data of patients with neuropathologically confirmed Alzheimer's disease (AD) and patients with or without other neurological disease

	Postmortem ventricular CSF	
	AD	OND/CO
n (M/F)	12 (6/6)	12 (3/9)
Age at death (y)	79.0 (3.5)	79.5 (3.8)
Age at onset (y)	73.2 (3.3)	—
Duration of illness (y)	5.8 (0.8)	—
Total CSF protein (mg/100 ml)	128.1 (30.1)	176.6 (32.5)
Brain weight (g)	1154.8 (50.4)	1161.8 (41.3)
Postmortem delay (h)	27.4 (3.8)	24.3 (4.2)
Ventricular CSF-tApoE-IR (µg/100 ml)	109 (19.9)	117 (23.9)

Values are means (SEM).

logical disorders (OND) without cognitive impairment (one intracerebral haemorrhage, six brain infarctions, four multiple sclerosis, two myelitis, two control taps after viral meningoencephalitis, two epilepsy, two multi-system atrophies, one meningioma); (3) and (4) 16 young (COY) and 10 elderly (COE) subjects without CNS disease in whom lumbar puncture was performed for exclusion purposes.

In addition, 24 postmortem CSF samples were obtained through ventricular tap, immediately centrifuged, and kept at -70°C until analysis. Twelve patients had neuropathologically confirmed Alzheimer's disease, 12 patients (OND/CO) had no disease or other neurological disease. Table 2 gives the clinical and neurochemical data.

Histological criteria for the diagnosis of Alzheimer's disease followed those outlined by the NIH/AARP Working Group¹² and the criteria of Tierney *et al*¹³ for "pure" Alzheimer's disease. The patients were also evaluated with the staging of Braak and Braak.¹⁴ Four patients met the criteria for limbic stage III-IV, eight had isocortical stage V-VI. Age at onset of dementia, defined as the earliest age at which behavioural changes were noticed, and qualitative assessment of the mental state before death were determined from the medical records, showing that all patients with

Alzheimer's disease were severely demented. The diagnoses of the OND/CO group were made using current pathological criteria (one Parkinson's disease, one Lewy body dementia, two vascular dementia, one multiple sclerosis, one Creutzfeldt-Jakob disease, two acute brain infarctions, one postanoxic parahippocampal sclerosis, and four brains of aged subjects showing nothing abnormal).

Total ApoE immunoreactivity (tApoE-IR) was measured with a specific symmetric non-competitive sandwich enzyme linked immunosorbent assay (ELISA) method slightly modified according to Carlsson *et al*.¹⁵ Instead of a polyclonal antibody, we used a monoclonal mouse antibody (Boehringer, Mannheim, Germany) as coating and biotinylated detection antibody. With this technique, there were no problems of steric hindrance and typical standard curves were obtained. For detection of antigen binding streptavidin-peroxidase was used, with 2,2-azino-di-[3-ethyl-benzthiazolin-sulphonic acid] (ABTS) as a chromogen. Absorption was read at 410 nm. A Wilcoxon two sample test and Spearman's rank correlation coefficient were used for statistical analysis.

Results

Tables 1 and 2 and fig 1 give the results of ApoE measurements in lumbar and ventricular fluid. In both compartments, there were no significant differences for tApoE-IR between the groups investigated (P > 0.05). The mean tApoE-IR in lumbar CSF of all patients investigated was 155 µg/100 ml and the mean serum concentration of all patients was 24.6 mg/l, resulting in a mean serum:lumbar CSF ratio of 15.9. There was no significant correlation (r = 0.067, P > 0.05) of MMSE scores of patients with Alzheimer's disease and their ApoE concentrations in lumbar CSF (fig 2).

Figure 1 Left: lumbar CSF total ApoE-IR (LCSF-tApoE-IR) of patients with "probable" Alzheimer's disease (AD), other neurological disorders (OND), young controls (COY), and elderly controls (COE). Right: ventricular CSF total ApoE-IR (VCSF-tApoE-IR) of patients with Alzheimer's disease (AD) confirmed by necropsy and in a group with or without other neurological disorders (OND/CO).

Figure 2 Lack of correlation of mini mental state examination (MMSE) scores with lumbar total ApoE-IR of patients with "probable" Alzheimer's disease (AD) ($r = 0.067$; $P > 0.05$).

Discussion

To our knowledge, this is the first study investigating ApoE concentrations in CSF which includes postmortem samples from patients with Alzheimer's disease confirmed by necropsy. The Alzheimer's disease group showed no significant differences in tApoE in either the lumbar or the ventricular compartment when compared with the OND group and the COY and COE controls. Our results corroborate recent in vivo findings in a heterogeneous patient group with degenerative, toxic, and metabolic brain disorders,¹⁵ but are at variance with the findings of Blennow *et al.*,¹⁶ who detected a significant decrease of lumbar tApoE in patients with Alzheimer's disease. Methodological differences could account for the overall higher tApoE concentrations in their study, but not for the observed difference between controls and patients with Alzheimer's disease. Blennow *et al.*¹⁶ give no information about the MMSE scores of their patients. We investigated a broad range of MMSE scores, and no correlation of MMSE with tApoE in lumbar CSF was found.

The mean tApoE serum:CSF ratio was 1:15.9; similar to the result of Carlsson *et al.*,¹⁵ who found a ratio of 1:12. Taking into account the molecular weight and hydrodynamic diameter of ApoE, it can be assumed that the main portion of ApoE in CSF stems from intrathecal synthesis, making the use of a quotient unnecessary.¹⁵

ApoE is involved in the regeneration and maintenance of myelin and neuronal membranes in both the peripheral and central nervous system and has been linked to synaptogenesis and, thus, synaptic plasticity.⁵ Therefore, the absence of an increase of ApoE in the CSF of patients with Alzheimer's disease could reflect poor re-innervation capacity or increased utilisation of ApoE in or adsorption to senile plaques and neurofibrillary tangles in brains of patients with Alzheimer's disease.

For patients with Alzheimer's disease, the

diagnostic value of ApoE-IR measurements in lumbar or ventricular fluid CSF as a single determination seems to be less than that of other markers, in particular hyperphosphorylated tau protein, which represents the main component of neurofibrillary tangles in Alzheimer's disease. Recently, raised total tau protein concentrations in lumbar CSF have been proposed as an important candidate for discriminating patients with Alzheimer's disease from elderly depressed patients as well as from young and elderly controls without mental illness.^{3,4,17} On the other hand, the absence of raised tApoE concentrations in CSF from patients with Alzheimer's disease could be meaningful as part of a neurochemical profile of the disease.

- Jellinger K, Danielczyk W, Fischer P, Gabriel E. Clinicopathological analysis of dementia disorders in the elderly. *J Neurol Sci* 1990;95:239-58.
- Galasko D, Hansen LA, Katzman R, *et al.* Clinical-neuropathological correlations in Alzheimer's disease and related dementias. *Arch Neurol* 1994;51:888-95.
- Hock C, Golombowski S, Naser W, Müller-Spahn F. Increased levels of tau-protein in cerebrospinal fluid of patients with Alzheimer's disease—correlation with degree of cognitive impairment. *Ann Neurol* 1995;37:414-5.
- Van de Voorde A, Vanmechelen E, Vandermeeren M, Dessaint F, Beckman W, Cras P. Detection of tau in cerebrospinal fluid. In: Iqbal K, Mortimer JA, Winblad B, Wisniewski HM, eds. *Research advances in Alzheimer's disease and related disorders*. John Wiley, 1995:189-95.
- Poirier J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. *Trends Neurosci* 1994;17:525-30.
- Namba Y, Tomanaga M, Kawasaki H, Otomo E, Ikeda K. Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain Res* 1991;541:163-6.
- Wisniewski T, Golabek A, Matsubara E, Ghiso J, Frangione B. Apolipoprotein E: binding to soluble Alzheimer's β -amyloid. *Biochem Biophys Res Comm* 1993;192:359-65.
- Wisniewski T, Frangione B. Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. *Neurosci Lett* 1992;135:235-8.
- Strittmatter WJ, Saunders AM, Schmechel D, *et al.* Apolipoprotein E: high avidity binding to β -amyloid and increased frequency of type 4 allele in late onset familial Alzheimer's disease. *Proc Natl Acad Sci USA* 1993;90:1977-81.
- Martinoli MG, Trojanowski JQ, Schmidt ML, *et al.* Association of apolipoprotein $\epsilon 4$ allele and neuropathologic findings in patients with dementia. *Acta Neuropathol* 1996 (in press).
- Folstein MF, Folstein SE, McHugh PR. "Mini mental state": a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12:189-98.
- Khachaturian Z. Diagnosis of Alzheimer's disease. *Arch Neurol* 1985;42:1097-105.
- Tierney MC, Fisher RW, Lewis AJ, *et al.* The NINCDS-ADRDA Work Group criteria for the clinical diagnosis of probable Alzheimer's disease. A clinicopathologic study of 57 cases. *Neurology* 1988;38:359-64.
- Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* 1991;82:306-15.
- Carlsson J, Armstrong VW, Reiber H, Felgenhauer K, Seidel D. Clinical relevance of the quantification of apolipoprotein E in cerebrospinal fluid. *Clin Chim Acta* 1991;196:167-76.
- Blennow K, Hesse C, Fredman P. Cerebrospinal fluid apolipoprotein E is reduced in Alzheimer's disease. *Neuroreport* 1994;5:2534-6.
- Rösler N, Wichart I, Jellinger KA. Total tau protein immunoreactivity in lumbar cerebrospinal fluid of patients with Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 1996;60:237.