

Tau protein concentrations in cerebrospinal fluid of patients with dementia of the Alzheimer type

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Abstract

Tau protein concentrations were measured in the CSF of 23 patients with dementia of the Alzheimer type (DAT), 36 patients with multi-infarct dementia (MID), and 23 control subjects. Tau protein concentrations were significantly higher in patients with DAT than in controls ($P < 0.001$) and patients with MID ($P < 0.001$). A significantly positive correlation between CSF tau protein and glucose concentrations ($r = 0.79$, $P < 0.001$) and evolution of disease ($r = 0.47$, $P < 0.05$), and a negative correlation with Folstein's mental state examination test ($r = -0.73$, $P < 0.001$) were found in patients with DAT.

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Keywords: cerebrospinal fluid; tau protein; MSE test; Alzheimer's disease; multi-infarct dementia

Dementia of Alzheimer type (DAT) is a brain disorder characterised by a progressive dementia that occurs in middle or late life. The neuropathological changes associated with DAT include the presence of neurofibrillary tangles, neuritic plaques, and granulovacuolar degeneration.^{1,2} Neurofibrillary tangles are neuronal inclusions that consist of dense accumulation of pathological paired helical filaments, of which the main component is a modified form of the microtubule associated tau protein.³ Although none of these changes are unique to Alzheimer's disease, their quantitative correlation with clinical dementia is well documented.⁴⁻⁶ Tau protein is known to be important for maintaining the stability of axonal microtubules involved in the mediation of fast axonal transport of synaptic constituents. In DAT, tau protein becomes abnormally phosphorylated, leading to a decreased stability of the microtubular network with concomitant impairment of the anterograde axonal transport system and to an increase in the amount of tau protein per neuron.^{3,7}

Because tau protein is normally an intracellular compound, the amount found in CSF is low. Quantitative determination of insoluble tau protein has been carried out post-mortem, in both the grey and white matter of brain regions.^{3,8} The development of specific monoclonal antibodies with high activity for tau protein has recently led to a commercial enzyme linked immunosorbent assay (ELISA)

test for detection of tau protein in brain tissue.⁹⁻¹² To our knowledge, however, there is only one report published about the quantitative detection of tau protein in CSF¹³ and the results obtained need to be confirmed by other studies.

There is evidence about peptidergic abnormality in DAT such as the loss of cortical somatostatin, which has been correlated with a decrease in CSF somatostatin.^{9,14,15} Reports on other neuropeptides such as substance P or neuropeptide Y are contradictory¹⁵⁻¹⁷ and a possible relation between these neuropeptides and tau protein in CSF has not yet been described.

The aim of this study was to measure tau protein in the CSF of patients with DAT Alzheimer type and patients with MID to clarify if its concentration was different in the two dementia types and age matched controls. We also aimed to establish whether its concentration in CSF was related to that of neuropeptide Y, somatostatin, and substance P, as well as with the physiological and biological state of patients with DAT.

Materials and methods

SUBJECTS

Patients with dementia and controls were in good general medical health and were from the neurology unit at La Paz Hospital. The control group comprised 23 subjects (13 men and 10 women with a mean (SD) age of 67 (11) years), in whom a lumbar puncture was performed for persistent cephalalgia to rule out a meningeal syndrome. None of them had any abnormality on physical examination and routine laboratory blood tests, and CSF was normal in biochemical, microbiological, and cytological analysis. Patients in the DAT group ($n = 23$, eight men and 15 women with a mean (SD) age of 70 (7) years) met the DSM-III-R and NINCDS-ADRA criteria for DAT. All patients showed brain atrophy and no sign of cerebral infarction or leukoaraiosis as determined by CT or MRI. Patients in the MID group ($n = 36$, 19 men and 17 women with a mean (SD) age of 70 (8) years) fulfilled the DSM-III-R criteria for dementia and all had undergone careful neuropsychological (Hachinski ischaemic score and Rosen score) and radiological evaluations (CT and MRI), which were in agreement with the clinical diagnosis of MID. Duration of disease was 41 (SD 27) months and 40 (SD 32) months in patients with DAT and those with MID respectively. Cognitive function was examined

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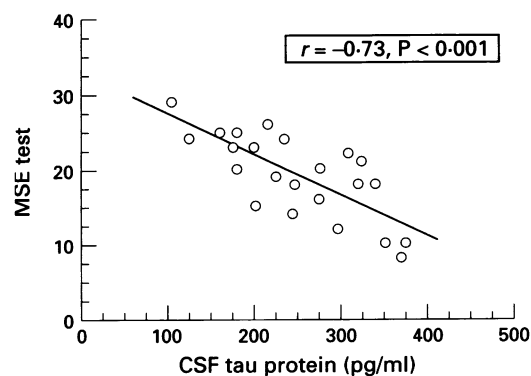
with Folstein's mental state examination (MSE) test and the Blessed score. Patients were mildly to moderately demented as measured by DSM III criteria (modified by Jorm and Henderson) and MSE. None of the patients were taking psychotropic (neuroleptics or antidepressants) or immunosuppressive medication and no symptoms or signs of major depressive disorders were found. The table shows the patients' characteristics.

ANALYTICAL METHODS

A sample of CSF was removed from the lumbar subarachnoid space between 9.30 and 10.30 am after an overnight fast and collected in plastic tubes containing EDTA (Merck) and aprotinin (Boehringer Mannheim). Cells were immediately removed by centrifugation and CSF samples were frozen at -40°C until analysis. At the same time as lumbar puncture blood samples were collected to obtain serum and the serum was stored at -40°C until assay.

Glucose and total protein concentrations in CSF were determined with standard methods using a Hitachi 704 autoanalyser (Boehringer Mannheim). Tau protein was measured in CSF with a commercial tau specific sandwich ELISA (Innogenetics). The sensitivity was <10 pg/ml and intra-assay variation $<8\%$. The ELISA measures total tau and does not distinguish between phosphorylated and unphosphorylated tau protein. Neuropeptide like immunoreactivities were measured by commercial radioimmunoassays for the quantitative determination of substance P (Peninsula Laboratories), neuropeptide Y (Peninsula Laboratories), and somatostatin (Bühlmann). The antibody for substance P does not show cross reactivity with neurokinin A and neurokinin B. The antibody for somatostatin shows 100% cross reactivity with somatostatin-28 and somatostatin-14. The antibody for neuropeptide Y shows $<0.02\%$ cross reactivity for human pancreatic polypeptide, vasoactive intestinal peptide, and peptide YY. All radioimmunoassays had an intra-assay variation $<6\%$ and an interassay variation $<10\%$. Sensitivity was 10 pg/ml for the substance P assay, 15 pg/ml for neuropeptide Y, and 8 pg/ml for somatostatin.

Statistical analysis was performed for untransformed and logarithmically transformed data with a one way analysis of variance (ANOVA), and the F Scheffe test for comparing between two groups. The same



Correlation between CSF tau protein and Folstein's mental test examination (MSE) in patients with Alzheimer's disease.

significances were found for both types of data. Correlations were examined with Pearson's correlation coefficient. Significance was set at $P < 0.05$.

Results

All patients were mildly to moderately demented and the mean duration of illness was similar in DAT and MID groups (table). There were no significant differences in age and sex between DAT and MID groups and controls.

The table shows the tau protein concentrations in CSF in the two groups of patients and controls. These were higher in patients with DAT than those with MID ($P < 0.001$) and control ($P < 0.001$) groups, whether expressed in pg/ml or in ng/g total protein.

The table shows the neuropeptide concentrations in CSF in the two groups of patients and controls. Substance P and somatostatin concentrations were lower in patients with DAT than in controls ($P < 0.01$), whereas neuropeptide Y concentration was not different between the three groups. Only somatostatin was significantly decreased in DAT compared with MID ($P < 0.01$).

In patients with DAT we found a good positive correlation between CSF tau protein and glucose concentrations ($r = 0.79$, $P < 0.001$). For cognitive function tests, there was a significant negative correlation between tau protein and the MSE test ($r = -0.73$, $P < 0.001$; (figure), but not with other neuropsychological tests (Blessed score, Rosen score). Moreover, a weak but significant correlation with the time course of DAT was found ($r = 0.47$, $P < 0.05$). We did not find significant correlations between CSF tau protein and somatostatin, substance P, or neuropeptide Y in patients with DAT, or between tau protein concentrations and age in the three groups studied.

Discussion

Because CSF offers the richest potential source of altered proteins in neurodegenerative diseases, we were interested in quantifying tau protein concentrations in CSF from

Patient's characteristics, and tau protein, neuropeptide (ng/l) and glucose (g/l) concentrations in CSF of patients with dementia and controls

	DAT	MID	Controls
MSE test	17.5 (7.4)	19.1 (9.1)	—
Blessed score	12.6 (5.3)	10.3 (5.8)	—
Hachinski score	0.8 (1.3)	8.2 (2.0)†	—
Rosen score	1.7 (1.0)	6.7 (3.1)†	—
Tau protein (ng/l)	279 (100)***‡	88 (61)*	26 (11)
Tau protein (ng/g total protein)	487 (175)***‡	147 (144)*	65 (12)
Neuropeptide Y	70 (16)	83 (50)	67 (16)
Substance P	86 (8)*	95 (37)	122 (64)
Somatostatin	16 (8)**‡	30 (8)	39 (10)
Glucose	0.64 (0.14)	0.62 (0.12)	0.61 (0.12)

Values are means (SD).

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ v controls; † $p < 0.001$ v DAT; ‡ $p < 0.001$ v MID.

patients with DAT. Our results indicate that significantly increased concentrations of CSF tau protein are present in such patients. These findings agree with those of Vandermeeren *et al.*,¹³ although their values are lower than ours. In addition, a large difference between patients with DAT and MID was found. Thus there was a threefold increase in CSF tau protein concentrations in DAT compared with MID.

Increased tau concentrations have been described in brain extracts of patients with DAT,^{11,18} this being most evident in associative brain regions involving particularly long corticocortical projections.^{3,8} This fact may be characteristic of DAT and it could be expressed clinically by functional cognitive deficits.¹⁹ Thus we have found a significant negative correlation between the MSE test, but not with the Rosen score and Blessed dementia scale, and CSF tau protein levels in DAT patients. Because the MSE test examines patient cognitive function, the measurement of CSF tau protein might provide a correlation with the psychological symptoms and severity of disease. As in other studies,^{6,19} the appearance of tangles within the neocortex is a reliable marker for the presence of DAT and higher neocortical tangle densities are associated with more severe dementia.

Some studies^{3,19} that quantify tau protein in brain tissues indicate that its relation is with severity of dementia, but not with age of patients with DAT, and that its concentrations could distinguish DAT from processes occurring during normal aging. Vandermeeren *et al.*¹³ have found increased CSF tau values in patients with DAT and controls older than 60, but not in patients who had other diseases of the CNS. We did not find any correlation between CSF tau protein concentrations and age in patients and controls.

A recent study²⁰ suggests that there are changes in the cytoskeleton of hippocampal neurons associated with age and they might be potentiated in Alzheimer's disease, leading to neurofibrillary tangle formation and cellular degeneration. This pattern of neuronal degeneration associated with DAT pathology can be present in mild and moderate Alzheimer's disease, and it seems to correlate with a validated instrument for staging severity of dementia.^{21,22} This neuronal degeneration could justify the high CSF tau concentrations found in this study in patients with mild and moderate DAT.

In regard to CSF neuropeptide concentrations, we have found a reduction in substance P in patients with DAT compared with controls. These data agree with those previously reported,^{9,14,15} and suggest that this reduction may be due to an unequal degradation on processing of substance P in patients with DAT.¹⁹ In agreement with several authors^{9,14} and our previous study,¹⁵ we have found a reduction in CSF somatostatin in patients with DAT compared with controls, and also with respect to the MID group. Immunohistochemical studies suggest that this loss may be due to a degeneration of

somatostatinergic neurons²³ in DAT. On the other hand, we have not found any relation between CSF neuropeptide concentrations and tau protein concentrations in DAT.

There is increasing evidence of a disrupted carbohydrate metabolism²⁴ and a reduction in cerebral glucose use in DAT, which is comparable in extent with hypoglycaemia.²⁵ This study reported a cerebral excess release of ammonia that may indicate an amino acid utilisation to substitute for a carbohydrate deficit in brains of patients with DAT. Decreases in brain glucose utilisation in patients with DAT can result in increased CSF glucose concentrations. It is important to point out that in our study CSF glucose concentrations correlated positively with the CSF tau protein concentrations.

In conclusion, increased concentrations of tau protein in CSF from patients with Alzheimer's disease were found. As tau protein is not found in blood or serum, its increased presence in CSF can reflect neuronal degeneration. Therefore, a simple test based on quantitative detection of tau protein in CSF fluid might be a useful marker in the diagnosis and assessment of severity in DAT. Further research is necessary to clarify the role of tau protein in patients with dementia and to establish the specificity and sensitivity of tau protein increases in DAT.

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