SHORT REPORT

Altered glycosylation of acetylcholinesterase in lumbar cerebrospinal fluid of patients with Alzheimer's disease

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Abstract

As clinical diagnosis of Alzheimer's disease is only 80%–90% accurate, there is a need to identify biochemical markers of Alzheimer's disease. Previous studies have shown an abnormality in the glycosylation of acetylcholinesterase (AChE) in the CSF collected postmortem from patients with Alzheimer's disease. This abnormality was very specific for Alzheimer's disease, as it was not detected in other illnesses causing dementia. We report here that the glycosylation of AChE is also altered in lumbar CSF collected antemortem. The altered glycosylation was due to increased concentrations of a minor AChE isoform that does not bind to concanavalin A (Con A). Glycosylation of AChE may eventually be of diagnostic value, especially when used in combination with other CSF markers.

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Alzheimer's disease is the most common form of dementia affecting elderly patients, with typical age of onset for sporadic forms being over 65.[1] Alzheimer's disease is currently diagnosed by clinical examination. However, this approach can be time consuming and is not always accurate.[2] Thus, there is a need to identify biochemical markers that can detect Alzheimer's disease at the earliest stages.[3] Recent studies have shown that the concentrations of tau and amyloid protein (Aβ) are altered in the CSF of patients with Alzheimer's disease,[4] although no marker fulfils the criteria of sensitivity or specificity for diagnosis. However, several markers may be used in combination to improve the accuracy of diagnosis. For this reason, the identification of new biochemical markers of Alzheimer's disease is important.

Acetylcholinesterase (AChE) is a key regulator of cholinergic activity in the nervous system.[5] The enzyme is encoded by a single gene, which undergoes some alternative mRNA splicing to generate two 70 kDa polypeptides which differ in their C terminal amino acid sequences.[6] Acetylcholinesterase exists in multiple forms that can be distinguished by their different molecular weights and hydrodynamic properties.[5] The expression of these different forms is controlled by mRNA splicing and by the coexpression of non-catalytic subunits that are the products of separate genes.[6]

The expression of cholinesterases is profoundly altered in the Alzheimer's disease brain.[7–9] We have recently used lectin binding to identify an abnormally glycosylated form of AChE in brains and CSF collected postmortem from patients with Alzheimer's disease.[10] We found that there was an increase in a form of AChE, which did not bind to concanavalin A (Con A), a lectin, which recognises specific carbohydrate structures on the surface of proteins. The glycosylation change was specific for Alzheimer's disease and was not found in non-AD dementias. The abnormal glycosylation was associated with amphiphilic monomeric ($G1^a$) and dimeric ($G2^a$) isoforms of AChE.[11 12]

The aim of the present study was to assess the suitability of AChE glycosylation as a diagnostic marker by examining whether AChE glycosylation is also altered in antemortem CSF taken by lumbar puncture.

Materials and methods

COLLECTION OF CSF

Patients at the Hospital Universitario San Carlos of Madrid were diagnosed for probable Alzheimer's disease according to NINCDS-ADRDA criteria[2] (table). Samples of CSF from 27 patients with probable Alzheimer's disease and 37 non-demented controls were collected. The duration of disease for the Alzheimer's disease group was (mean (SEM)) 2.8 (0.3) years and the average clinical dementia rating (CDR)[13] was 1.4 (0.1). Lumbar punctures were performed in lateral decubitus and the CSF stored immediately at −40°C. No drugs were taken 12 hours before the collection of CSF. None of the patients received cholinesterase inhibitor therapy.

LECTIN BINDING

The CSF samples were thawed at 4°C, centrifuged (1000×*g*, 15 min) and aliquots (0.3 ml) added to 0.1 ml (hydrated volume) of Sepha-

Table 1 Level and glycosylation of lumbar CSF AChE in various CNS diseases

Group	*Age (y)*	*Sex (M/F)*	*% Bound to Con A*	*Total AChE (nmol/min/ml)*
Controls (no CNS disease)	65.8 (3.9)	5/9	1.57 (0.20)	12.52 (0.81)
Probable AD	65.8 (1.6)	5/22	4.12 (0.89)*	10.88 (0.97)
All CNS diseases (except AD)	69.8 (3.1)	16/7	2.04 (0.27)	11.35 (1.07)
Cerebrovascular accident	73.4 (2.2)	4/1	1.98 (0.31)	12.46 (3.34)
Benign intracranial hypertension	51.0 (5.8)	1/2	1.93 (1.69)	8.87 (0.94)
Normal pressure hydrocephalus	81.0 (3.8)	3/3	2.45 (0.62)	12.51 (2.65)
Epilepsy	72.9 (4.4)	6/1	1.97 (0.28)	10.99 (1.55)
Optic neuritis	43	1/0	1.30	10.65
Multiple sclerosis	45	1/0	1.50	9.54

Values are means (SEM). Controls are cases without CNS disorder (two healthy, five migraine, one breast cancer, five polyneuropathy, one peripheral neuropathy). *Significantly different from all non-AD cases and from no CNS disease controls (Student's *t* test, p<0.05).

rose conjugated lectins (Sigma-Aldrich Pty Ltd, Seven Hills, Australia), either *Canavalia ensiformis* (Con A) Sepharose or *Triticum vulgaris* (WGA) Sepharose, incubated at 4°C and unbound AChE assayed at 22°C as previously described[10 11] by a modification of the method of Ellman.[14]

SEDIMENTATION ANALYSIS

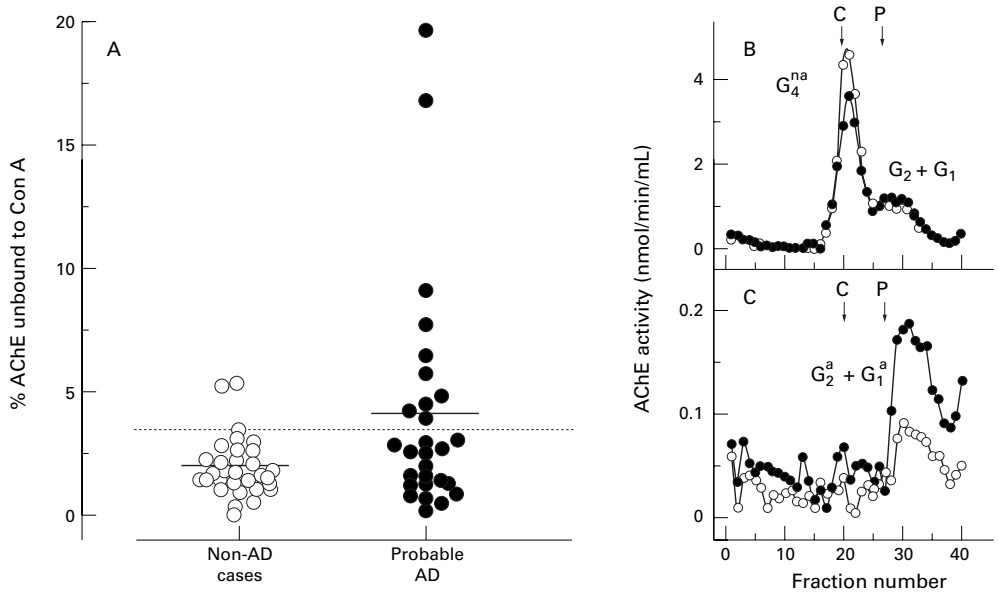
Isoforms of AChE were analysed by ultracentrifugation at 150 000 *g* in a continuous sucrose gradient (5%–20% w/v) for 18 hours at 4°C in a Beckman SW40 rotor. Gradients contained 50 mM $MgCl_2$, 0.5 M NaCl, and 0.5 % (w/v) Triton X-100, which allowed the separation of amphiphilic and non-amphiphilic AChE species as previously described.[11 12] About 40 fractions were collected and assayed for AChE activity.[14] Isoforms of AChE were identified by their sedimentation coefficients using bovine liver catalase (11.4S) and *E coli* alkaline phosphatase (6.1S) as markers.

Results

LECTIN BINDING

To examine whether the glycosylation of AChE is altered in lumbar CSF collected antemortem, the ability of AChE to bind to Con A was determined. The CSF was incubated with Con A-Sepharose and the amount of activity remaining unbound was measured. The percentage AChE unbound to Con A was increased in the Alzheimer's disease group; 60% of patients with probable Alzheimer's disease were above a value of 2.5% AChE unbound (fig). Only 20% of all controls were above this value. No significant increase in the percentage AChE unbound to Con A was seen in other CNS disorders including cerebrovascular accident, benign intracranial hypertension, normal pressure hydrocephalus, and epilepsy (table) (p=0.23).

In our previous report on postmortem CSF,[10] we found that the percentage of AChE unbound to Con A was best reported as a ratio (C/W=(% AChE unbound to Con A)/(% AChE unbound to WGA)). The measurement of binding to WGA, which binds carbohydrates that are different in structure from those bound by Con A, controlled for postmortem proteolysis in the sample. In the present study, the C/W ratio did not sufficiently discriminate between controls and the Alzheimer's disease group (C/W controls=0.69 (0.09), n=37; C/W Alzheimer's disease group=0.78 (0.08), n=27), because of the variability between samples in the amount of AChE unbound to WGA. The percentage AChE unbound to WGA in control groups (3.2 (0.7)%) was not significantly



Analysis of AChE concentrations and glycosylation in CSF by binding to Con A and by sucrose density gradient sedimentation analysis. (A) The percentage CSF AChE which does not bind to Con A is shown for all control (n=37) and the probable Alzheimer's disease (n=27) groups. Bars show mean values for each group. The dotted line shows the value (3.1) below which most controls clustered. (B) Sucrose density gradient sedimentation analysis of total CSF AChE isoforms. Samples (1.0 ml), obtained by pooling CSF from four control or four Alzheimer's disease cases, were applied to 5%–20% sucrose density gradients. (C) Analysis of CSF AChE isoforms that do not bind to Con A after preabsorption of whole CSF with Con A-sepharose. AChE isoforms were identified by comparison with the position of molecular weight markers catalase (C, 11.4S) and alkaline phosphatase (P, 6.1S). The figure shows that a minor G_1^a isoform which does not bind to Con A is increased in the Alzheimer's disease CSF.

different from the Alzheimer's disease group (5.5 (1.2)%, n=27).

ANALYSIS OF AChE ISOFORMS

Previous studies indicate that minor amphiphilic dimers (G_2^a) and monomers (G_1^a) are differentially glycosylated in the Alzheimer's disease frontal cortex.[11] To determine whether the change in AChE glycosylation in lumbar CSF was due to a change in the ratio of molecular weight isoforms, AChE isoforms in CSF were separated by sucrose density gradient centrifugation. In lumbar CSF, the increase in AChE unbound to Con A in the Alzheimer's disease CSF was due to increased concentrations of minor G_2^a and G_1^a isoforms that are not recognised by Con A (figure), similar to that reported previously for the frontal cortex.[11]

Discussion

This study shows that the glycosylation of AChE is altered in lumbar CSF collected antemortem from patients with Alzheimer's disease. The change in glycosylation was due to increased expression of minor amphiphilic dimeric and monomeric isoforms. It is not yet possible to predict what differences there are in carbohydrate structure between the abnormally glycosylation isoform and other AChE isoforms, as lectins are known to bind a wide variety of different types of carbohydrate. Although the origin of these isoforms is unknown, they may be identical to the isoforms that are increased in association with amyloid deposits in the Alzheimer's disease brain.[8 9] This possibility is consistent with our previous finding that the level of abnormally glycosylated AChE isoforms is increased in the frontal cortex of patients with Alzheimer's disease where amyloid deposits are found, but not increased in the cerebellum, where the deposits are rarely found.[11] This possibility is also consistent with the finding that similar isoforms are increased in transgenic mice that overexpress human amyloid protein (Aβ).[12]

The distribution of percentage AChE unbound values in the Alzheimer's disease group was much greater than in the control group, although there was also considerable overlap between the groups. However, the degree of overlap is probably less than that of Aβ1–42(43), which is a candidate diagnostic marker for Alzheimer's disease.[4] Although another putative diagnostic marker (tau) may provide greater discrimination between patients with Alzheimer's disease and healthy controls,[4] tau is increased in the CSF of some dementing and non-dementing illnesses.[15] Thus AChE glycosylation is probably more sensitive than CSF Aβ1–42(43) and more specific (but less sensitive) than CSF tau. In addition, the true degree of overlap between controls and patients with Alzheimer's disease may be less than that shown, because of the inherent uncertainty of clinical diagnosis. As the mean age of all controls was about 70, a small percentage (perhaps 5%–10%) of people in this group may have early preclinical Alzheimer's disease.[16] Interestingly, although most of the control values clustered between 0%–3.1% AChE unbound, two values in the control group were greater than the mean value for the Alzheimer's disease group. Whether these two higher values were due to the inaccuracy of the method or whether they represent early preclinical cases of Alzheimer's disease needs to be examined. Although obvious cases of vascular dementia were excluded from the probable Alzheimer's disease group, we cannot rule out the possibility that a percentage (10%–20%) of the patients in this group were misdiagnosed.[17]

The specificity and sensitivity of the glycosylation of AChE as a marker of Alzheimer's disease can only be accurately determined when diagnosis can be confirmed by pathology. The present study demonstrates that there is no alteration in AChE glycosylation in a range of non-dementing central nervous system disorders. Our previous report[10] based on an analysis of AChE glycosylation from postmortem CSF with confirmed neuropathology suggested that the glycosylation of AChE provides a marker which is 80% sensitive and almost 100% specific for Alzheimer's disease. However, this previous study used a ratio (% AChE bound to Con A /% AChE bound to WGA) as an index of glycosylation rather than percentage AChE bound to Con A as used in this study. Therefore, a prospective study of AChE glycosylation in CSF samples with confirmed diagnosis obtained by postmortem examination will be necessary to determine the true specificity and sensitivity of the assay method.

In summary, our results suggest that the glycosylation of AChE in CSF may have value for the diagnosis of Alzheimer's disease, based on the finding that AChE glycosylation is not altered in some other neurological diseases (this study) and including other dementias.[10] However, because of the sensitivity of the method, the use of other diagnostic markers in CSF such as Aβ1–42(43) and tau[4] in combination with AChE glycosylation may be needed for this approach to replace clinical examination.

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