Re-Evaluation of Cerebrospinal Fluid Angiotensin-Converting Enzyme Activity in Patients with 'Probable' Alzheimer's Disease

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Summary: Angiotensin-converting enzyme activity was measured in lumbar cerebrospinal fluid from patients with 'probable' Alzheimer's disease (n = 17) and age-matched controls (n = 19), using a spectrofluorimetric method. In contrast to a previous finding, no statistically significant difference in the mean (specific) angiotensin-converting enzyme activity was found between the two groups. No correlation existed between (specific) enzyme activity and severity of dementia in the Alzheimer's disease patients. We conclude that angiotensin-converting enzyme in cerebrospinal fluid does not appear to be useful as a potential antemortem marker for Alzheimer's disease.

Introduction

Angiotensin-converting enzyme (EC 3.4.15.1) is a dipeptidylcarboxy-peptidase which catalyses the release of dipeptides from a variety of substrates. Its main physiological substrate is the decapeptide angiotensin I from which it generates the vasoactive compound angiotensin II by the removal of the C-terminal dipeptide histidyl-leucine (1). However, other physiological peptides, structurally unrelated to angiotensin I, may also act as a substrate for angiotensin-converting enzyme. These include the neuropeptides enkephalin, neurotensin and substance P (2).

Angiotensin-converting enzyme is mainly a membrane-bound enzyme, and it has been detected in many human tissues (2, 3). In addition, a soluble (non-membrane bound) form of the enzyme is found in body fluids, for example in blood and broncho-alveolar fluid (4), and in low activity in cerebrospinal fluid (CSF) (5).

In the normal human central nervous system, angiotensin-converting enzyme has been localized (by immunohistochemistry) not only in areas where angiotensin or angiotensin receptors are detected, but also in high activity in the epitheloid cells of the choroid plexus, in the basal ganglia (caudate nucleus, putamen) and the substantia nigra pars reticularis, with lower activity in the cortex and hippocampus (6, 7). The high activity of the enzyme in the basal ganglia suggests that the enzyme may be involved in processing neuropeptides that occur in high concentration in these structures.

Alzheimer's disease is a progressive neurodegenerative disease leading to dementia. Two reports on an alteration in angiotensin-converting enzyme activity in some brain regions of patients with definite Alzheimer's disease have been published. Arregui et al. (7) claimed a significantly higher angiotensin-converting enzyme activity (this included both soluble and membrane bound enzyme) in whole tissue homogenates of the caudate nucleus and the frontal cortex. Barnes et al. (8), using a different method of assessment, showed an increase (70%) in the density of recognition sites for [3H]ceranapril (a selective angiotensin-converting enzyme inhibitor) in the temporal cortex from patients with Alzheimer's disease. The location of this abnormality is of special interest, since in Alzheimer's disease the temporal cortex is rich in intra-neuronal neurofibrillary tangles and extracellular amyloid plaques (9, 10).

As CSF bathes the cerebrum, CSF angiotensin-converting enzyme activities may therefore be an indicator of degenerative changes in Alzheimer brains.

To our knowledge, there is only one report which deals with angiotensin-converting enzyme activity in the CSF of live Alzheimer's disease patients (11). Using 4C-labelled glycine-hippuryl-histidyl-leucine as a substrate, the authors observed a significantly lower mean (specific) angiotensin-converting enzyme activity than in controls. We now report a similar study, using different methodology.

Material and Methods

Study groups

Seventeen patients with 'probable' Alzheimer's disease were included in the study (6 men and 11 women; 51-78 years of age; mean ± SD age 64.1 ± 8.1 years). The diagnosis was made according to current research criteria (12) after exclusion...
of all other causes of dementia by means of clinical investigation, laboratory screening and computed tomography or magnetic resonance imaging of the brain. The mean ± SD degree of cognitive impairment for the Alzheimer group, graded by Mini-Mental State Examination (13), was 15 ± 3.9 (normal is 24–30). The controls were matched for age (mean age 66.3 ± 11.6; 9 men and 10 women) and comprised mainly subjects in which no neurological deficit could be established. Two persons had a herniating disc and two an essential tremor. All controls showed a normal ratio of the concentration of CSF albumin to serum albumin ("albumin ratio", which serves as an index of the integrity of the blood-CSF barrier), and a normal CSF protein concentration.

Methods

Samples of lumbar CSF were collected from patients and controls with a 25 gauge needle at L4–L5 level between 8.00 and 10.00 am after an overnight bed rest, subjects lying in a left-sided position. Informed consent was obtained from all of the subjects or their relatives. The first 4 ml of CSF were used for routine analysis and the next 20 ml were gently mixed and used for other determinations, including angiotensin-converting enzyme. After collection, CSF samples were kept on water-ice for a maximum of 1 h, centrifuged at 1600 g for 10 min and stored at −70 °C until analysed.

The angiotensin-converting enzyme activity was determined by the method of Schweisfurth & Schöberg-Schiesgmtz, in a Kontron SFM 23 spectrofluorometer, using benzylxycarbonyl-phenylalanyl-histidyl-leucine (165 μmol/l in the assay) as the substrate (14). The enzyme was assayed in duplicate in 100 μl of cell-free, colourless CSF. We did not consider CSF blanks because a pilot study indicated that these values were similar to the reagent blank values.

Histidyl-leucine (dissolved in 20% iso-propanol) was used as the standard and incubated in the same way as the CSF samples. Angiotensin-converting enzyme activity (U/l) is expressed as μmol histidyl-leucine released per min per litre CSF. The lower limit of detection in the assay was 0.06 U/l.

Patient and control (pooled CSF) samples were assayed in two series with an intra-assay and inter-assay variation coefficient of 4.0% and 8.6%, respectively.

CSF protein was determined with an automated benzethonium chloride method (15) and CSF albumin and serum albumin were quantitated with an automated nephelometric method (16).

Statistics

The results are presented as means ± SD and range. Comparisons between groups were made using Student's two-tailed t-test, and p < 0.05 was considered significant. Correlations between variables were assessed by the Pearson correlation coefficient (r) (17).

Results

The results for CSF total protein, albumin ratio and CSF angiotensin-converting enzyme activity (U/l) in patients with Alzheimer's disease and controls are shown in table 1. In our study, the reference range (0.55–1.21 U/l) for angiotensin-converting enzyme is very similar to that (0.47–1.16 U/l) reported earlier (14).

There was no statistically significant difference between groups for total protein, albumin ratio or angiotensin-converting enzyme activity. Because cerebral atrophy in Alzheimer's disease may cause an increase in CSF volume, the angiotensin-converting enzyme activities were expressed relative to protein concentration (specific activity, U/g). Mean angiotensin-converting enzyme specific activity in the Alzheimer group did not differ significantly from the control group. No correlation was found between Mini-Mental State Evaluation and angiotensin-converting enzyme (specific) activity in the Alzheimer group.

Discussion

Our study revealed that mean CSF angiotensin-converting enzyme (specific) activity in the Alzheimer's disease patients did not differ from that of age-matched controls. Thus, our study cannot support the findings of Zubenko et al. (11), who claimed both a statistically significant lower (41%) angiotensin-converting enzyme activity (U/l) and a lower (27%) specific activity (U/g) in their Alzheimer group (n = 13) when compared with controls (n = 30).

In view of these conflicting results, the following points should be noted concerning the blood-CSF barrier, severity of dementia, age of onset and difference in kinetics of angiotensin-converting enzyme isoforms. Angiotensin-converting enzyme activity in blood is about 50–100 fold higher than the activity in CSF (4). Its high molecular mass (M, 147000) prevents angiotensin-converting enzyme from crossing the intact blood-brain barrier. However, a disturbance in the blood-brain barrier may considerably increase both the albumin ratio and the angiotensin-converting enzyme activity in CSF.

In the present study, controls showed a normal albumin ratio (upper limit of normal 9.0). The albumin ratios of two Alzheimer patients were 10.7 and 12.8; their angiotensin-converting enzyme (specific) activities were 0.85 (1.35) and 0.72 (1.50), respectively.

Zubenko et al. (11) did not report the albumin ratios in their samples. Possibly, in some of their controls, the albumin ratio exceeded the normal upper limit. This would explain the higher mean angiotensin-converting enzyme (specific) activity in their controls, compared with diseased subjects.

Concerning the severity of dementia, the difference in the Mini-Mental State Examination of Zubenko's patients and our patients is prominent.

Our Alzheimer patients showed considerable cognitive impairment (15 ± 3.9; 0 = worst; 30 = best), while in Zubenko's study patients were mildly demented (score, 23.3 ± 3.6). This makes comparison between the two studies difficult. Clinically, Alzheimer's disease does not fit a uniform pattern (18).
Subgrouping the disorder on the basis of age at onset may be reflected in biochemical differences observed in CSF (19). This is another factor that may underly the conflicting results.

Finally, different molecular forms of angiotensin-converting enzyme appear to be present in pooled CSF (20). If such isoforms do exist in Alzheimer’s disease, they may show different kinetics with the substrates used in Zubenko’s study (11) and in the present procedure. This could partly account for the discrepancy between our results and those of Zubenko et al.

Conclusion
The present study does not confirm the previous finding (11) that (specific) angiotensin-converting enzyme activity is decreased in CSF of live patients with Alzheimer’s disease, and therefore questions the validity of the enzyme as a biological marker in this disease. The role of angiotensin-converting enzyme in the pathophysiology of Alzheimer’s disease has yet to be clarified.

References