Brain Choline Acetyltransferase and Mental Function in Alzheimer Disease

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Objective: To determine whether higher brain levels of choline acetyltransferase (ChAT) are associated with improved neuropsychological function in patients with Alzheimer disease (AD).

Design: Case series with single-blind post hoc analysis of biopsy specimens.

Setting: Urban hospital and medical school.

Patients: A consecutive sample of 8 patients with AD undergoing brain biopsy and surgical implantation of intraventricular pumps for administration of potential chemotherapeutic agents.

Interventions: Brain biopsy, surgical implantation of intraventricular pumps, and, in 1 patient, ventriculo-peritoneal shunt placement.

Main Outcome Measures: All patients underwent neuropsychological testing no more than 2 weeks before surgical biopsy. Levels of ChAT were determined in fresh brain tissue from biopsy samples.

Results: Significant positive correlations were found between ChAT levels and 2 neuropsychological test scores, Mini-Mental State Examination and the Logical Memory subtest of the Wechsler Memory Scale.

Conclusion: Degeneration of the cholinergic system in vivo correlates with decreasing cognitive function in patients with AD.

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Since the discovery of Alzheimer disease (AD) in 1897, there has been controversy concerning the cause of the loss of cognitive, intellectual, and social function seen in patients with this disorder. Many have argued that the number of plaques seen in the brain parenchyma of patients with AD correlates directly with loss of function, whereas others have suggested that the degree of cerebral atrophy is a more useful measure. Others have postulated specific systems to be selectively dysfunctional. These have included the hypothalamic-pituitary-adrenal axis, the adrenergic system, and the cholinergic system.

Previous human data have been obtained mainly from autopsy studies, in which there has often been a lapse of a number of hours before tissue could be harvested. This makes determination of specific neurotransmitter levels problematic, as there is often a deterioration of chemical activity seen within minutes of death. Our study was undertaken to more clearly delineate the relationship between neuropsychological function and alteration in the cholinergic system. We used brain biopsy material from patients who underwent complete neuropsychological assessment no more than 2 weeks before surgical brain biopsy. Information from these tests was correlated with neurotransmitter values in fresh brain tissue.

RESULTS

Histopathological analysis confirmed the diagnosis of AD in all 8 patients.

Neuropsychological scores are shown in Table 1, and correlations are described in Table 2. Significant positive correlations were found between ChAT levels and 2 neuropsychological test scores (Figure). Patients demonstrating higher Mini-Mental State Examination scores also had higher ChAT levels (P = .03). Higher Wechsler Memory Scale Logical Memory scores were also associated with higher ChAT values (P = .05).

COMMENT

The most striking alteration seen in neurotransmitters in AD occurs in the cholinergic system. Clinical trials using cholinergic agents have shown promising reduction of the mental impairment seen in AD. The consistency with which this biochemical disruption is seen in...
SUBJECTS AND METHODS

SUBJECTS

This study was reviewed and approved by the institutional review boards of Baylor College of Medicine, Houston, Tex, and The Methodist Hospital, Houston. Five women and 3 men were entered into the study. The average age was 60 years (range, 54-83 years), and the average duration of illness was 3.5 years (range, 1.5-5 years). Informed consent was obtained after the nature of the procedure was fully explained. A complete medical history was obtained from the patient and/or spouse. Previous medical records were reviewed, and a physical examination, including a thorough neurologic evaluation and mental status assessment, was performed. Each patient had received a diagnosis of AD with no evidence of depression by a board-certified neurologist (S.H.A) using National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders Association criteria.14 Extensive laboratory studies were performed as well as a computed tomographic or magnetic resonance imaging scan, or both. Patients with any other medical condition that could explain the dementia, including multiple infarct states, were excluded from the study. One patient included in the study had a history of normal pressure hydrocephalus and underwent placement of a ventriculoperitoneal shunt. This patient's dementia was thought to be unrelated to the normal pressure hydrocephalus, and, indeed, did not change after the shunt, although his gait and urinary incontinence improved dramatically.

BIOPSY

Patients underwent dural and cortical brain biopsy in the right frontal region. In some patients, this was a prelude to placement of a ventricular catheter for a shunting procedure or as part of an experimental protocol to infuse cholinergic agonists intraventricularly. In others, a brain biopsy alone was performed.

A burr hole was placed just in front of the coronal suture, 2.5 cm from the midline. After the dura was opened and excised for biopsy, an underlying 10 mm² of cortex was excised, which was divided longitudinally. One half of this specimen was immediately placed in liquid nitrogen for analysis of choline acetyltransferase (ChAT) levels. The other half of the specimen was handed off the field and placed in gluteraldehyde for histological verification of AD. Pa
tients were observed in the hospital for several days after the procedure and then returned home.

HISTOPATHOLOGICAL VERIFICATION OF AD

Senile plaque counts were performed on Bielschowsky-

stained, paraffin-embedded, 9-mm-thick sections. Ten fields from each case were counted at magnification ×200 corresponding to a field area of 0.9 mm². The histopathological analysis was performed without knowledge of the patient's clinical status or the results of biochemical studies.

ChAT ASSAY

The ChAT assay was adapted from Schrier and Shuster.15 Biopsy material was homogenized in 10 volumes of homogenization buffer (0.05 mol/L sodium phosphate [pH, 6] containing 0.2% alkylaryl polyether alcohol [Triton X-100; Union Carbide Corporation, Danbury, Conn]) and incubated for 10 minutes at 37°C. The reaction was terminated by incubation at 95°C for 5 minutes and then kept at 0°C until separated on ion exchange resin (Dowex 1; Dow Chemical Company, Midland, Mich) (30-100 mesh chloride form). The incubation included 3 mmol acetyl-CoA radiolabeled with hydrogen 3, 50 mmol/L choline chloride, 0.88 mmol/L physostigmine, and 140 mmol/L sodium chloride in 200 mL homogenization buffer. Protein was determined using the method of Lowry et al.16 Enzyme activity, determined in triplicate, was expressed as nanomoles per minute per milligram of protein.

NEUROPSYCHOLOGICAL SCORING

The following neuropsychological procedures were administered to all subjects before the brain biopsy: Mini-Mental State Examination; Global Deterioration Scale; the Digit Symbol, Digit Span, and Block Design subtests of the Wechsler Adult Intelligence Scale–Revised; the Logical Memory and Visual Reproduction subtests of the Wechsler Memory Scale with 30-minute delayed recall (1945 version); The Trail Making Test (1985 version); Controlled Oral Word Association Test (1983 version); an abbreviated version of the Selective Reminding Test involving the acquisition and retention of 8 unrelated, common words across 10 trials; and an abbreviated version of the Boston Naming Test consisting of 15 drawings of objects that the subjects were required to identify by name.

STATISTICAL ANALYSIS

Statistical analysis of the results of the neuropsychological and biochemical assessments was performed using the Spearman rank correlation test. Differences were considered significant when P<.05.

AD and its association with mental impairment have led to the general acceptance and refinement of the cholinergic hypothesis of dementia in AD.10,11,21,24 Indeed, the animal models that have been developed to simulate AD focus on disruption of the cholinergic system.25,26

To date, the human tissue analysis that has verified cholinergic involvement in AD has been performed on postmortem tissue.12 As neurotransmitter levels can change rapidly following death, it is likely that postmortem tissue levels are different from those in premorbid brain.13

Our study analyzed fresh tissue from biopsy samples. In our patients, lower levels of cortical ChAT were negatively associated with neuropsychological performance. Our results indicate that in patients with biopsy-confirmed AD, there is an in vivo loss in ChAT level that correlates with severity of disease measured by loss of neuropsychological function.

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Bartus RT, Dean RL III, Beer B, Lippa AS. The cholinergic hypothesis of geriatric material. Levels of ChAT had a significant positive correlation (r = 0.77) with the MMSE score (P = .03). Right, Correlation of Wechsler Memory Scale Logical

Research Foundation, Houston, Tex; and the Merit Review Board of the Department of Veterans Affairs, Washington, DC (all to Dr Baskin).

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REFERENCES


Table 1. Neuropsychological Assessment Scores*

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<th>WMS-LMD</th>
<th>WMS-VRD</th>
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* MMSE indicates Mini-Mental State Examination; GDS, Global Deterioration Score; WMS-LM, Logical Memory subtest of the Wechsler Memory Scale; WMS-LMD, Logical Memory Delayed subtest of the WMS; WMS-VRD, Visual Reproduction Delayed subtest of the WMS; BN, Boston Naming Test; and ellipses, not done.

Table 2. Correlation of MMSE With Other Neuropsychological Tests*

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*Abbreviations are explained in the footnote to Table 1. † Indicates P value of correlation.