Early and Widespread Cholinergic Losses Differentiate Dementia With Lewy Bodies From Alzheimer Disease

Pietro Tiraboschi, MD; Larry A. Hansen, MD; Michael Alford, BA; Annette Merdes, MD; Eliezer Masliah, MD; Leon J. Thal, MD; Jody Corey-Bloom, MD, PhD

Background: Reductions in cholinergic function occur in Alzheimer disease (AD) and dementia with Lewy bodies and correlate with cognitive decline. However, whether such alterations appear in early-stage disease is unclear.

Objective: To examine the timing of cholinergic deficits in AD and dementia with Lewy bodies.

Methods: Autopsy series of 89 patients with AD and 50 patients with the Lewy body variant of AD (LBV). Stage of disease was stratified according to results of the last Mini-Mental State Examination (MMSE) before death as mild, moderate, severe, or very severe. We analyzed choline acetyltransferase (ChAT) activity in the midfrontal, superior temporal, and inferior parietal cortices.

Results: Although compared with a normal control group ChAT activity was decreased in the AD and LBV cohorts, ChAT activity reduction for the LBV cohort was much greater. Moreover, although the decline in ChAT activity in the AD cohort compared with the normal control group was significant only for patients in later stages of the illness, the decline in the LBV cohort was significant for those who died with mild-stage disease. When less impaired patients in each cohort (MMSE, ≥10) underwent separate analysis, the relationship of ChAT activity with the MMSE score was strong and significant for the LBV cohort alone.

Conclusions: Although cholinergic deficits are seen in both AD and LBV, loss of ChAT activity is less severe and occurs later in the clinical course of AD. Conversely, in LBV, loss of ChAT activity is already prominent in the earliest stages of the illness, suggesting that cholinergic replacement therapy may be more effective in LBV than in AD, especially in mild-stage disease.

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kinsonism.11,12 Neuropsychologically, patients with DLB may display a different pattern of cognitive decline, with worse performance on tests of visuospatial activity, initiation, and perseveration.13 Furthermore, the progression of their deterioration on global measures of dementia (eg, the Mini-Mental State Examination [MMSE])14 and the Mattis Dementia Rating Scale)15 may be faster than that observed in patients with AD.16,17 Neuropathologically, in most brains of patients with DLB, accompanying AD pathologic changes in the form of neocortical diffuse plaques, neuritic plaques, and modest numbers of neurofibrillary tangles are found in the medial temporal lobe (ie, the Lewy body variant of AD [LBV]).18 A few brains, however, have no more AD pathologic changes than do age-matched controls. Neurochemically, although the decline of ChAT activity has been shown to be much greater in patients with DLB than in those with AD,19 the strength of its association with cognitive decline has been more controversial.20-22

Apart from a small number of autopsy studies that included cases with mild impairment,23-25 descriptions of neurochemical changes in AD and DLB have been generally based on findings in patients with late-stage disease. Moreover, few studies have been performed using biopsy specimens of living patients with mild to moderate dementia.22,23 Finally, most of these studies considered mixed populations of patients with AD and DLB, since they were performed before DLB had been well defined. Consequently, how early in the course of each of these diseases neurochemical alterations occur remains relatively unclear.

In the present study, we sought to determine whether cholinergic dysfunction was an early event in AD and LBV by examining the activity of ChAT in autopsy specimens of these prospectively observed cohorts who died at different stages of their illness (classified clinically as mild, moderate, severe, and very severe). In addition, we investigated the relationship between the decline in ChAT activity and dementia severity in AD and LBV.

PATIENTS AND METHODS

PATIENTS

The patients included in the present study were observed clinically at the University of California–San Diego Alzheimer’s Disease Research Center, Seniors Only Care, San Diego, or in the private practices of its senior clinicians. They represent all patients who underwent autopsy between 1985 and the present with a clinical diagnosis of AD or LBV or normal control (NC) for whom ChAT activity was available, and where cognitive testing was administered within 24 months prior to death.

Eighty-nine patients with AD and 50 with LBV met the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition for a clinical diagnosis of dementia (eg, the Mini-Mental State Examination [MMSE]) or of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association for probable or possible AD.23 In addition, they met the criteria of the National Institute on Aging for a pathological diagnosis of AD24 and of the Consortium to Establish a Registry for Alzheimer’s Disease for a diagnosis of probable or definite AD.25 Patients with LBV also met the criteria of the Consortium on DLB for a pathological diagnosis of DLB.26,27 All patients with AD and LBV were stratified according to their last MMSE score before death as having mild (MMSE score, ≥20; n=14 for the AD cohort and n=7 for the LBV cohort), moderate (MMSE score, 10-19; n=20 for the AD cohort and n=10 for the LBV cohort), severe (MMSE score, 1-9; n=29 for the AD cohort and n=11 for the LBV cohort), and very severe disease (MMSE score, 0; n=26 for the AD cohort and n=22 for the LBV cohort).

The NC group consisted of 18 patients with no evidence of cognitive impairment or any other neurologic or psychiatric disorder. Twelve of these patients were also considered neuropathologically normal. The remaining 6 patients had only sufficient senile plaques to meet the National Institute on Aging criteria for AD or neuritic plaques to meet the criteria of the Consortium to Establish a Registry for Alzheimer’s Disease for possible AD, but did not display any cognitive impairment on results of extensive neuropsychological testing administered in these cases less than 1 year before death.

NEUROPSYCHOLOGICAL EXAMINATION

Mean scores are reported for the following 3 commonly used measures of global cognitive status: the MMSE, the Blessed Information-Memory-Concentration test,26 and the Mattis Dementia Rating Scale. Most of the patients had also received the comprehensive neuropsychological battery administered through the Alzheimer’s Disease Research Center as part of their annual evaluation.27

NEUROPATHOLOGICAL EXAMINATION

Pathological assessment was performed by one of us (L.A.H.). Autopsy was performed within 12 hours of death using a protocol described by Terry et al.30 The left half of the brain was fixed by means of immersion in 10% formalin for 5 to 7 days, at which time blocks were taken for paraffin embedding from the midfrontal (MF), rostral superior temporal (ST), and inferior parietal (IP) areas of the neocortex, hippocampus, entorhinal cortex, basal ganglia/substantia innominata, mesencephalon, and pons. The neocortical areas correspond to Brodmann areas 46, 38, and 39. The parafin blocks of neocortex were cut at 7-μm thickness for hematoxylin-eosin and thionin fluorescence S staining. Total plaque, neuritic plaque, and neurofibrillary tangle counts were determined by the same examiner (L.A.H.) using the same criteria consistently. Lewy bodies were detected using hematoxylin-eosin and/or anti-ubiquitin immunostaining, as recommended by the Consortium on Dementia with Lewy Bodies.11,12 Specifically, to receive a pathological diagnosis of LBV, 1 or more Lewy bodies had to be present in both the neocortex and brainstem in addition to significant AD pathologic changes.

NEUROCHEMISTRY

Samples were taken from the MF, ST, and IP areas of frozen, unfixed cortex from the right half of the brain and homogenized in 1mM EDTA (pH, 7.0) containing 0.1% Triton X-100 (Sigma-Aldrich Corp, St Louis, Mo). Analysis of ChAT activity was performed by means of the modified Fonnum technique.31,32 The coefficient of variation was 3%, with an intra-assay variability of 7.9%.

STATISTICAL ANALYSIS

Mean values among groups (AD vs NC and LBV vs NC) were compared using 1-way analysis of variance (ANOVA). In case of inequality of group variances (P<.05, Bartlett test), the ANOVA was preceded by logarithmic transformation of raw data.
The present study investigated the timing and distribution of ChAT activity decline during the course of AD and...
LBV and the relationship between reduction of ChAT activity and dementia severity in these illnesses. To explore this, we used (1) large and well-characterized cohorts of patients with AD and LBV with the last neuropsychological assessment reasonably close to death, and (2) tissue samples from multiple neocortical areas of the patients.

Although definite conclusions about longitudinal changes occurring in individual patients can hardly be drawn from a cross-sectional study, our data strongly support the view that the integrity of cholinergic neurotransmission is affected at different points in the course of these diseases. Cholinergic dysfunction appears to occur early in LBV, whereas in AD, it is seemingly severely reduced only in the later stages of the illness. Furthermore, although this neurochemical deficit may be fairly generalized beginning with early-stage disease in LBV, there may be region-specific differences, with the temporal cortex more susceptible to the loss of ChAT activity than the frontal and parietal cortices, in AD. In fact, ST ChAT activity was significantly decreased in patients with moderate AD at death, whereas MF and IP ChAT activities were significantly reduced only in the AD cohort with more severe impairment. This is in contrast to a recent study by Davis et al, in which no regional differences in ChAT activity decline were observed, and suggests that the progression of deterioration in AD may depend on the involvement of an increasing number of specific neocortical regions as the disease advances.

In contrast to AD, the pattern of ChAT activity decline in LBV—early and more extensive, involving all neocortical areas—may contribute to its characteristic clinical profile. In particular, the higher prevalence of psychotic symptoms (visual hallucinations and delusions), severe visuospatial dysfunction, and prominent deficits in ex-

### Table 2. Demographic, Neuropsychological, and Neurochemical Variables in LBV*

<table>
<thead>
<tr>
<th>Cognitive test, final score</th>
<th>MMSE</th>
<th>BIMC§</th>
<th>DRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at death, y</td>
<td>77.4 ± 11.2</td>
<td>24.4 ± 3.1</td>
<td>91.2 ± 32.4</td>
</tr>
<tr>
<td>Male/Female</td>
<td>10/8</td>
<td>4/3</td>
<td>8/6</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>15.7 ± 3.1</td>
<td>11.5 ± 4.3</td>
<td>79.3 ± 24.0</td>
</tr>
<tr>
<td>Test-death interval, mo</td>
<td>9.5 ± 4.8</td>
<td>11.9 ± 10.3</td>
<td>8.1 ± 5.4</td>
</tr>
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</table>

Results of neurochemistry, nmol per 100 mg/h

| ST ChAT                  | 304.8 ± 113.5 | 164.4 ± 101.8 | 56.4 ± 20.1 |
| IP ChAT                  | 204.4 ± 89.4  | 55.8 ± 46.2   | 33.0 ± 15.0 |
| MF ChAT                  | 214.1 ± 61.2  | 74.7 ± 31.6   | 52.5 ± 32.2 |

*Unless otherwise indicated, data are given as mean ± SD. LBV indicates Lewy body variant of AD. Other abbreviations are given in the first footnote to Table 1.

†Determined by means of analysis of variance.

‡Differences were significant (P<.05 to P=.001) for each of the pairwise comparisons (Tukey-Kramer multiple comparisons test).

§Score indicates the number of incorrect responses.

### Table 3. Correlations Between MMSE Scores and ChAT Activity in AD and LBV*

<table>
<thead>
<tr>
<th>Patients, Impairment</th>
<th>AD Cohort, r and P Values</th>
<th>LBV Cohort, r and P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST</td>
<td>IP</td>
</tr>
<tr>
<td>Mild (MMSE, ≥20)</td>
<td>0.44; .21</td>
<td>-0.32; .42</td>
</tr>
<tr>
<td>Moderate (MMSE, 10-19)</td>
<td>0.20; .33</td>
<td>-0.04; .83</td>
</tr>
<tr>
<td>Very Severe LBV</td>
<td>0.35; .02</td>
<td>0.33; .03</td>
</tr>
<tr>
<td>Mild and moderate</td>
<td>0.47; 0.001</td>
<td>0.35; .005</td>
</tr>
</tbody>
</table>

*Correlations were performed using Pearson product moment procedure. Normal control subjects were excluded from analyses. Sample sizes and abbreviations are given in the first footnote to Tables 1 and 2.
ective function, attention, and literal fluency reported for patients with mild- to moderate-stage LBV11-13 may be related to earlier and greater reductions of ChAT activity in the temporal, parietal, and frontal cortices, respectively.

Loss of ChAT activity has been shown to correlate with cognitive decline in patients with AD by several investigators,2,6-8 who reported correlation coefficients ranging from 0.46 to 0.82. Although these discrepancies across studies may have several explanations, such as heterogeneity of populations (demented patients alone vs mixtures of patients with and without cognitive impairment), tissue samples (biopsy vs autopsy specimens), and brain regions considered for biochemical assay (eg, allocortical vs neocortical areas), different disease severity of the cohorts examined was likely the most important reason. In our AD sample, the strength of correlation between ChAT activity decline and cognitive impairment increased with increasing severity of dementia of the patients included in the analyses. When the whole AD cohort and the NC group were considered, our results ($r = 0.52$ [MF and IP cortices]; $r = 0.59$ [ST cortex]) were quite consistent with those found by other investigators.7,8

The relationship between ChAT activity and cognitive performance has been more controversial in DLB, in which correlated correlation coefficients range from 0.25 to 0.90.18 The same reasons raised for discrepancies in studies of AD and, in particular, the different disease severity of the patients examined, might have contributed to this great variability, since the magnitudes of correlation coefficients in previous studies were considerably reduced when more deteriorated patients were included.20 Moreover, the present study clearly shows that in LBV, unlike AD, the relationship between ChAT activity decline and cognitive impairment weakens as the severity of dementia of the patients included in the analyses increases.

Our findings may have several therapeutic implications. First, our data may provide a rationale for the greater responses reported for patients with more advanced AD in experimental trials of cholinesterase inhibitors, implying that even patients with severe AD retain the capacity to respond to cholinergic treatment. Second, and more important, these results suggest that cholinergic replacement therapy may be particularly effective in LBV, beginning with the earliest stages. Cholinergic replacement therapy has been postulated to play an important role in the treatment of LBV after 3 patients with combined LB-AD pathology and very low neocortical ChAT activity were reported to show a substantial response to tacrine hydrochloride treatment.33 Evidence has also shown that cholinesterase inhibitors may have neuro- psychiatrict benefits,34 including reduction of agitation, delusions, and hallucinations, which are highly prevalent in LBV.11,12 Although a recent pharmacological trial35 has only partially supported these findings, the present study strongly suggests that cholinergic replacement therapy may have greater benefits, especially early, for patients with LBV than for those with AD. This observation should be further assessed in well-designed, randomized clinical trials.

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REFERENCES


