Neurochemical Markers Do Not Correlate With Cognitive Decline in the Lewy Body Variant of Alzheimer Disease

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Background: Reductions in neocortical synapses and cholinergic function occur in patients with Alzheimer disease (AD) and in patients with the Lewy body variant of AD (LBV). The relation between these losses and cognitive decline has been reported frequently in patients with AD but remains unclear for patients with LBV.

Objectives: To investigate the relation between clinical markers of disease progression and choline acetyltransferase activity or synaptic density, measured by synaptophysin (Syn) level, in patients with LBV, and to investigate the relation of these neurochemical markers with one another.

Methods: Brain specimens of 41 patients with autopsy-confirmed (National Institute on Aging criteria for AD) LBV were examined. The last Mini-Mental State Examination and Blessed Information-Memory-Concentration test scores before death were reviewed. Midfrontal synapse counts were quantified by a dot-immunobinding assay for Syn. Choline acetyltransferase activity of the midfrontal cortex was assayed by established protocols.

Results: The last Mini-Mental State Examination score before death did not correlate significantly with Syn level \((n = 25, r = 0.25, P = .24)\); however, there was a trend toward significance for the relation between last Mini-Mental State Examination score and choline acetyltransferase activity \((n = 39, r = 0.31, P = .05)\). The last Blessed Information-Memory-Concentration test score did not correlate with either Syn level \((n = 24, r = -0.17, P = .44)\) or choline acetyltransferase activity \((n = 39, r = -0.16, P = .33)\). Finally, there was only a modest correlation between Syn level and choline acetyltransferase activity \((n = 25, r = 0.38, P = .06)\), which did not reach statistical significance.

Conclusion: Unlike AD, neurochemical markers do not appear to correlate well with cognitive decline in LBV.

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SUBJECTS AND METHODS

SUBJECTS

The subjects with LBV in the present study were followed up clinically at the University of California, San Diego, Alzheimer’s Disease Research Center; the Senior’s Only Care, a program sponsored by the University of California, San Diego; or the private practices of its senior clinicians.

Brain specimens of 41 patients with autopsy-confirmed LBV were examined. These specimens, with brainstem or neocortical Lewy bodies, met National Institute on Aging27 and Consortium to Establish a Registry for Alzheimer’s Disease28 criteria for definite or probable AD and clinically fulfilled Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition30 criteria for a diagnosis of dementia; most of them also fulfilled National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association29 criteria for a clinical diagnosis of probable or possible AD. The last MMSE31 and BIMC test scores2 were used to assess dementia severity. Patients were excluded if they had not undergone a clinical examination within 24 months of death.

NEUROPATHOLOGIC EXAMINATION

The postmortem interval varied from 2 to 24 hours. The autopsy was performed using a protocol described by Terry et al.8,32 The left hemibrain was fixed by immersion in 10% formaldehyde solution for 5 to 7 days, at which time blocks were taken for paraffin embedding from the midfrontal (MF), rostral superior temporal, and inferior parietal areas of the neocortex; hippocampus; basal ganglia or innominate substance; mesencephalon; and pons. The cortical areas correspond to Brodmann areas 46, 38, and 39. The paraffin blocks of the isocortex were cut at 7-µm thickness for hematoxylin-eosin staining. Sections (10 µm thick) were prepared for thioflavine S staining. Quantification of Lewy bodies was performed by methods previously described.10,33,34

ChAT MEASUREMENTS

Samples were taken from MF areas of frozen unfixed right hemibrain isocortex and homogenized in EDTA, pH 7.0, 1 mmol/L, containing 0.1% alkaryl polyether alcohol (Triton X-100). Analysis of ChAT activity was performed in triplicate by the modified Fonnum technique.35-37 The coefficient of variation was 3%, with an intra-assay variability of 7.9%.

SYNAPSE DENSITY MEASUREMENTS

Synaptic density measurements from the right MF cortex were performed by the dot-immunobinding assay for Syn immunoreactivity described by Alford et al.38 Briefly, this is a technique that uses immunocytochemical labeling of the synapse-associated protein, Syn, coupled with quantification by optical density measurement. Particulate fractions were prepared, and the pellet was resuspended and sonicated. After total protein determination, samples were diluted to a uniform concentration of 40 µg of protein per milliliter. Samples were then blotted in a microsample filtration manifold (Schleicher & Schuell, Keene, NH) on a 0.45-mm pore size nitrocellulose membrane, drawn through by vacuum, and then dried. Mouse monoclonal Syn antibody was incubated with the samples overnight followed by consecutive incubations of rabbit anti–mouse IgG and iodine 125–protein A for 2 hours each. Autoradiography was then performed on these preparations. Each sample has 6 replications, with an intra-sample coefficient of variation of 7.9%.

STATISTICAL ANALYSIS

Correlation analyses for patients with LBV were performed by Pearson product moment correlations.

RESULTS

The mean values for the demographics, clinical indexes, and biochemical results are summarized in Table 1. The correlations between presynaptic markers and global mental test scores for the subjects with LBV are summarized in Table 2. The correlation between last MMSE score before death and Syn level was not statistically significant; however, there was a trend toward significance for the correlation between last MMSE score and ChAT activity. The last BIMC test scores did not correlate with either Syn level or ChAT activity.

There was only a modest correlation between Syn level and ChAT activity, which approached but did not reach statistical significance.

COMMENT

The present study examines the relation between the presynaptic markers ChAT and Syn in a well-characterized cohort of subjects with LBV. Furthermore, it assesses the relation between these presynaptic markers and global measures of dementia severity in patients with LBV.

A relation between global measures of dementia severity and synapse loss has been established in patients with AD.6,38 Using electron microscopy of biopsy specimens, DeKosky and Scheff8 reported a correlation coefficient of 0.69 between scores on the MMSE and number of synapses in Brodmann area 9 in 8 patients with AD. Likewise, Terry and coworkers8 and Samuel and co-workers9 reported correlation coefficients of 0.73 for the
The patients included 23 men and 18 women. LBV indicates Lewy body variant of Alzheimer disease; MMSE, Mini-Mental State Examination; BIMC, Blessed Information-Memory-Concentration test; ChAT, choline acetyltransferase; and Syn, synaptophysin.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value†</th>
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<tbody>
<tr>
<td>Age at death, y</td>
<td>78.5 ± 8.1 (57-103)</td>
</tr>
<tr>
<td>Disease duration, y‡</td>
<td>7.2 ± 2.9 (1-16)</td>
</tr>
<tr>
<td>Interval from last examination to death, mo</td>
<td>10.9 ± 9.1 (1-24)</td>
</tr>
<tr>
<td>Final MMSE score‡</td>
<td>7.8 ± 8.6 (0-28)</td>
</tr>
<tr>
<td>Final BIMC score‡</td>
<td>24.6 ± 8.1 (7-33)</td>
</tr>
<tr>
<td>MF ChAT level‡§</td>
<td>49.9 ± 32.2 (27-335)</td>
</tr>
<tr>
<td>MF Syn level¶</td>
<td>88.7 ± 32.5 (28.6-143.2)</td>
</tr>
</tbody>
</table>

*The patients included 23 men and 18 women. LBV indicates Lewy body variant of Alzheimer disease; MMSE, Mini-Mental State Examination; BIMC, Blessed Information-Memory-Concentration test; ChAT, choline acetyltransferase; and Syn, synaptophysin.
†Data are given as mean ± SD (range).
‡n = 40.
§The number of incorrect responses.
¶Measured as number of arbitrary units per microgram of protein.
†Measured as number of nanomoles of acetylcholine per hour per 100 mg of protein.

Choline acetyltransferase activity has been shown to correlate with cognitive decline in patients with LBV. However, in the present study using subjects with LBV, synaptic loss did not correlate with level of cognitive decline, which was quite surprising since mean Syn counts were akin to those seen in patients with AD. In a smaller cohort of subjects with LBV (n = 12), Samuel et al also found no correlation between synaptic loss and cognitive decline.

Choline acetyltransferase activity has been shown to correlate with cognitive decline in patients with AD by several investigators. Perry et al first demonstrated a relation between ChAT activity and the BIMC test score in 1978. A correlation coefficient of 0.82 was reported by these researchers, but careful examination of their data reveals that this high correlation resulted from a mixture of patients with dementia and patients with depression. A significant correlation between ChAT activity and measures of dementia severity was also reported in a mixed population of patients with dementia and patients without dementia in another series. Using a clinical index of dementia severity that ranged from 0 to 9, Francis et al found a correlation of 0.63 between ChAT levels and cognitive impairment in 17 young patients with AD who underwent cortical biopsy. Their correlation may be somewhat influenced by selection bias and the younger age of their subjects. A recent report by Bierer et al examining the relation between ChAT activity in the temporal cortex and the Clinical Dementia Rating scale reported a correlation coefficient of 0.46.

For LBV, the relation between ChAT activity and dementia severity has not been clear. Using a large sample of well-characterized subjects with LBV, we did not find a correlation between ChAT activity and BIMC test score and observed only a weak correlation between ChAT activity and MMSE score. This is in contrast to the previous study by Samuel et al from our institution using a smaller cohort (n = 17) of mixed subjects with diffuse Lewy body disease and subjects with LBV in which correlation coefficients of 0.62 (MMSE) and −0.33 (BIMC) were reported. When their analysis was limited to patients with LBV (n = 9), MF ChAT activity continued to correlate with MMSE (r = 0.67) and BIMC test (r = −0.60) scores. Likewise, the small study of 8 subjects with senile dementia of the Lewy body type by Perry et al reported a strong correlation (n = 8, r = 0.9, P < .01) between ChAT activity and BIMC test scores before death. Using a considerably larger, but possibly more demed, cohort of well-characterized subjects with LBV, we were unable to confirm these findings with either the BIMC test or the MMSE. Only 6 subjects overlapped with the previous report from our institution, and their test death interval was considerably longer. Hypothetically, the severity of dementia in our sample might have affected our ability to demonstrate correlations owing to the presence of floor or ceiling limitations on the MMSE and the BIMC test in many subjects (about one third). Nevertheless, when we excluded from analysis subjects who had achieved a floor score on the MMSE, the correlation did not change appreciably (ChAT vs MMSE, r = 0.36; P = .07).

An additional finding of our study is that there is only a modest correlation between ChAT activity and neocortical synaptic density measurements in the MF cortices of subjects with LBV. For AD, this correlation in a small study of 12 subjects was somewhat stronger (r = 0.54, P < .05), although hardly robust. Since studies of rat hippocampus had shown that ChAT is membrane bound to synaptic vesicles that contain Syn, we had expected that there might be a tight correlation between the 2. Our finding of only a modest correlation between ChAT and Syn may have several explanations. First, not all synapses labeled by Syn are cholinergic. Synaptophysin labels noradrenergic, dopaminergic, and γ-aminobutyric acid neuronal populations in the brain. Second, the Syn immunobinding assay used in our study may not be as accurate as evaluation of Syn staining by laser confocal microscopy. While clearly easier to perform, the dot blot assay does not have the anatomic accuracy of examining tissue sections; however, the dot blot Syn immunobinding assay has a correlation coefficient of 0.82 to the measurement of Syn by laser confocal microscopy. In the present study, we sought to examine the relations between neurochemical markers and several clinical indexes of dementia severity in a relatively large, well-characterized cohort of subjects with autopsy-proved LBV. The relations between these markers and cognitive decline have been reported frequently in patients with AD.
but remained unclear in patients with LBV. The lack of correlation between the presynaptic markers ChAT and Syn and global cognitive measures, the MMSE and BIMC test scores in the present study, suggests that other factors, which are not operative in patients with AD, may be operative in determining dementia in patients with LBV; these factors include greater neuronal loss and the presence and number of Lewy bodies.23

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REFERENCES


