Preservation of Neurons of the Nucleus Basalis in Subcortical Ischemic Vascular Disease

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Objective: To investigate loss of neurons in the nucleus basalis (NB) of Meynert in patients with subcortical ischemic vascular disease (SIVD) compared with healthy controls, patients with Alzheimer disease (AD), and patients with mixed AD and SIVD.

Design: Autopsied cases drawn from a longitudinal observational study of patients with SIVD, patients with AD, and healthy controls.

Setting: Multi center, university-affiliated, program project neuropathology core.

Patients: Patients with pathologically defined SIVD (n=16), AD (n=20), and mixed AD and SIVD (n=10) and healthy controls matched by age and educational level (n=17) were studied.

Main Outcome Measures: The NB neuronal cell counts in each group and their correlation with the extent of magnetic resonance imaging white matter lesions and Clinical Dementia Rating (CDR) scores closest to death.

Results: No significant loss of neurons was found in SIVD patients compared with age-matched controls in contrast to the AD and mixed groups, who had significant neuronal loss. A significant inverse correlation between NB neurons and CDR scores was found in the AD group but not in the SIVD and mixed groups. The NB cell counts were not correlated with either the extent of white matter lesions or cortical gray matter volume in the SIVD or AD groups.

Conclusions: These findings inveigh against primary loss of cholinergic neurons in SIVD patients but do not rule out the possibility of secondary cholinergic deficits due to disruptions of cholinergic projections to cerebral cortex.


Several randomized, placebo-controlled clinical trials have shown beneficial effects of acetylcholine esterase inhibitors in patients with vascular dementia (VaD). However, cognitive impairment in vascular origin is highly heterogeneous, and the mechanisms underlying the positive effects of acetylcholine esterase inhibitors are not clear. Possible explanations include inclusion of mixed cases of Alzheimer disease (AD) and VaD, disruption of cholinergic projection pathways by ischemic white matter lesions (WMLs), or primary degeneration in the nucleus basalis (NB) of Meynert. Among the 4 cholinergic neuron groups in the forebrain, neurons of NB of Meynert (also known as Ch4+) project to the cerebral cortex through the medial and lateral cholinergic pathways. It is well known that neurons of the NB degenerate and their cholinergic transmissions are interrupted in AD. However, whether there is loss of cholinergic neurons in pure VaD remains unclear.

Subcortical VaD is a subtype of vascular cognitive impairment attributed to ischemic brain injury (eg, infarcts and white matter changes). Subcortical ischemic vascular disease (SIVD) refers to evidence of ischemia in subcortical regions based on magnetic resonance imaging (MRI) or neuropathologic findings without reference to cognitive status. We hypothesized that WMLs can produce retrograde degeneration of NB neurons. In this study, we assessed NB neurons in autopsied patients enrolled in a longitudinal, prospective, multicenter ischemic vascular dementia program project (IVD project, P01-AG12435). We compared the numbers of neurons in patients with pathologically defined SIVD with healthy controls matched by age and educational level, patients with AD, and patients with mixed AD and SIVD. We also correlated NB neuronal loss with the extent of WMLs measured on MRI and Clinical Dementia Rating (CDR) scores.
STUDY PARTICIPANTS

Patients were recruited to participate in a large, multicenter longitudinal study to examine relative contributions of SIVD and AD to cognitive impairment. Patients with SIVD, AD, and mixed SIVD and AD and cognitively normal elderly individuals were enrolled. Cognitively impaired patients or patients with dementia were recruited mainly from university-affiliated memory clinics, whereas healthy controls were recruited from the community. Samples described in this article comprise 191 autopsy cases (included in the December 2010 neuropathology database) drawn from a total of 738 cases, of whom 278 were deceased (autopsy rate, 68.7%). The research project was reviewed and approved by appropriate institutional review boards, and written informed consent was obtained from the study participants or their legal representatives.

Inclusion criteria at the time of enrollment included age older than 55 years, English speaking, cognitively normal, or cognitively impaired (CDR score ≤2) due to either SIVD or AD. SIVD was defined clinically by the presence in proton density MRI of discrete gray matter hyperintensities greater than 2 mm in diameter, which were operationally defined as lacunes. Exclusion criteria included severe dementia (CDR score >2), history of alcohol or substance abuse, history of head trauma with loss of consciousness longer than 15 minutes, severe medical illness, neurologic or psychiatric disorders except dementia, or currently taking medications likely to affect cognitive function. Patients with evidence of cortical infaracts, hemorrhage, or structural brain disease other than atrophy, lacunes, or WMLs were excluded.

Comprehensive clinical information including medical history, physical, and neurologic examination, Mini-Mental State Examination (MMSE) score, and laboratory findings were collected from all participants. Apolipoprotein E (ApoE) genotype was also obtained using a polymerase chain reaction–based assay. Serial neuropsychological tests and quantitative MRI measures were obtained in this prospective, longitudinal study. Although the clinical diagnosis of probable or possible AD or SIVD was made based on published criteria, pathologic diagnoses were used to define comparison groups in this study.

NEUROPSYCHOLOGICAL EVALUATION

All study participants received a standardized battery of neuropsychological tests within 6 months of the MRI. Besides MMSE and CDR for standard clinical assessments of global cognitive function, 4 domain-specific composite scores were calculated: global cognition, verbal memory, nonverbal memory, and executive function. The procedures and validation of these scores have been previously described. The composite scores were calculated and transformed to a measurement scale with a mean of 100 and an SD of 15.

NEUROPATHOLOGIC PROCESSING, DIAGNOSIS, AND QUANTITATIVE ANALYSIS

At the time of autopsy, brains were weighed and fixed in 10% neutral-buffered formalin for at least 2 weeks. Each cerebral hemisphere was sectioned coronally at 5-mm thickness using a rotary slicer and examined for macroscopic lesions. All macroscopic infarcts were measured, photographed, and blocked for microscopic examination. Tissue was obtained from standardized regions in 1 hemisphere according to a standardized protocol. The standard protocol includes sections from both anterior and posterior white matter in addition to combined sections recommended by AD and dementia of Lewy body consortium groups. Each case was reviewed at a consensus neuropathology conference, which included 2 neuropathologists who were masked to the clinical information.

The severity of cerebrovascular ischemic injury was rated using a cerebrovascular parenchymal pathology score (CVDPS) as previously described. Subscores for cystic infarcts, lacunar infarcts, and microinfarcts were created by summing the individual scores across all brain regions and normalizing to a scale of 0 to 100. The 3 subscores were then summed to give a total CVDPS (range, 0-300). Acute strokes near the time of death were noted but were not included in the CVDPS. For neurodegenerative lesions, Braak and Braak (BB) stage, Consortium to Establish a Registry for Alzheimer’s Disease plaque score, and Lewy body scores based on the criteria of McKeith et al were recorded.

PATHOLOGIC DIAGNOSIS

To define and categorize subgroups pathologically, cutoff scores were chosen for CVDPS and BB stage. We used a CVDPS of 20 or higher as the cutoff score for cerebrovascular disease and a BB stage of IV or higher to indicate AD as described in our previous publications. Patients with a CVDPS of 20 or higher and a BB stage less than IV were classified as having SIVD, those with a BB stage of IV or higher and a CVDPS of less than 20 as having AD, and those with a BB stage of IV or higher and a CVDPS of 20 or higher as having mixed AD and SIVD. Patients with intact cognition (CDR score of 0) without significant AD or CVDPS disease were classified as healthy controls.

NB NEURON COUNTS

Serial, 15-µm-thick sections containing NB tissue were stained with cresyl violet and reviewed at a low magnification field as described in our previous publication. One slide from each case was chosen for counting. The total number of magnocellular NB neurons was counted in the Ch4 region containing the site of maximum neuronal density. In addition, nucleolated neurons were identified and counted under 200 magnification. Each section was counted 3 times. Although NB was only available from 1 hemisphere, it is bilaterally symmetric in healthy controls and AD patients. Counts were averaged for the 3 trials and expressed as the total number of neurons or the number of nucleolated neurons per section.

MRI ACQUISITION AND PROCESSING METHODS

Acquisition and segmentation methods for MRI data have been described in previous publications. The MRI variables of interest were computerized measures of volumes of WMLs and cortical gray matter (CGM). The WML volumes were assessed based on semiautomated segmentation using T1-weighted, T2-weighted, and proton density images. The WML volume was defined as hyperintense regions on proton density and T2-weighted MRIs and anatomically located in white matter regions. All volumes were expressed as the percentage of total intracranial volume (ICV).

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was used to compare continuous variables of the demographic characteristics (age, years of education, and duration of illness), total and nucleolated NB neuron counts, and the MMSE and 4 domain-specific composite cognitive scores among the 4 pathologic diagnosis groups (SIVD, AD, mixed, and control groups). The Fisher exact test
was applied for categorical variables (sex, race, presence of stroke risk factors, and ApoE allele). The MRI measures were corrected for ICV and expressed as the percentage of the ICV. Spearman correlation coefficients were used to examine the associations between NB neuron count and MRI measures and the correlations between neuron cell counts and global cognitive function (CDR scores closest to death). Analyses were 2-tailed with significance set at <.05 and were performed with the SPSS statistical software, version 17 (SPSS Inc).

### RESULTS

The number of autopsied cases (n=191) comprised 19 SIVD cases, 81 AD cases, 16 mixed AD and SIVD cases, 44 normal or minimal disease cases (18 with a CDR score of 0 and 26 with a CDR score of ≥0.5), 11 dementia of Lewy body cases, 2 frontotemporal dementia cases, 2 progressive supranuclear palsy cases, and 16 cases pending consensus diagnosis. For this study, we selected 16 SIVD cases with complete neuropsychological, MRI, and NB sections. We then selected comparison groups matched on age and educational level: 20 patients with AD, 10 patients with mixed AD and SIVD, and 17 healthy controls (CDR score of 0). The groups did not significantly differ on duration of illness, sex, or race (Table 1). Whereas a history of stroke was more frequent among SIVD patients (P=.02), the presence of an ApoE4 allele was more frequent in the AD and mixed groups (P=.03).

### COGNITIVE FUNCTION BY PATHOLOGIC DIAGNOSIS

Table 2 gives the mean cognitive scores by pathologic group for all 63 study participants. The MMSE score and all cognitive summary scores (global cognition, verbal memory, executive function, and nonverbal memory) differed significantly among the groups (all P < .001, ANOVA). Mean scores of all cognitive variables were the highest in the control group and the lowest in the mixed group.
Table 3 summarizes the BB stages and CVDPSs for the SIVD cases and details the location of the infarcts. All had infarcts in the basal ganglia, thalamus, or subcortical white matter. However, many of them had cystic and microinfarcts in the cerebral cortex as well. Two cases (Nos. 1 and 14) had large infarctions in the distribution of major arteries.

PATHOLOGIC CHARACTERISTICS OF THE SIVD GROUP

The severity of NB neuronal loss varied considerably among groups (Figure 1). Although the SIVD group showed no NB neuronal loss, both the total and nucleolated cell counts were significantly lower in the AD and mixed groups compared with the control and SIVD groups (P < .001, ANOVA). Among SIVD patients, the mean counts of total and nucleolated cells were similar to those in healthy controls (P = .99 in the post hoc analyses) and significantly higher than those of the AD and mixed groups (P = .001 and P < .001 for the AD group and P = .005 and P = .02 for the mixed group, respectively). The SIVD group showed no morphologic evidence of retrograde degeneration, such as chromatolysis.

NEUROPATHOLOGY OF THE NB

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Table 3. Size and Location of Infarcts in Subcortical Ischemic Vascular Disease Cases

<table>
<thead>
<tr>
<th>Case No.</th>
<th>BB Stage</th>
<th>CVDPS (Range, 0-300)</th>
<th>Cystic (Range, 0-100)</th>
<th>Lacune (Range, 0-100)</th>
<th>Micro (Range, 0-100)</th>
<th>Location of Infarcts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>II-III</td>
<td>29</td>
<td>17</td>
<td>8</td>
<td>4</td>
<td>Thalamus, Frontal, occipital, posterior cerebral artery infarction</td>
</tr>
<tr>
<td>2</td>
<td>III</td>
<td>79</td>
<td>33</td>
<td>17</td>
<td>29</td>
<td>Thalamus, Amygdala, frontal, parietal, occipital</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>180</td>
<td>72</td>
<td>75</td>
<td>33</td>
<td>Putamen, caudate, Frontal, insula</td>
</tr>
<tr>
<td>4</td>
<td>II</td>
<td>89</td>
<td>39</td>
<td>25</td>
<td>25</td>
<td>Caudate, putamen, Frontal, parietal</td>
</tr>
<tr>
<td>5</td>
<td>III</td>
<td>102</td>
<td>6</td>
<td>67</td>
<td>29</td>
<td>Thalamus, putamen, globus pallidus, Occipital, hippocampus</td>
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<tr>
<td>6</td>
<td>0</td>
<td>42</td>
<td>17</td>
<td>0</td>
<td>25</td>
<td>Caudate, white matter, Parietal, temporal, insula</td>
</tr>
<tr>
<td>7</td>
<td>I</td>
<td>39</td>
<td>6</td>
<td>25</td>
<td>8</td>
<td>Caudate, thalamus, white matter, Hippocampus</td>
</tr>
<tr>
<td>8</td>
<td>III</td>
<td>75</td>
<td>0</td>
<td>50</td>
<td>25</td>
<td>Caudate, globus pallidus, frontal white matter, occipital white matter</td>
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<tr>
<td>9</td>
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<tr>
<td>10</td>
<td>II</td>
<td>21</td>
<td>0</td>
<td>8</td>
<td>13</td>
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</tr>
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<td>11</td>
<td>III</td>
<td>58</td>
<td>33</td>
<td>17</td>
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<td>Thalamus, Frontal, occipital</td>
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<tr>
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<td>I</td>
<td>84</td>
<td>17</td>
<td>50</td>
<td>17</td>
<td>Putamen, thalamus, white matter, Parietal, occipital</td>
</tr>
<tr>
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<td>II</td>
<td>44</td>
<td>11</td>
<td>8</td>
<td>25</td>
<td>Caudate, putamen, globus pallidus, thalamus, Frontal</td>
</tr>
<tr>
<td>14</td>
<td>I</td>
<td>133</td>
<td>83</td>
<td>50</td>
<td>0</td>
<td>Caudate, putamen, globus pallidus, thalamus, white matter, Frontal, parietal, middle cerebral artery infarction</td>
</tr>
<tr>
<td>15</td>
<td>III</td>
<td>25</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>Frontal white matter, parietal white matter</td>
</tr>
<tr>
<td>16</td>
<td>III</td>
<td>42</td>
<td>0</td>
<td>42</td>
<td>0</td>
<td>Putamen, white matter</td>
</tr>
</tbody>
</table>

Abbreviations: BB, Braak and Braak; CVDPS, cerebrovascular parenchymal pathology score.
CORRELATIONS OF NB COUNTS WITH STRUCTURAL MRI DATA AND CDR

The number of nucleolated NB neurons was not significantly correlated with either the extent of WMLs ($r = -0.204, P = .48$) or the volume of CGM ($r = 0.147, P = .62$) evaluated in the SIVD group. Similar results were obtained using total NB cell counts. Similarly, in the AD group, no significant correlations were found between NB cell counts and WML ($r = -0.183, P = .44$) or CGM ($r = 0.028, P = .91$). The association between nucleolated NB neuronal loss and CDR scores (closest to death) differed by group (Figure 3). Neuronal loss in NB was significantly correlated with CDR scores in AD (Spearman $r = -0.866, P < .001$) but not in the SIVD ($r = -0.096, P = .77$) or mixed ($r = 0.026, P = .94$) groups.

COMMENT

In this autopsy study, we report preservation of NB neurons in cases of SIVD compared with healthy controls while confirming significant neuronal loss in cases with AD and mixed AD and SIVD. Although all of our cases had subcortical infarcts, many of them also had cystic infarcts and microinfarcts in the neocortex (Table 3). Loss of NB neurons in AD is well established in the literature but has not been well studied in SIVD or mixed AD and SIVD. Our findings are consistent with a previous study of cases with multi-infarct dementia but extend the findings of NB sparing to the SIVD (subcortical) end of the vascular brain injury spectrum.

Only a few studies have examined NB and cholinergic projections in subcortical VaD, and the status of the network as a whole remains unsettled. Whereas a single case report of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) showed pathologically intact NB neurons, another report of 9 patients with CADASIL and 14 age-matched controls showed cholinergic neuronal deficits in cerebral cortex with possible retrograde degeneration of NB. In a study of 6 patients with sporadic Binswanger syndrome, a discrepancy was noted be-
the status of cholinergic projections to cerebral cortex in patients with severe white matter disease (CADASIL or Binswanger syndrome). Because of limitations imposed by tissue fixation, we were unable to assess the status of cholinergic projections to cerebral cortex. However, unlike previous studies, we obtained MRI measures of WMLs, with volumes reaching an ICV of up to 11%. We hypothesized that severity of WMLs would correlate with retrograde degeneration of NB in patients with SIVD, whereas severity of cortical atrophy would correlate with the number of NB neurons in patients with AD. Contrary to our expectation, cell counts of NB in the SIVD group were not correlated with either volume of WMLs or CGM. The absence of a correlation casts doubt on the hypothesis that WMLs cause retrograde degeneration of NB. However, our conclusions should be tempered by the limited sample size and the possibility that retrograde changes might still occur when WMLs reach an extreme threshold, such as in CADASIL.

Also, contrary to our hypothesis, no correlations were found in the AD group between NB cell counts and CGM. Cullen et al reported that the number of NB (Ch4) neurons in AD patients was coupled to the volume of CGM. They conducted the study using pathologic specimens to measure CGM volume, whereas we used MRI segmentation methods to measure CGM volume. In addition, the range used to define the AD group (BB stage ≥IV) and the small number of participants in our study group may also be limiting factors.

The association between number of NB neurons and severity of dementia differed by group. Whereas the SIVD and mixed groups showed no correlation, the CDR scores were inversely correlated with the number of NB neurons in the AD group. In our study, the comparison groups were defined by severity of disease pathology (not by CDR scores). Correlations between NB number and CDR score were not shown in a previous study of Cullen et al. in which the AD patients all had advanced dementia (CDR scores of 4 and 5). A broad range of CDR scores in the AD group was more evenly distributed in the present study (CDR score of 0 in 2 patients, CDR score of 0.5 in 2 patients, CDR score of 1 in 4 patients, CDR score of 2 in 6 patients, and CDR score of ≥3 in 6 patients). Iraizoz et al. also reported correlations between NB cell counts and clinical dementia measures, in which stages ranged from stage 1 to III using their own rating system. Because we found no evidence of NB loss in SIVD, the absence of correlation with CDR in SIVD is not surprising. There may not have been sufficient power to detect similar expected correlations between NB and CDR in the mixed AD and SIVD group, where severity of dementia (CDR) is determined by a combination of AD (where NB loss is expected) and SIVD (where no NB loss occurs).

The present study has several major strengths. The participants in this study were all enrolled in a standardized longitudinal study. All were evaluated under a single neuropathology protocol that involved extensive evaluation and rating of vascular and neurodegenerative lesions at a consensus case conference. In addition, all clinical, radiologic, neuropsychological, and pathologic data were collected prospectively under a single protocol. To our knowledge, no previous studies have included volumetric MRI measures in concert with clinicopathologic correlations of NB in SIVD.

Several limitations in the sample and method of counting neurons should also be mentioned. The study material came from a convenience rather than population-based sample, so the 4 groups in this study may not be representative. The comparison groups were defined by cutoff scores, which are often used in the literature but are to some extent arbitrary. Because the number of cases in each group was relatively small (it is difficult to find pure cases of VaD in autopsy studies from AD centers), replication in a larger sample is still warranted. In addition, we used representative sections of NB rather than an unbiased stereologic method for counting neurons. Our method was simi-
lar to some other studies, and a meta-analysis stated that studies that used a nonstereologic method showed similar results compared with those that used a stereologic method. We report numbers of total neurons and numbers of nucleolated neurons in the complete medial to lateral extent of the nucleus basalis. Thus, our estimates of the number of neurons would not be affected by atrophy of the NB in the coronal plane. Our method, however, would not be sensitive to neuronal loss should atrophy occur selectively in the sagittal plane.

In this study, the neurons of NB in SIVD were preserved irrespective of severity of cognitive deterioration and extent of MRI-defined WMLs, whereas definite neuronal degeneration that strongly correlated with CDR scores was observed in AD patients. These findings confirm the absence of primary degeneration of NB in SIVD. Our study is consistent with the possibility that the response to acetylcholine esterase inhibitors in VaD reflect a mixed AD condition. Our study does not rule out the possibility of distal cholinergic deficiency due to discrete lesions in cholinergic pathways, without primary neuronal degeneration of NB.

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REFERENCES


Correction

Error in Byline. In the article titled “Heterogeneity of Coenzyme Q$_{10}$ Deficiency: Patient Study and Literature Review,” published in the online April 9, 2012, issue of the Archives (doi:10.1001/archneurol.2012.206), there was an error in the byline. Dr López’s name should have been listed as Luis C. López instead of Luis López. This article was corrected online.