Cerebral Cortical Arteriolar Angiopathy, Vascular Beta-Amyloid, Smooth Muscle Actin, Braak Stage, and APOE Genotype

Edward G. Stopa, MD; Parag Butala, MD; Stephen Salloway, MD; Conrad E. Johanson, PhD; Liliana Gonzalez, PhD; Rosemarie Tavares, BSc; Virginia Hovanesian, BSc; Christine M. Hulette, MD; Michael P. Vitek, PhD; Ronald A. Cohen, PhD

Background and Purpose—We examined the associations among the vascular β-amyloid levels, smooth muscle actin, wall thickness, and lumen diameter to achieve greater understanding of the arteriolar changes that accompany Alzheimer disease (AD).

Methods—Post-mortem pathology brain specimens from 76 patients with AD and 19 non-AD age control subjects were studied. We analyzed arterioles of the frontal cortex (Brodmann area 10) by immunohistochemistry and morphometry, and derived measures of vascular β-amyloid level, smooth muscle actin (SMA) volume, and arteriolar wall thickness and lumen diameter. APOE genotype was determined for each case.

Results—Overall, there was a striking reciprocal relationship between arteriolar β-amyloid volume and smooth muscle actin (P<0.0001). In addition, there was a strong positive association between progressively accumulating vascular β-amyloid and augmentations in both wall thickness (P<0.0001) and lumen width (P<0.0001). In comparison with non-AD control subjects, smooth muscle actin was decreased in patients clinically diagnosed with AD and was reduced >10-fold in cases with AD pathology (Braak I to VI) compared with those lacking AD neuropathology. Significantly altered composition and structure of cortical vessels in pre-Braak stages corroborated our hypothesis that arterioles are devastated early in the AD pathological process. Smooth muscle actin, arteriolar wall thickness, and luminal diameter did not vary with Braak stage severity (P>0.05), indicating that substantial arteriolar damage may precede at least some of the interstitial plaques and neuronal tangles. Moreover, the structural and biochemical arteriolar abnormalities did not vary as a function of APOE genotype (P>0.05).

Conclusion—We postulate that in elderly patients, the continually progressing β-amyloid-associated angiopathy, at the arteriolar level, harms the contractile apparatus and cerebral blood flow autoregulation, thereby making the downstream capillaries vulnerable to damage. Collectively, our observations lend further support to the idea that microvascular damage has a role, perhaps relatively early, in the onset of major AD pathology. (Stroke. 2008;39:814-821.)

Key Words: Alzheimer disease • APOE • arteriolar thickness • beta-amyloid • Braak stage • frontal cortex • smooth muscle actin (SMA)

The role of vascular risk factors in the etiology of Alzheimer disease (AD) has been the subject of increased interest and debate over the past decade.1–10 Although stroke has been recognized as a risk factor in epidemiological studies of AD, cerebrovascular disease has generally not been viewed as a primary determinant of AD pathology. Abnormalities related to β-amyloid11–15 and the protein Tau16–20 in the brain have been the subject of much greater focus, and these factors are thought to play a more central role in AD.21–27 Yet, there continues to be support from various lines of clinical research for a link between AD and cerebral microvascular disease as well.2,6,8,28–36 Cerebrovascular abnormalities associated with AD have received only limited neuropathological consideration. Past investigations have shown increases in luminal diameter and wall thickness of the large cerebral arteries of patients with AD at autopsy.37 Smooth muscle actin (SMA) is also decreased in AD with these vascular alterations associated with increases in β-amyloid levels in arterial walls.38 Furthermore, there are also now data showing abnormalities of cerebral capillary function and structure in AD that also relate to β-amyloid levels in these capillaries.39–41 These findings suggest that...
cerebrovascular morphometry and function are compromised relatively early, although how AD pathology influences or is affected by these changes is not entirely clear.

An intriguing commonality between AD and vascular disease emerges when one considers the APOE. The APOE 4,4 genotype is known to be strongly associated with AD risk, pointing to a genetic basis for this disorder. Yet, at a cellular level, APOE plays a major role in lipid function and specifically the metabolism of cholesterol. Given the well-recognized role of lipids, including cholesterol, in vascular disease, it seems reasonable to question whether variations in metabolic function related to APOE share common influences in AD as well. Recent studies have demonstrated abnormalities of cholesterol metabolism, lending support to this possibility. However, relatively little data exist on the interrelationship among APOE genotype, cerebrovascular pathology, and specific AD factors.

One possible explanation for these cerebrovascular abnormalities in AD is that amyloid deposition has toxic effects on cytoskeletal/actin integrity in the vasculature. As actin expression diminishes, the atrophying smooth muscle is replaced with scar tissue, which thickens the wall. Reductions in contractile function (as evidenced by reduced SMA on staining) leads to reduced vascular tone and arteriolar dilation. This could impair cerebral blood flow autoregulation with consequent downstream damage to capillaries.

In previous studies, we found a relationship between agrin immunoreactivity and the structural integrity of the capillary basement membrane of AD. The APOE 4,4 genotype was associated with greater loss of agrin, suggesting that this genotype influences microvascular changes in AD that may contribute to compromise of the blood–brain barrier. This finding raises the possibility that APOE genotype influences microvascular angiopathy associated with AD and perhaps microvascular function. Yet, microvascular autoregulation is strongly influenced by arteriolar function, so the functional significance of these microvascular abnormalities cannot be fully determined by examining capillary morphometry. Cerebral arteriolar function plays a potentially important role, because arterioles provide a gating mechanism for perfusion of the cerebral capillaries. Yet, to date, there is little known about cerebral arteriolar disturbances associated with AD, which motivated the current study.

We measured cerebral arteriolar SMA area, arteriolar wall thickness, and luminal diameter from multiple brain sections of patients who had died with AD and compared them with control subjects with systemic disease to provide measures of cerebrovascular abnormality. These indices were examined in relation to APOE genotype and AD markers, including levels of β-amyloid in the arteriolar wall and the Braak stage. In view of the potential contribution of vascular factors to AD, we hypothesized that patients with AD would differ with respect to SMA area, arteriolar wall thickness, and luminal diameter as a function of APOE genotype. Moreover, we postulated that β-amyloid levels would also relate to these vascular indices but that this relationship would be more dependent on overall severity of AD pathology.

Clinical Sample
All brains were obtained within 2 to 24 hours of death. Neuropathology data were derived from 2 sources. Seventy-six patients donated their brains to the Kathleen Price Bryan Brain Bank and Alzheimer’s Disease Research Center at Duke University Medical Center. Another 19 patients donated their brains to the Rhode Island Hospital Brain Bank of Brown Medical School.

Subjects enrolled in the Joseph and Kathleen Price Alzheimer Disease Research Program, in existence since 1985, were assessed prospectively and followed until death according to protocols approved by the Duke University Medical Center Institutional Review Board. At the time of death, brain autopsy was performed and tissue evaluated neuropathologically according to standard protocols. Tissue, blocks, and slides were banked.

Demographic, clinical, and neuropathological data were stored according to established protocols. As previously reported, patients with AD died as a result of pneumonia, urinary tract infection, or cardiac arrest. Cause of death of patients with AD was confirmed at general autopsy for 40 patients. Cause of death was extrapolated from clinical records for the remaining patients with AD. Duke cases were supplied as 5-μm paraffin-embedded sections of the frontal cortex (Brodmann area 10).

Control brain neuropathology data were derived from 19 cases from the Rhode Island Hospital (RIH) Brain Bank. Causes of death for the RIH control subjects included sickle cell anemia, cardiac arrest, acute myocardial infarction, acute respiratory distress syndrome, ventricular tachycardia, and diverticulitis, all based on autopsy. No control patient had clinical evidence of cognitive or functional impairments that met criteria for dementia before death. SMA and arteriolar β-amyloid were the only measures obtained for the control subjects.

Diagnosis of AD was made in accordance with accepted National Institute of Aging–Reagan criteria. The total sample included 47 patients who had a diagnosis of probable AD, whereas the remainder did not have dementia at the time of death. The diagnosis of AD was made on the basis of clinical evidence of dementia as well as a Braak stage >II. Accordingly, there were cases with AD pathology based on Braak stage that did not have clinical symptoms of dementia (n = 29). Data presented in tables and text varied in sample size based on data availability and whether the analysis was for the total sample based on Braak stage or based on AD clinical diagnosis. AD staging was determined with the criteria of Braak and Braak for all cases. APOE genotype was determined by previously established methods. To test hypotheses regarding differences in vascular and AD pathology as a function of APOE, we examined the distribution of patients in each APOE genotype to determine whether it reflected the reported distribution of APOE in AD (see Table 1).

Vascular Measures
Immunofluorescent double labeling was done using the RIH immunohistochemistry laboratory protocol. Slides were double-labeled for SMA and β-amyloid. Monoclonal mouse anti-human SMA antibody (Sigma-Genosys, The Woodlands, Texas) was used for labeling SMA, and a polyclonal rabbit anti-human antibody (DAKO Corp., Carpinteria, Calif) was used for amyloid.

A minimum of 10 arterioles was imaged for each case. The Brodmann 10 locations analyzed were randomly chosen with images taken of both the SMA and amyloid labeling for each site. Because arterioles are defined as vessels with diameter between 10 and 50 μm, we used this range of diameters for characterizing arterioles in our analyses. A total of 925 arterioles of patients with AD was imaged. At least 10 arterioles were imaged for each control case. Images of arterioles were acquired for computer analysis with the RIH Digital Image Laboratory Nikon E800 microscope (Nikon Inc., Melville, NY) having a Spot II digital camera (Diagnostic Instruments, Sterling Heights, Mich) and a 60× PlanApo objective lens.

Arterial analysis was conducted using Scion Image for Windows, version beta 4.0.2 (Scion Corp). Arteriolar images were calibrated with a stage micrometer. Because a random cross-section...
of an arteriole is elliptical and does not reveal the true diameter but overestimates it, the diameter was taken as the minimum axis of the ellipse, which is the arteriolar minor axis. Wall thickness of the medial layer was determined by measuring the external minor axis and the arteriolar minor axis and then taking half of the difference.52,53

The area (in microns squared) of SMA and amyloid staining was measured by intensity thresholding the staining of the filtered image at a user-defined level and converting the image to binary for area calculation.54 Filtering was performed to remove electronic noise in the image. Furthermore, one user made all calculations to ensure consistency. Although no predetermined threshold end point was selected due to variations in arteriolar staining, no statistically significant difference was found (P > 0.05) in test measurements to determine user consistency. All measurements were performed blind to APOE genotype and Braak stage.

### Statistical Analysis

As discussed previously in the description of the clinical sample, analyses were conducted based on both the clinical history of dementia and also according to the Braak stage of AD pathology. Descriptive statistics were calculated for all variables of interest. For analyses involving comparisons of the clinical and pathology groupings with control subjects, age was entered as a covariate. For analyses involving comparisons of the clinical and pathology groupings with control subjects, age was entered as a covariate. A log transformation was performed on the amyloid data to adjust for the nonnormal nature of the distribution of values. Regression analyses, analyses of variance, and analyses of covariance were performed to examine the relationships among Braak stage, APOE genotype, SMA area, arteriolar wall thickness, and luminal diameter and arteriolar amyloid levels.

Between-group comparisons were conducted using multivariate analyses of covariance. Arteriolar amyloid levels, SMA immunoreactivity, arteriolar wall thickness and luminal diameter, APOE genotype, Braak stage, and clinical diagnosis (AD versus non-AD) were analyzed. The Braak stage analyses were conducted initially with all participants included and then with only those participants who showed evidence of AD pathology (Braak I to VI).

### Results

#### Clinical Characteristics

Of the 76 Duke University neuropathology cases with a Braak stage > 0, most at Braak stage I or II (n=23) did not meet criteria for diagnosis of dementia. In contrast, the majority of patients (only 4 of 4 of the cognitively normal Duke control subjects) had a Braak stage III. All of the Brown Medical School control subjects (n=19) had a Braak stage of 0. A majority of cases in the sample had a clinical diagnosis of probable AD (n=47), whereas the remainder did not have a clinical diagnosis of AD at death.

The mean age of the total sample was 76.7±9.7 years (Duke+RIH). The RIH control sample was younger than the Duke University sample (Duke: 77.9±9.04 years; control subjects: 69.5±10.7 years; P<0.05). Because of this difference, age was treated as a covariate in analyses comparing the RIH control subjects with the Duke patients who had plaque and tangle neuropathology. The sample consisted of 38 males and 57 females. There was not a significant difference in age between men and women in the sample nor did men and women differ in distribution of APOE, vascular β-amyloid, or other vascular indices.

For the Duke cases with plaque and tangle neuropathology (ie, Braak > 0), there was not a significant difference in the number of cases at each Braak stage (Stage I to II=23; Stage III to IV=28; Stage V to VI=25). There was not a significant difference in age among patients by Braak stage. There was a difference in gender as a function of Braak stage (P=0.006), because Braak stage I to II had a greater percentage of men than expected, whereas Braak stages III to IV and V to VI had a higher of percentage of females than expected. The gender effect was also present when RIH control subjects were included in the analysis (P=0.01).

#### Arteriolar Measurements

SMA area varied as a function of age, because older patients had smaller SMA areas (r = -0.32, P<0.01). Therefore, age was entered as a covariate in analyses of SMA for the various between-group comparisons. There were no significant associations found between arteriolar wall thickness or luminal diameter and age (P>0.10).

Arteriolar wall thickness and luminal diameter were strongly correlated (r = -0.65, P<0.001). SMA area was not significantly associated with arteriolar wall thickness but approached a significant negative relationship with arteriolar luminal diameter (r = -0.22, P=0.06). Descriptive data regarding vascular characteristics of the sample as a function of AD diagnosis and Braak stage are provided in Table 2.

To test our hypothesis that accumulating β-amyloid in the arteriolar wall results in atrophy of the smooth muscle contractile apparatus, we pooled non-AD and AD data to ascertain if there was an inverse relationship between SMA and vascular β-amyloid. This postulate was strengthened by the finding of a highly significant (P<0.0001) negative correlation (r = -0.631) between the depleted SMA (dependent variable) and retained β-amyloid (Figure 1).

#### Vascular Characteristics and Alzheimer Disease Diagnosis

An overall difference in vascular findings occurred between patients with a clinical diagnosis of probable AD compared with patients without AD at time of death when, on the multivariate analyses of covariance, SMA, luminal diameter,
and arteriolar wall thickness were simultaneously entered as dependent variables with age treated as a covariate (P<0.01). The largest difference between patients with AD and non-AD patients was evident with respect to SMA (P<0.0001) after correcting for age. SMA decreased as a function of age (P=0.003). As shown in Figure 2, the magnitude of this difference was considerable, because the patients with AD exhibited a greater than 10-fold reduction in SMA compared with the control subjects.

Arteriolar wall thickness also varied as a function of AD diagnosis. Patients with AD had greater wall thickness than patients without an AD diagnosis (P<0.001). Arteriolar luminal diameter did not differ significantly between patients with AD and non-AD patients.

**Vascular Findings by Braak Stage**

A significant difference was observed in SMA area between the AD neuropathology group (Braak I to VI) and the control subjects (Braak=0), because patients with AD pathology had much smaller SMA areas than the control subjects (P<0.0001). Excluding the control subjects from the analysis indicated that SMA area did not differ as a function of Braak stage (P>0.30). Therefore, although patients with AD pathology at any Braak stage had reduced SMA areas compared with patients without any AD pathology, SMA area did not decrease as a function of AD disease severity as defined by Braak stage.

There were not significant differences in arteriolar luminal diameter (P>0.20) or wall thickness (P>0.20) among patients across the 3 Braak stages, suggesting that these vascular findings occur independent of the severity of other AD pathology that comprise the Braak staging. Effect sizes and power for these tests are given in Table 3.

Analyses comparing patients as a function of Braak stage and both gender and age also failed to reveal significant effects, suggesting that SMA area, arteriolar luminal diameter, and wall thickness do not vary as a function of interactions among age, gender, and Braak stage.

**APOE Genotype**

The 3 APOE genotype groups did not differ significantly in sample size (APOE 3,3=42.7%, APOE 3,4=26.7%; APOE 4,4=30.6%). Furthermore, the proportion of the APOE-epsilon 4 genotypes in the sample (57.3%) did not differ significantly from previously reported frequencies (50%, Z=1.2124, P>0.20) of these genotypes in AD populations, including the Duke University cohort, based on a Z-test for comparison of proportions. APOE status was not available for one participant. There were no significant differences with respect to age and gender. Chi square analysis did not reveal a significant difference in the frequencies of the Braak stages as function of APOE genotype (see Table 1). None of the vascular measures (SMA, arteriolar luminal diameter, or arteriolar wall thickness) differed significantly as a function of APOE genotype. Furthermore, neither absolute nor log-transformed vascular β-amyloid levels differed as a function of APOE genotype.

**Table 1. Cerebral Arteriolar Morphometry and β-Amyloid Findings**

<table>
<thead>
<tr>
<th></th>
<th>AD Clinical Diagnosis</th>
<th>Non-AD Diagnosis</th>
<th>Total Duke Sample</th>
<th>Control Subjects (no AD pathology)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA area, μm²</td>
<td>8.72 (13.03)</td>
<td>46.11 (53.82)</td>
<td>9.55 (12.18)</td>
<td>104.56 (46.46)</td>
</tr>
<tr>
<td>Luminal diameter, μm</td>
<td>27.81 (6.17)</td>
<td>25.29 (4.38)</td>
<td>27.08 (5.79)</td>
<td>N/A</td>
</tr>
<tr>
<td>Wall thickness, μm</td>
<td>4.26 (1.42)</td>
<td>3.88 (0.87)</td>
<td>4.15 (1.29)</td>
<td>N/A</td>
</tr>
<tr>
<td>β-amyloid, μm²</td>
<td>19.62 (26.33)</td>
<td>9.04 (25.65)</td>
<td>16.51 (26.41)</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

Note: Luminal diameter was not determined for the RIH control subjects (patients without AD pathology). Means for SMA among the non-AD patients includes both patients without a diagnosis of AD from the Duke sample and the control subjects from the RIH sample. Means for luminal diameter, wall thickness, and β-amyloid for non-AD patients in the table are for patients from the Duke sample only. β-amyloid was below detectable limits for all controls. Numbers in parentheses are SDs.

N/A indicates not available.
Arteriolar Beta-Amyloid and Alzheimer Disease Pathology

Levels of the log of β-amyloid in the arteriolar wall varied as a function of AD diagnosis (P<0.01). Patients with a clinical AD diagnosis at time of death had much higher levels of β-amyloid compared with those without such a diagnosis. Only trace levels of arteriolar β-amyloid were found in patients without AD pathology. Among patients with AD pathology (Braak >0), significant differences in the log of arteriolar β-amyloid levels were evident as a function Braak severity (P=0.01). As AD severity increased as measured by Braak stage, so did the quantity of β-amyloid found in the arteriolar wall.

Relationships Among the Vascular Indices and Alzheimer Disease Status

The relationships among the AD variables and the 3 vascular indices were examined in separate hierarchical regression analyses in which age was entered as the first independent variable in step one followed by log-transformed β-amyloid level, Braak stage, and APOE genotype.

Only cases with AD pathology (Braak I to VI) were considered when examining the relationship among the 3 vascular indices and arteriolar β-amyloid levels. The log-transformed arteriolar β-amyloid levels were significantly associated with arteriolar wall thickness (r=0.54, P<0.0001) and with arteriolar luminal diameter (r=0.47, P<0.0001). When the 3 vascular indices were entered into a regression analysis as independent variables with log-transformed β-amyloid level as the dependent variable, a strong association was again found (P<0.0001). Arteriolar wall thickness emerged as the strongest correlate of vascular β-amyloid levels (P<0.01), although arteriolar luminal diameter also contributed to this association (P<0.05). SMA area was not retained as a significant correlate of vascular β-amyloid level (P>0.20). Neither APOE genotype nor Braak stage was retained as significant correlates of any of the 3 vascular indices in these analyses of covariance (P>0.20).

Discussion

The current findings for frontal cortex arterioles provide insight on the association among the vascular β-amyloid level, wall thickness, lumen width, and the APOE genotype of AD and non-AD subjects. Significant loss of arteriolar SMA was found in clinically diagnosed patients with AD. SMA was also dramatically reduced in patients with AD (Braak >0) compared with those lacking AD pathology. The attenuated SMA was substantial (>10-fold), suggesting significant pathology. Along with reduced SMA were increases in arteriolar wall width and luminal diameter in patients with significant AD pathology versus those without AD and also in subjects with clinical AD compared with nondemented patients. This altered structure of cerebral arterioles fits our a priori hypotheses and implicates dysfunction at this vascular level.

Abnormalities in AD occur across large arteries, arterioles, and capillaries. Previous studies demonstrated altered cerebral capillary structure and function in AD.37–40,55–57 Disturbed capillary function compromises blood flow to neurons. Normal arteriolar function is essential for healthy cerebral perfusion. Arterioles engage in hemodynamic gating, ensuring that blood volume and pressure to capillaries are adequate. On the other hand, arteriolar dysfunction may cause excessive mechanical pressure on capillaries. Thus, arteriolar

Table 3. Power on the Dissociation Between Severity of AD Neuropathology as Measured by Braak Stages and Indices of Vascular Abnormality

<table>
<thead>
<tr>
<th>Arteriolar Indices</th>
<th>F &lt;sub&gt;2,72&lt;/sub&gt;</th>
<th>P Value</th>
<th>Effect Size</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA</td>
<td>0.509</td>
<td>0.603</td>
<td>0.014</td>
<td>0.131</td>
</tr>
<tr>
<td>Luminal diameter</td>
<td>1.487</td>
<td>0.233</td>
<td>0.040</td>
<td>0.307</td>
</tr>
<tr>
<td>Wall thickness</td>
<td>1.350</td>
<td>0.266</td>
<td>0.036</td>
<td>0.282</td>
</tr>
</tbody>
</table>

Note: The F-values in the first column are the test statistics values and the second column gives the significance level associated with these values. The effect size indicates the proportion of total variability of the vascular index attributable to Braak stages. Lastly, the power of the tests is calculated using noncentral F-distributions and computations assuming P=0.05.
loss of actin and accompanying vasodilation presumably compromises blood flow autoregulation and probably leads to a greater degree of arterial pressure transmittal to downstream capillary beds. These factors would predispose to microvessel damage.

In the current study, the primary abnormality in aging and AD was a substantially reduced SMA volume (Figures 1 and 2) that almost certainly alters vasoreactivity when contractile elements are obliterated. Another arteriolar finding was augmented luminal diameter in AD. Such dilation seems counterintuitive if one considers the reduced cerebral perfusion in AD. Yet, when viewed hemodynamically, a greater luminal diameter in AD likely reflects the breakdown of arteriole wall structure and less ability to respond adaptively to changes in perfusion. Such arteriolar luminal expansion likely manifests severely disrupted contractile mechanisms that normally regulate cerebrovascular resistance.

Another noteworthy finding was a striking dissociation between severity of AD neuropathology (Braak stage) and arteriolar β-amyloid levels in the relationship to vascular structural abnormalities evident on neuropathology. Vascular β-amyloid levels were strongly associated with the arteriolar indices among the AD brains. In contrast, the 3 vascular indices did not differ with Braak stage, suggesting that with progressive worsening of AD, there was not a concomitant exacerbation of arteriolar pathology.

Other findings highlight the significance of the dissociation between arteriolar structure and Braak stage advancement. Patients with AD pathology (ie, Braak stages I to VI) differed from individuals without established AD pathology in respect to SMA volume, wall thickness, and lumen size. When considered together with the lack of vascular structural differences across Braak stages, these observations suggest a linkage between vascular neuropathology and AD. This relationship between angiopathy and AD seems to occur independently of disease progression once the patient has been identified as having AD. In summary, vascular structural changes occur early in AD but yet do not worsen with the increasing number of neurofibrillary tangles and other findings that constitute Braak staging. Although clinical AD is accompanied by cerebrovascular changes, particularly in arterioles, the fact that these vascular changes (enhanced luminal diameter and wall thickness) do not worsen with Braak stage, whereas vessel amyloid levels continue to increase, provides support for the idea that vascular structural alterations contributes early to AD pathogenesis. Thus, near-maximal damage to the arteriole may occur before the appearance of plaques and tangles.16,17

When vascular β-amyloid levels, Braak stage, and APOE genotypes are simultaneously examined in relationship to the vascular structural indices, only arteriolar β-amyloid emerges as a significant correlate of vascular abnormality. That the amount of vascular β-amyloid was strongly associated with both arteriolar wall thickness and luminal diameter in patients with AD is noteworthy and supports a link between vascular amyloid and altered arteriolar integrity.56–58 This relationship also suggests that the vascular abnormalities found in patients with AD (Braak stages I to VI) were not entirely independent of amyloid deposition, which is generally presumed to be a critical AD-associated abnormality.11–15 Although a causal relationship between these factors cannot now be established, a pathophysiological relationship between vascular amyloid deposition and cerebrovascular integrity is suggested. Our findings intimate that either vascular amyloid is contributing to alterations in blood vessel structure and/or, conversely, that vascular dysfunction (manifested by greater arteriolar wall thickness and luminal diameter) contributes to the accumulation of vascular amyloid.

The neuropathological events underlying angiopathy in AD have been widely studied. Cerebral amyloid angiopathy (CAA) results from the deposition of various β-pleated sheet amyloid proteins in the walls of arteries and arterioles and, less commonly, veins and capillaries.55 Most CAA is sporadic, resulting from β-amyloid deposits in the cerebral vasculature of cognitively normal elderly individuals as well as in those with pathologic evidence of AD. Several rare familial forms of CAA, however, are characterized by the deposition of distinct forms of β-amyloid and non-β-amyloid proteins.55

A link between CAA and AD neuritic plaque/neurofibrillary tangle pathology has been debated. β-amyloid deposition in capillaries (dyshoric angiopathy) has long been recognized, but the effects of β-amyloid on capillary structure and function in the aging brain need elucidation.58 Recent studies point to a role for capillary β-amyloid deposition in AD.57,58 Distinct types of sporadic CAA have been identified. In type I CAA, β-amyloid deposited on cortical capillaries, whereas in type II CAA, it did not. Curiously, the APOE4 allele, a known risk factor for AD, is >4 times more common in type I CAA than in type II.57 In another study, only capillary β-amyloid deposition correlated significantly with recognized Consortium to Establish a Registry for Alzheimer’s Disease, Braak, and National Institute on Aging-Reagan Institute criteria for AD.58 The current data lend additional support for an important role of microvascular injury in AD pathogenesis.

Arteriolar amyloid levels did not vary as a function of APOE genotype. Furthermore, the vascular indices (SMA, arteriolar luminal diameter, and wall thickness) did not differ by APOE genotype. Such findings indicate that the relationship between APOE genotype and arteriolar impairments in AD is indirect but may be mediated by vascular amyloid. This provides support for the idea that vascular amyloid has a role in the vascular dysfunction accompanying AD. The lack of an association between APOE genotype and arteriolar wall thickness and luminal diameter differs from the strong relationship previously observed between APOE and capillary agrin.40 Reasons for this differential relationship between vascular indices and APOE, in arterioles versus capillaries, need to be clarified. Whether APOE has different influences depending on the type of cerebral arteriole warrants also further investigation.

The fact that the arteriolar effects, both structural (wall and lumen) and biochemical (↑ β-amyloid and ↓ SMA), occurred at the earliest stages of AD pathology suggests that primary vascular abnormalities reflect processes that precede other aspects of cortical AD pathology (plaques and tangles). Although the arteriolar structural damage may be nearly
complete before the onset of major interstitial and neuronal degeneration, the “downstream” capillary damage may continue to worsen during the later Braak stages. Alternatively, these vascular findings may not reveal progression that mirrors the overall disease advancement because they follow a less intensive course and are eventually overwhelmed by an avalanche of cortical neuronal abnormalities as AD burgeons. Taken as a whole, recent findings, including those in the current study, suggest complex relationships among vascular amyloid, cerebrovascular integrity, APOE genotype, and progression of AD as reflected by Braak stage. In the elderly, the marked attenuation of SMA in arterioles (which may be the consequence of vascular β-amyloid toxicity) could be a pivotal event that leads to disruptions in the optimal perfusion of capillaries and solute transport at the blood–brain barrier; such altered homeostasis at the downstream neurovascular unit would have dire effects on cortical neuronal networks. Although it appears that APOE genotype does not directly account for breakdown of arteriolar integrity in AD, the extent to which cerebrovascular disease (including capillary damage) is influenced by the APOE 3 and 4 alleles needs to be examined in future investigations.

Acknowledgments

E.G.S., P.B., and R.A.C. contributed equally to this study.

Sources of Funding

This study was supported by funding provided by the National Institute of Aging RO1 AG01797 (to R.A.C.) at Brown University, by the National Institute of Health support (to C.M.H.) for the Duke University Brain Bank, and by the National Institute of Aging RO1 AG027910 (to C.E.J.) to study blood–brain barrier function. This study was supported by funding provided by the National Institute of Aging RO1 AG01797 (to R.A.C.) at Brown University, by the National Institute of Aging RO1 AG027910 (to C.E.J.) to study blood–brain barrier function.

Disclosures

None.

References


Cerebral Cortical Arteriolar Angiopathy, Vascular Beta-Amyloid, Smooth Muscle Actin, Braak Stage, and APOE Genotype

Edward G. Stopa, Parag Butala, Stephen Salloway, Conrad E. Johanson, Liliana Gonzalez, Rosemarie Tavares, Virginia Hovanesian, Christine M. Hulette, Michael P. Vitek and Ronald A. Cohen

Stroke. published online February 7, 2008;

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://stroke.ahajournals.org/content/early/2008/02/07/STROKEAHA.107.493429.citation