Subacute Ischemic Stroke Is Associated With Focal $^{11}$C PiB Positron Emission Tomography Retention But Not With Global Neocortical Aβ Deposition

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Background and Purpose—Conflicting evidence exists as to whether focal cerebral ischemia contributes to cerebral amyloid deposition. We aimed to look at Aβ deposits, detected by N-methyl-2-(4'-methylaminophenyl)-6-hydroxybenzothiazole (PiB) positron emission tomography, in patients with recent ischemic stroke. Specifically, we hypothesized that patients with recent ischemic stroke have higher local and neocortical PiB positron emission tomography retention and that this may be associated with major vascular risk factors.

Methods—Ischemic stroke patients were studied using PiB positron emission tomography within 30 days and compared to age-matched controls. Distribution volume ratio maps were created using Logan graphical analysis with the cerebellar cortex as a reference.

Results—Among the 21 ischemic stroke patients (median age, 76 years; interquartile range, 68–77), the ipsilateral peri-infarct region PiB retention was higher compared to the contralateral mirror region, with a PiB distribution volume ratio difference of 0.29 (95% CI, 0.2–0.44; $P<0.001$) at median 10 (interquartile range, 7–14) days after stroke. Two patients also had higher PiB retention within the infarct compared to the contralateral side. There was no difference in the neocortical PiB retention elsewhere in the brain among ischemic stroke patients compared with 22 age-matched normal controls ($P=0.22$). Among the risk factors in the ischemic stroke patients, diabetes was associated with a higher neocortical PiB retention (Spearman Rho=0.48; 95% CI, 0.28–0.72).

Conclusions—PiB retention was higher in the peri-infarct region among patients with recent ischemic stroke. This did not translate into a higher global neocortical PiB retention except possibly in patients with diabetes. The cause of the focal PiB retention is uncertain and requires further investigation. (Stroke. 2012;43:1341-1346.)

Key Words: amyloid ■ beta amyloid ■ brain imaging ■ cerebral amyloid ■ cognitive impairment ■ diabetes ■ ischemic stroke ■ positron emission tomography

Stroke has an enormous impact on individual patient and public health as a leading cause of death and permanent disability. Significant cognitive impairment is frequently observed after stroke and increases the probability of long-term disability and mortality. Although the relationship between Alzheimer disease (AD) and stroke with cognitive impairment is well-established, the mechanism remains complex and controversial. Recent animal and human studies assessing the association between cerebral amyloid and brain ischemia also have been conflicting.1-4

N-methyl-[11C]2-(4'-methylaminophenyl)-6-hydroxybenzothiazole (11C-PiB) is a derivative of thioflavin-T and has been developed as a ligand for imaging cerebral β-amyloid (Aβ).5 Positron emission tomography (PET) has been successfully used to detect and quantify Aβ deposition in the living human brain, in AD, other dementias, and cerebral amyloid angiopathy-related hemorrhage.6-9 This ability to detect and quantify Aβ deposition in humans allows us to study a wide range of clinical associations with cerebral amyloidosis. Hence, this study aimed at assessing patients presenting with recent ischemic stroke using PiB PET to evaluate the relationship of acute ischemic stroke and Aβ deposition. Specifically, we tested the hypotheses that among patients with ischemic stroke, PiB retention is higher in the peri-infarct regions compared to the contralateral side, higher in the ipsilateral neocortical regions than in the contralateral

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neocortical regions, and higher in the global neocortical region when compared to normal aged-matched population. We also hypothesized that vascular risk factors among the ischemic stroke patients may affect PiB retention.

Subjects and Methods
Patients with first-ever ischemic stroke within 30 days of onset were recruited from the Austin Hospital. Patients with history of cerebral infection, dementia, or other neurodegenerative conditions were excluded, as were those with previous intracerebral hemorrhages, severe head trauma, tumor, or brain surgery. Age-matched healthy normal control subjects were randomly selected from a cohort of subjects participating in the longitudinal Healthy Aging Study at the Mental Health Research Institute of Victoria and were not known to have any cardiovascular risk factors as previously described. The study was approved by the Human Research Ethics Committee of the Austin Hospital and all participants gave written consent to be included in the study.

Imaging
All subjects underwent a brain 11C-PiB PET scan and a T1-weighted MRI scan for anatomic coregistration. Subjects also underwent fluid-attenuated inversion recovery and diffusion-weighted image MRI sequences.

Imaging Acquisition
PET data were acquired using Phillips Allegro PET camera with a resolution of ~4.4 mm full-width at half-maximum (FWHM). A thermoplastic facemask was used for head fixation. All subjects underwent short transmission scans to ensure correct head positioning and attenuation correction. This was followed by a 70-minute/90-minute dynamic 11C-PiB PET scan performed in 3-dimensional mode after intravenous injection of 370 MBq 11C-PiB. List-mode raw data were converted into 26 to 28 dynamic frames of 4×30-second, 9×1 minute, 3×3 minutes, and 10×6 minutes ±2×10 minutes frames, and were reconstructed using 3-dimensional row-action maximum likelihood algorithm.

Image Analysis
To avoid arterial blood sampling, a simplified reference tissue model was used. Parametric images were generated by applying the Logan analysis on a voxel-by-voxel basis (35–70 minutes) to create distribution volume ratio (DVR) maps from the dynamic PET data using the cerebellar cortex as a reference region. MRI and PET images were coregistered to individual PET images using SPM5 (Statistical Parametric Mapping) software. Because of the heterogeneous nature of the infarcts, PET images were not normalized. Regions of interests were defined on individual coregistered MRI images. These included neocortical regions comprising of the frontal, cingulate, parietal, lateral temporal, occipital, and primary visual cortices, ensuring areas of stroke (based on either diffusion-weighted image or fluid-attenuated inversion recovery) were excluded to minimize the effect of potential tissue distortion attributable to edema. The peri-infarct region of interest (PIR) was drawn visually around the fluid-attenuated inversion recovery or diffusion-weighted image of the MRI. A mirror region of interest of PIR was created for comparison in the contralateral side (Figure 2), ensuring as much as visually possible to exclude cerebrospinal fluid. Thus, peri-infarct and its mirror image, as well as ipsilateral and contralateral neocortical regions, were generated. The regions of interest defined on the coregistered MRI were used to sample the DVR PET images for comparative analysis. All analyses were performed blinded to the clinical details of the patients or controls.

Statistical Analysis
Among the ischemic stroke patients, Wilcoxon sign-rank test was used to compare ipsilateral and contralateral hemispheric neocortical PiB retention, PIR, and the contralateral region of interest. For the global neocortical PiB retention, the Wilcoxon-Mann-Whitney rank-sum test was used to test differences between the normal control individuals and ischemic stroke patients, with corresponding effect sizes estimated by Hodges-Lehmann shift parameter. Because the overall sample size was not sufficient to perform an adjusted association analysis, the strength of unadjusted individual association between various risk factors and PiB retention was quantified with Spearman rank correlation coefficient.

Table. Demographic Details

<table>
<thead>
<tr>
<th></th>
<th>Normal Controls (N=22)</th>
<th>Ischemic Stroke (N=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (IQR)</td>
<td>73 (IQR, 66–76)</td>
<td>76 (IQR, 68–77)</td>
</tr>
<tr>
<td>Days from stroke</td>
<td>N/A</td>
<td>10 (IQR, 7–14)</td>
</tr>
<tr>
<td>Sex</td>
<td>14 M, 8 F</td>
<td>14 M, 7 F</td>
</tr>
<tr>
<td>Hypertension</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Smoker/ex-smoker</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>
| F indicate female; IQR, interquartile range; M, male; N/A, not applicable.

Results
Twenty-four ischemic stroke patients who were eligible for study together with 22 control subjects were recruited. Three patients could not complete PET imaging and thus were excluded. In total, there were 21 ischemic stroke patients and 22 controls available for PiB PET for analysis, with a median age of 76 years (interquartile range, 68–77) and 73 years (interquartile range, 66–76), respectively. The median time from stroke onset to PET scan was 10 days (interquartile range, 7–14; Table).

Among the stroke patients, all but 1 had a relative higher PiB retention in the ipsilateral PIR compared to the contralateral mirror region, with an overall median PiB DVR difference of 0.29 (95% CI, 0.2–0.44; P = 0.001; Figure 1). Visually, this appears to be greater in the white matter around the infarct. Specifically, 3 patterns of PIR PiB retention were discernable. Ten patients had a relative higher PiB retention in the PIR only. Nine also had relative higher PiB retention but absence of PiB retention within the infarct.
region, “photopenic region.” Two patients had relative higher PiB retention in the PIR, but also within the infarct region (Figure 2). One patient with no relative higher PiB retention in ipsilateral PIR had bilateral infarcts.

The overall median PiB DVR difference, excluding focal areas of peri-infarct retention, between the ipsilateral neocortical DVR compared to contralateral neocortical DVR was 0.01 (95% CI, 0–0.1; \( P = 0.06 \); Figure 1). This local higher PiB retention, however, did not translate to a higher global neocortical PiB retention in stroke patients compared to normal controls with median global neocortical PiB DVR of 1.38 and 1.32, respectively (\( P = 0.22 \); Figure 3). There was no significant difference between the hemispheric neocortical DVR among the normal controls (0.04; 95% CI, −0.01 to 0.09; \( P = 0.12 \)).

Among the stroke patients, univariate analysis of the independent risk factors and global neocortical PiB retention indicated diabetes as the only risk factor associated with higher neocortical PiB retention (Spearman Rho=0.48; 95% CI, 0.28–0.72; \( P = 0.02 \); Bonferroni-corrected significance threshold=0.008; Figure 4).

**Discussion**

There are some associations between ischemic stroke and AD, with both sharing common risk factors, including age and elevated serum markers, including homocysteine and apolipoprotein E status. However, whereas cognitive impairment is associated with stroke, it remains unclear whether ischemic stroke contributes to AD. Several animal and earlier human studies have suggested that the ischemic brain may contribute to cerebral amyloidogenesis. To explore this relationship further, we utilized PiB PET to assess cerebral Aβ after recent stroke onset. This has the obvious advantage of providing an in vivo cross-sectional assessment of the global and regional pattern of Aβ deposition distribution in real time.
Patterns of Focal PiB Retention

We found all but 1 of the ischemic stroke patients had a higher PiB retention within the ipsilateral peri-infarct region compared to the contralateral side, particularly in the white matter around the infarct region. This is an intriguing finding and there may be several explanations for its presence.

Among the patients with relative focal higher PiB retention, we observed 3 specific patterns: higher retention in the peri-infarct region only, with dramatically reduced retention within the infarct (“photopenic region”), and within the infarct and surrounding peri-infarct region. It is not known what factors influence these patterns, but they may reflect the perfusion state at the time of scan. Speculatively, the “photopenic” pattern that appears to be related to larger cortical infarcts may be related to total occlusion and an absence of perfusion within the infarct core, whereas the higher PiB within the infarct region may reflect higher reperfusion injury.

The latter would lend support for the argument that the observed PiB retention within the infarct region may not represent true Aβ deposition but merely leakage of free PiB across the blood–brain barrier damaged by the ischemic process and reperfusion injury. In contrast, patients with higher retention in the peri-infarct region only and with dramatically reduced retention within the infarct (photopenic region) did not have higher PiB retention within the infarct region but did have mild relative increase in the peri-infarct region. Whether this peri-infarct PiB retention is also a product of PiB leakage across the blood–brain barrier remains to be proven. Unfortunately, contrast MR perfusion scans at time of the PET studies were not available but, if performed, may provide further useful information.

Alternatively, the higher PiB retention may be a true reflection of Aβ accumulation in the peri-infarct region in postischemic stroke patients. This would concur with several previous studies in humans in which a relationship between ischemic stroke and focal cerebral amyloidogenesis has been suggested. In a postmortem study of 131 brains, the investigators found punctate Aβ deposits in the cerebral cortex in association with small vessel cerebral vascular disease and other ischemic-susceptible zones, such as arterial border zones. This has been further supported in several rat models in which acute occlusion of the middle cerebral artery has been accompanied by both increased amyloid precursor protein (APP) and Aβ immunoreactivity in the “penumbral region” of infarct 1 week later. It has been suggested that this local ischemic effect on Aβ accumulation may relate to an upregulation and alteration of APP processing favoring beta and gamma peptide pathways, with increased β-secretase activity observed in rats after induced transient ischemic attack. In contrast with AD, in which plasma and cerebrospinal fluid Aβ levels are lower, serum and cerebrospinal fluid beta amyloid levels have been found to be higher in patients with acute ischemic stroke, suggesting that this increase may be derived from the brain as a consequence of ischemic insult. Higher plasma Aβ was also associated with more lacunar infarcts and white matter lesions in a cross-sectional association study of 1077 participants within the population-based Rotterdam Scan Study and with diffuse small vessel disease subtypes. Plasma Aβ concentration also has been found to be independently associated with white matter hyperintensity in patients with AD, mild cognitive impairment, or cerebral amyloid angiopathy (CAA).

Conversely, recent studies have shown that silent infarcts are relatively frequent after CAA-related hemorrhage and are associated with higher overall hemorrhage burden, perhaps because of occlusive small vessel arteriopathy. These findings raise the possibility that the observed focal PiB retention in our ischemic cohort may reflect preexisting Aβ accumulation, which may have contributed to the infarct. However, no overall increase in the neocortical PiB retention was observed in our patients, a finding typically seen symmetrically with temporal and occipital predominance in patients with CAA. Furthermore, patients with previous intracerebral hemorrhage and dementia were excluded from our study. The pattern of peri-infarct PiB retention also is different from other reported conditions that have been associated with focal PiB retention. Patients with presenilin-1 mutation carrier have been reported to have increased focal striatal and moderate neocortical PiB retention. Focal PiB retention also have been observed in an otherwise well subject within the sagittal and transverse venous sinuses. The relevance of this remains unclear.

Further observations from rat models of acute middle cerebral artery territory infarction showing a transient increase in APP, Aβ, and reactive astrocytes in peri-infarct regions lend support to a third hypothesis suggesting that the focal Aβ accumulation represents an acute inflammatory response produced by focal ischemic stress. Reactive astrocytes with APP immunoreactivity can be observed in the periphery of acute infarcts from day 3 after middle cerebral artery occlusion. Aβ peptide immunoreactivity has been seen from day 7 up to day 30, and disappearing by day 60 in rat models of cerebral ischemia, suggesting that Aβ peptide was derived from processing of APP in reactive astrocytes acutely and stopped at day 60. Further support for the acute phase reactant theory comes from a PET study utilizing PiB and (R)-PK11195 (marker of activated microglia) in patients with
Global Neocortical PiB Retention (Excluding Infarct Region)

Despite the focal peri-infarct PiB retention described, we did not find a significant overall global neocortical increase in PiB retention. Although this may reflect the limitation of sample size, it is of interest that similarly no such cortical increase in Aβ deposition was found in a study of 484 postmortem brains with verified cerebrovascular lesions (but no neurodegenerative pathology) compared to 57 age-matched controls. However, unlike in the study by Jendroska et al, the areas surrounding the cerebrovascular lesions (ie, the peri-infarct or penumbral regions) were not assessed for amyloid. Interestingly, of those who had evidence of acute cerebral lesions, there was a trend (though statistically not significant), toward higher cortical Aβ.

Although no immediate effect on neocortical PiB retention was observed in our patients overall, it remains unclear whether the focal PiB retention will translate into a higher cortical cerebral Aβ burden over time. Repeat PiB scans in some of our patients are planned, and they would help shed some light on this issue. Any effect on global PiB retention with time, however, likely would depend on the clearance of cerebral Aβ. Although the dynamics of cerebral amyloidosis remains complex, in normal human brain the production and elimination of Aβ is thought to be maintained in a balanced milieu so that no excess Aβ is formed. This is thought to be achieved by drainage of cerebral Aβ through the extracellular space via a perivascular route. The factors influencing the clearance of Aβ are not known, although evidence suggests that it may involve various Aβ receptor transport across the blood–brain barrier, enzymes such as insulin-degrading enzymes and various peptides, microglia, and astrocytes. There is some evidence to suggest that cerebral vascular disease itself may affect the elimination of Aβ protein because of loss of pulsations in thomboembolic or arteriosclerotic arteries required for drainage.

Effect of Diabetes on PiB Retention

Interestingly, among our acute ischemic stroke patients there was a trend for higher global neocortical PiB retention in patients with diabetes compared to those without. Our sample size was small, with 7 patients having diabetes and 14 without diabetes; hence, chance alone is always a possibility. Although there has been clinical and epidemiological association with diabetes and AD and CAA, Aβ burden was not found to be higher in a postmortem study of aged individuals with and without diabetes. However, it has to be noted that none of the subjects in this particular study had recent ischemia. More recent studies do suggest an exacerbation of Aβ amyloidogenesis and astrocytic activation in acute ischemic diabetic mice, and that this amplification colocalizes with autophagosomes. Similarly, in a middle cerebral artery occlusion model with rats fed a high-fat diet for 2 months and streptozotocin-induced type 2 diabetes showed exacerbated cognitive impairment and additively higher Aβ burden compared to control ischemic rats. Furthermore, insulin-degrading enzymes soluble fraction taken from human postmortem brain tissue using a monoclonal antibody was shown to remove >85% of the Aβ degrading activity. Insulin-degrading enzymes are responsible for the breakdown of insulin but also have been found to regulate Aβ degradation in mice. Mutations leading to the partial loss of function of these enzymes induce diabetes and also impair Aβ degradation.

The long-term effect and other potential factors influencing the production and clearance of Aβ may be at play but remain unclear. Further longitudinal and more detailed topographical PiB studies may shed more light on this complex process of cerebral amyloidosis and ischemic stroke. How the various vascular risk factors influence cerebral amyloidogenesis in the absence of ischemic stroke also remains unclear and will need further evaluation.

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Disclosures

None.

References


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