Carbon 11–Labeled Pittsburgh Compound B and Carbon 11–Labeled (R)-PK11195 Positron Emission Tomographic Imaging in Alzheimer Disease

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**Background:** Alzheimer disease (AD) is defined neuropathologically by the presence of neurofibrillary tangles and plaques associated with tau and β-amyloid protein deposition. The colocalization of microglia and β-amyloid plaques has been widely reported in pathological examination of AD and suggests that neuroinflammation may play a role in pathogenesis and/or progression. Because postmortem histopathological analyses are limited to single end-stage assessment, the time course and nature of this relationship are not well understood.

**Objective:** To image microglial activation and β-amyloid deposition in the brains of subjects with and without AD.

**Design, Setting, and Participants:** Using two carbon 11 (\(^{11}\text{C}\) )–labeled positron emission tomographic imaging agents, Pittsburgh Compound B (PiB) and (R)-PK11195, we examined the relationship between amyloid deposition and microglial activation in different stages of AD using 5 control subjects, 6 subjects diagnosed with mild cognitive impairment, and 6 patients with mild to moderate AD.

**Results:** Consistent with prior reports, subjects with a clinical diagnosis of probable AD showed significantly greater levels of \(^{11}\text{C}\)PiB retention than control subjects, whereas patients with mild cognitive impairment spanned a range from control-like to AD-like levels of \(^{11}\text{C}\)PiB retention. Additionally, 2 asymptomatic control subjects also exhibited evidence of elevated PiB retention in regions associated with the early emergence of plaques in AD and may represent prodromal cases of AD. We observed no differences in brain \(^{11}\text{C}\)(R)-PK11195 retention when subjects were grouped by clinical diagnosis or the presence or absence of β-amyloid pathological findings as indicated by analyses of \(^{11}\text{C}\)PiB retention.

**Conclusions:** These findings suggest that either microglial activation is limited to later stages of severe AD or \(^{11}\text{C}\)(R)-PK11195 is too insensitive to detect the level of microglial activation associated with mild to moderate AD.


**Definitive Diagnosis of Alzheimer Disease (AD)**

A definitive diagnosis of Alzheimer disease (AD) relies on the demonstration of β-amyloid (Aβ) plaques and neurofibrillary tangles at autopsy. Microglial activation frequently accompanies Aβ deposition, although the time course of their relationship is unclear. While the exact time course of Aβ deposition in AD has not been elucidated, evidence gained through a postmortem study of Down syndrome (a condition in which Aβ deposition is always present by age 40 years and dementia is common) and an increasing number of reports using the positron emission tomographic (PET) Aβ imaging agent carbon 11 (\(^{11}\text{C}\) )–labeled Pittsburgh Compound B (PiB) suggests that Aβ deposition precedes development of the clinical symptoms of dementia. Compared with control subjects, patients with AD show marked retention of \(^{11}\text{C}\)PiB in areas of brain association cortex known to contain large amounts of Aβ deposits in AD. Levels of \(^{11}\text{C}\)PiB retention comparable to those seen in some subjects with AD have been observed in the cognitively normal elderly population. These and other studies suggest that Aβ deposition precedes significant cognitive decline, although more definitive longitudinal studies in healthy elderly persons are needed to fully characterize the relationship between Aβ deposition and cognitive decline.

The colocalization of activated microglia with Aβ-containing neuritic plaques that are characteristic of AD was highlighted by McGeer et al. There is much debate surrounding the central question of whether the role of activated microglia in the pathophysiology of AD is one of neuroprotec-
tion or neurotoxicity; this issue has been the subject of many recent reviews. Together, these investigations implicated microglia as at least a participant in the AD process.

Several groups have investigated the utility of selective ligands of the peripheral benzodiazepine receptor (PBR) as surrogate markers for activated brain microglia (as reviewed by Venneti et al11). Unlike the central benzodiazepine receptor, the PBR is expressed at low levels in the healthy brain. During neuroinflammation associated with AD and a variety of other central nervous system diseases, binding of the PBR radioligand [11C](R)-PK11195 increases in the brain.11 We have confirmed specific binding of tritium-labeled (R)-PK11195 to simian and human microglia using ex vivo autoradiography in lentiviral encephalitis and AD.12 Additionally, we have demonstrated increased radiotracer retention in the simian brain consistent with postmortem histopathological confirmation of microglial activation.13

The goal of our work was to use PET radiotracers to label cerebral Aβ deposits and activated microglia and assess their potential temporal relationship using a cross-sectional design. The presence of significant elevations of [11C]PiB retention in asymptomatic elderly subjects suggests that significant Aβ aggregation precedes detectable cognitive dysfunction by many years.14 Therefore, if microglia are involved in the deposition of Aβ, one would expect these cells also to be activated years before cognitive dysfunction is detected clinically. One hypothesis is that the brain’s immune response to fibrillar Aβ deposits in the form of activation of microglia results in the secretion of a plurality of neurotoxic factors that contribute to synaptic loss and cognitive impairments. The correlation of the time course of microglial activation with the time course of Aβ deposition will help to elucidate the role of neuroinflammation in the pathophysiology of AD.

**METHODS**

Seventeen subjects (5 control subjects, 6 patients with mild cognitive impairment [MCI], and 6 patients with mild to moderate AD) were studied with fully dynamic [11C]PiB and [11C](R)-PK11195 PET scans (Table). This study encompasses all of the subjects who were scanned with both [11C]PiB and [11C](R)-PK11195 at our institution. In addition, volumetric magnetic resonance imaging was performed in all of the subjects for anatomical coregistration and volume-of-interest placement. All of the subjects were recruited after diagnostic evaluation at the University of Pittsburgh Alzheimer Disease Research Center in a protocol approved by the University of Pittsburgh Internal Review Board. Each subject received an extensive clinical evaluation, scored normally on neuropsychiatric evaluations, and received the Alzheimer Disease Research Center consensus diagnosis of healthy control. No exclusion was made for treatment with anticholinesterase drugs as this factor did not affect PiB studies in the initial Swedish cohort of subjects with AD. Informed consent was obtained and the investigator’s certification statement was signed (prior to initiating research procedures) by one of the physician investigators (C.A.W.). All of the groups were matched for age (within 2 years), sex, and level of education. High-specific-activity [11C]PiB was synthesized as described previously. Magnetic resonance–PET image alignment was performed using an automated algorithm for image alignment, reslicing, and region-of-interest (ROI) determination, and indices of radiotracer binding were determined for both [11C]PiB and [11C](R)-PK11195 using previously described methods. As this study did not involve the placement of arterial lines for discrete arterial sampling for input function determination, simplified methods of analysis were applied. For [11C]PiB, the regional standardized uptake value (SUV) measure normalized to the cerebellar SUV determined over the 40- to 90-minute postinjection interval (SUV ratio) was selected as the index of [11C]PiB radiotracer retention. For [11C](R)-PK11195, 2 methods of analysis were applied to the data set. The first was a basis-function implementation of the simplified reference tissue model using the whole cerebellum as a reference for estimation of the [11C](R)-PK11195 binding potential (BP). The second was a tissue ratio method whereby the regional radioactivity concentration was summed over the 10- to 60-minute interval and normalized to that determined for subcortical white matter (ROI to subcortical white matter ratio).

Due to the potential confounding effects of cerebral atrophy on the study of elderly subjects with PET, all PET data obtained in this project were corrected for partial volume effects using a validated magnetic resonance imaging–based method. Analyses of variance with posttest Bonferroni correction were used to analyze data between control, MCI, and AD groups to compare [11C](R)-PK11195 and [11C]PiB retention data with...
and without atrophy corrections. Nonparametric Mann-Whitney tests were used to compare data between [11C]PiB-positive and [11C]PiB-negative groups and to compare [11C]PK11195 and [11C]PiB retention data with and without atrophy corrections. Correlational analyses were performed to quantify the relationship between [11C]PK11195 and [11C]PiB retention. Results from correlational analyses are represented by \( r \), the Pearson correlation coefficient. Confidence intervals at the 95% level were used to analyze data, and \( P < .05 \) was considered statistically significant.

## RESULTS

### CEREBRAL ATROPHY CORRECTION

Average regional cerebrospinal fluid correction factors showed statistically significant differences only between the AD and control groups, and then only in the mesial temporal cortex (MTC) \( (P = .004) \). Significant differences \( (P < .05) \) in the degree of cerebral atrophy were observed in the MTC and the sensorimotor cortex when subjects were grouped by [11C]PiB retention status (PiB positive or negative).

### [11C]PiB PET IMAGING

All of the subjects were evaluated for [11C]PiB retention status using criteria established through statistical analysis of a larger cohort of subjects \( (n = 62 \) control subjects) who underwent an essentially identical [11C]PiB imaging study.\(^{22}\) As applied to our study, a cutoff of 1.65 units in the regional SUV relative to cerebellum over 90 minutes of scanning was used for the frontal cortex and the precuneus region.\(^2\) Any subject with an SUV ratio over 90 minutes that was greater than 1.65 units in either the fron-
tal cortex or the precuneus region was classified as PiB positive for the purpose of this analysis.

As a group, the control subjects showed rapid entry and clearance of \(^{11}\text{C}\)PiB in all cortical and subcortical gray matter areas, including cerebellar cortex. In contrast, the patients with AD showed approximately 2-fold greater retention of \(^{11}\text{C}\)PiB than control subjects in areas of the brain known to contain large amounts of A\(\beta\) deposits in AD \((P < .05)\), such as parietal and frontal cortices as well as the posterior cingulate gyrus and precuneus region (Figure 1 C and D). Subjects with MCI showed patterns of \(^{11}\text{C}\)PiB retention that were intermediate between control and AD groups. Reclassification of subjects into PiB-positive and PiB-negative groups based on the aforementioned criteria for determining \(^{11}\text{C}\)PiB positivity showed a very clear delineation between groups expected to have significant A\(\beta\) pathological findings and those without \((P < .01)\) (Figure 2).

In general, the observed pattern of \(^{11}\text{C}\)PiB retention was consistent with the pattern of A\(\beta\) plaque deposition described in postmortem studies of AD brain\(^{22,24}\) as well as other published reports using \(^{11}\text{C}\)PiB to image brain A\(\beta\)-amyloidosis in AD.\(^{3-8}\) In this study, 12 of 17 subjects studied were classified as PiB positive (6 of 6 patients with AD, 4 of 6 patients with MCI, and 2 of 5 control subjects). Most PiB-positive subjects showed evidence of increased \(^{11}\text{C}\)PiB retention in all brain areas, but 2 of the 12 PiB-positive subjects showed more focal \(^{11}\text{C}\)PiB retention (Figure 2B).

\[^{11}\text{C}\](R)-PK11195 PET IMAGING

\(^{11}\text{C}\)(R)-PK11195 exhibited similar levels of uptake and rates of clearance in the brains of both PiB-positive and PiB-negative subjects as well as diagnostic groups (Figure 1). Retention of \(^{11}\text{C}\)(R)-PK11195 during the 10- to 60-minute postinjection interval did not differ signifi-

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**Figure 2.** Carbon 11–labeled \((^{11}\text{C})\) Pittsburgh Compound B (PiB) standardized uptake value ratio over 90 minutes (SUVR90) outcome measures corrected for cerebral atrophy for the mesial temporal cortex (MTC), sensorimotor cortex (SMC), frontal cortex (FRT), parietal cortex (PAR), and posterior cingulate or precuneus region (PRC). Data are classified by clinical diagnosis (control, mild cognitive impairment [MCI], or Alzheimer disease [AD]) (A) and \(^{11}\text{C}\)PiB retention (B). Indices of \(^{11}\text{C}\)PiB retention in patients with AD show significantly higher values relative to control subjects in cortical regions \((P < .05)\). Also, when classified on the basis of amyloid pathological findings (PiB positive or PiB negative), PiB-positive subjects showed indices of retention that were approximately 2-fold higher than in PiB-negative subjects \((P < .01)\) in regions where A\(\beta\)-amyloid pathological findings are expected.

**Figure 3.** Carbon 11–labeled \((^{11}\text{C})\) (R)-PK11195 region of interest to subcortical white matter ratio (ROI/SWM) outcome measures corrected for cerebral atrophy for the cerebellum (CER), mesial temporal cortex (MTC), sensorimotor cortex (SMC), frontal cortex (FRT), parietal cortex (PAR), and posterior cingulate or precuneus region (PRC). Data are classified by clinical diagnosis (control, mild cognitive impairment [MCI], or Alzheimer disease [AD]) (A) and \(^{11}\text{C}\) Pittsburgh Compound B (PiB) retention (B). No statistically significant differences were noted between diagnostic groups or \(^{11}\text{C}\)PiB retention groups (PiB positive or PiB negative).
sively in any brain region among the AD, MCI, and control groups \( (P = 0.25) \) or between PiB-positive and PiB-negative groups \( (P > 0.10) \), even in the precuneus region where \(^{11}\text{C}\)PiB retention was most prevalent (Figure 3). The BP estimates of regional \(^{11}\text{C}\)(R)-PK11195 retention derived using the simplified reference tissue method varied with a mean near 0, suggesting that voxelwise BP estimates were most likely attributable to statistical noise and not signal from activated microglia (data not shown). The use of a late-scan tissue ratio (ROI to subcortical white matter retention ratio) without application of the cerebral atrophy correction showed slightly lower ratios in patients with AD relative to both control and MCI groups, suggesting a trend toward decreased binding of \(^{11}\text{C}\)(R)-PK11195 in PiB-positive subjects compared with PiB-negative subjects (Figure 4). However, these differences did not reach the level of significance and only approached trend-level differences in the MTC \( (P = 0.14) \). Correction of the ROI to subcortical white matter ratio for atrophy negated any apparent differences in the retention of \(^{11}\text{C}\)(R)-PK11195 and did not yield any significant differences between diagnostic groups \( (P = 0.46) \) or Aβ status \( (P = 0.46) \) (Figure 3). No brain region showed a significant correlation between indices of \(^{11}\text{C}\)(R)-PK11195 and \(^{11}\text{C}\)PiB retention, most notably in regions with consistent PiB-positive status such as the frontal cortex and the precuneus region, where significant levels of Aβ deposition were expected in the AD cases (Figure 5).

**COMMENT**

The PBR ligand PK11195 has undergone development as an agent for assessing macrophage and microglial activation in neurological disease for nearly 2 decades.\(^{1, 12, 25-28}\) More than a dozen years ago, Groom et al\(^{29}\) used PET with fludeoxyglucose F 18 and \(^{11}\text{C}\)(R,S)-PK11195 to compare PBR expression and cortical glucose metabolism in 8 patients with AD vs those of a con-

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Figure 4. Carbon 11–labeled \((^{11}\text{C}\)) \((R)\)-PK11195 outcome measures (region of interest to subcortical white matter ratio [ROI/SWM]) demonstrating the effects of a magnetic resonance–based correction for cerebral atrophy. Measures are shown as atrophy corrected classified by clinical diagnosis (control, mild cognitive impairment [MCI], or Alzheimer disease [AD]) (A), atrophy corrected classified by \(^{11}\text{C}\) Pittsburgh Compound B (PiB) retention (B), uncorrected classified by clinical diagnosis (C), and uncorrected classified by \(^{11}\text{C}\)PiB retention (D). Uncorrected measures of \(^{11}\text{C}\)(R)-PK11195 retention exhibit a trend toward lower levels of \(^{11}\text{C}\)(R)-PK11195 retention in subjects with AD and PiB-positive subjects, particularly in the mesial temporal cortex (MTC) where atrophy is the greatest in AD \( (P = 0.14) \). Indices of \(^{11}\text{C}\)(R)-PK11195 retention show no significant differences between subject groups \( (P = 0.46) \) or Aβ status \( (P = 0.46) \) when data are corrected for the dilutional effects of atrophy. CER indicates cerebellum; SMC, sensorimotor cortex; FRT, frontal cortex; PAR, parietal cortex; PRC, posterior cingulate or precuneus region; and error bars, SD.

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control subject. These investigators found low levels of specific binding of $[11\text{C}]$(R,S)-PK11195 in regions of fludeoxyglucose F 18 hypometabolism but no increase in the $[11\text{C}]$(R,S)-PK11195 BP in AD compared with the single control subject.

Six years after the initial report by Groom and colleagues, Cagnin et al.\textsuperscript{16} reported a similar study using the more active $R$-enantiomer of $[11\text{C}]$PK11195, comparing 15 control subjects, 8 patients with AD (5 mild and 3 moderate), and 1 patient with MCI. They observed small BP increases bilaterally in the fusiform, inferior, and middle temporal gyri and in the left parahippocampal gyrus, amygdala, and posterior cingulate gyrus of patients with AD. Further, they noted an age-related increase in $[11\text{C}]$(R)-PK11195 BP in the thalamus that was not specific to AD. Versijpt et al.\textsuperscript{31} described the utility of a radioiodinated analog of PK11195 for single-photon emission computed tomographic imaging, $[123\text{I}]$iodo-PK11195, which showed increased uptake in nearly all neocortical regions of 10 patients with AD compared with 9 control subjects, but this only achieved significance in the frontal and right MTC regions, which are notably free of $[11\text{C}]$ plaques in mild to moderate AD. Other neurodegenerative diseases have also been assessed with $[11\text{C}]$(R)-PK11195 (eg, Huntington disease and multiple system atrophy), but there was no simple correspondence between clinically involved regions and $[11\text{C}]$(R)-PK11195 binding.\textsuperscript{32,33} The observed distribution of $[11\text{C}]$PiB in patients with mild to moderate AD in this study is consistent with prior reports showing the highest $[11\text{C}]$PiB retention in the posterior cingulate gyrus, parietal cortex, and frontal cortex but relatively low binding in the MTC regions such as the amygdala and hippocampus.\textsuperscript{46,48} This characteristic distribution of in vivo $[11\text{C}]$PiB retention reflects the known distribution of Aβ plaques in AD, which are most abundant in the neocortical regions but relatively absent from the MTC regions.\textsuperscript{24} Interestingly, the prior literature suggests an evaluation of $[11\text{C}]$(R)-PK11195 retention in the MTC regions in AD,\textsuperscript{30,31} an area characterized by low densities of neuritic plaques but high densities of neurofibrillary tangles,\textsuperscript{24} suggesting an incongruity between microglial activation as assessed by $[11\text{C}]$(R)-PK11195 retention and the known pattern of Aβ deposition. This incongruity may have several important implications. The first is the possibility that microglial activation in AD is not a specific indicator of Aβ plaque load. The second is that the previously observed regional increases in binding of $[11\text{C}]$(R)-PK11195 arise from processes that are not directly involved in the pathogenesis of AD. The third is that the methods applied to the study of $[11\text{C}]$(R)-PK11195 may not fully assess the distribution of activated microglia in the brain.

Krogholler et al.\textsuperscript{19} recently reported a trend toward increases in $[11\text{C}]$(R)-PK11195 binding in elderly control subjects compared with young subjects and further elevations in patients with clinically characterized MCI. Interestingly, patients with AD showed a declining trend in the $[11\text{C}]$(R)-PK11195 BP relative to MCI. None of these findings, however, reached the threshold of statistical significance. The data from our study indicate a similar trend in the ROI data prior to applying atrophy correction (Figure 4). However, when atrophy correction factors are applied to our indices of $[11\text{C}]$(R)-PK11195 retention, the trend is no longer apparent. These trends mirror the group differences in atrophy correction factors, with the MTC as the region with the greatest component of atrophy. Because the data reported by Krogholler and colleagues did not appear to incorporate an atrophy correction for the loss of PET signal, it is possible that the apparent trends in their data are influenced by varying degrees of cerebral atrophy. When corrected, our data show no differences in the retention of $[11\text{C}]$(R)-PK11195 and presumably no significant differences in the degree of microglial activation between diagnostic groups or the presence and absence of $[11\text{C}]$PiB retention. A recently published Hun-

**Figure 5.** Transaxial and sagittal parametric images of the carbon 11–labeled ($[11\text{C}]$) Pittsburgh Compound B (PiB) standardized uptake value ratio (SUVR) and the $[11\text{C}]$(R)-PK11195 region of interest to subcortical white matter ratio (ROI/SWM) in a PiB-negative control subject (subject C-1) and the patient with the most advanced Alzheimer disease (AD) in our study (patient A-5). Areas of significantly elevated $[11\text{C}]$PiB retention are evident in the patient with AD in a manner consistent with the known pattern of amyloid deposition in AD. No similar pattern is noted in the $[11\text{C}]$(R)-PK11195 binding potential images.
Huntington disease study\textsuperscript{33} suggested that \textsuperscript{[\textsuperscript{11}C] (R)-PK11195 detected microglial activation in 11 presymptomatic disease gene carriers. That study showed low but significantly increased regional \textsuperscript{[\textsuperscript{11}C] (R)-PK11195 BP values in Huntington disease gene carriers relative to healthy control subjects. Our similarly sized study of AD did not detect significant differences in \textsuperscript{[\textsuperscript{11}C] (R)-PK11195 BP between patients with AD and control subjects, but after atrophy correction, it showed a trend toward decreased binding in AD.

Considering the postmortem literature showing a spatial relationship between A\textbeta pathological burden and microglial activation, the failure of this study to detect increases in microglial activation using \textsuperscript{[\textsuperscript{11}C] (R)-PK11195 in subjects where considerable A\textbeta pathological burden is presumably manifested (PiB-positive subjects) can be interpreted in several ways. The first is that A\textbeta deposition precedes microglial activation and is itself the principal substrate of neuronal degeneration and cognitive impairment in AD. In this interpretation, microglial activation is not involved early in the AD disease process but becomes more of a feature of the disease many years later in its terminal stages. This would seem to argue that microglial activation is more of an inert product than a significant participant of the neurodegenerative processes involved in AD. A second interpretation is that microglial activation is a feature of early AD, perhaps even presymptomatic AD, but that \textsuperscript{[\textsuperscript{11}C] (R)-PK11195 lacks the sensitivity to detect it. Because no patients with AD in our study showed significant \textsuperscript{[\textsuperscript{11}C] (R)-PK11195 retention despite a mean Mini-Mental State Examination score of 19 (and 1 subject with a Mini-Mental State Examination score of 13), we favor the latter interpretation.

The advent of anti-A\textbeta therapies clearly demonstrates a pressing need for technology that would allow for non-invasive in vivo quantitation of A\textbeta and microglia in the human brain. One theory of how anti-A\textbeta therapies clear A\textbeta deposition is through A\textbeta opsonization and microglial-mediated destruction. The small number of autopsy reports from the AN 1792 A\textbeta immunization study showed decreased A\textbeta load and increased microglial activation,\textsuperscript{34,35} suggesting that the increased microglial activation attributable to A\textbeta immunization resulted in the significant clearance of brain amyloid deposits. However, because we have no measure of pretreatment amyloid load, it is not possible to definitively document the therapeutic effect in humans.\textsuperscript{36} Antiamyloid therapies such as passive immunization with anti-A\textbeta antibodies are promising avenues in the treatment and prevention of AD. The ability to quantify A\textbeta load and microglial activation prior to treatment and then follow the effects of treatment is critical to the efficient development of this therapy. If these therapies become an effective treatment for AD, it also will be critical to have a means of evaluating clinical success, particularly in mildly impaired subjects with few clinical symptoms to follow. Finally, the ability to follow the natural history of A\textbeta deposition and its relationship to microglial activation beginning in the presymptomatic stage should yield important pathophysiological insights regarding the accuracy of the amyloid cascade hypothesis of AD. To accomplish this, a marker demon-

strated to be both a reliable and robust indicator of microglial activation in vivo will be essential.

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