PET of Peripheral Benzodiazepine Binding Sites in the Microgliosis of Alzheimer's Disease

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Animal and human autoradiographic studies have shown increased in vitro binding of the peripheral benzodiazepine binding site antagonist PK 11195 in areas of microgliosis, including the temporal association cortex of patients with Alzheimer's disease. To further elucidate the role of cellular inflammation and microgliosis in Alzheimer's disease during life, we used PET and [^{11}C]PK 11195, a peripheral benzodiazepine receptor ligand known to bind avidly to microglia. **Methods:** Eight patients with a diagnosis of probable Alzheimer's disease underwent PET of the brain using [^{11}C]PK 11195 and, for comparison, with [^{18}F]FDG to determine cerebral glucose metabolism. Uptake of [^{11}C]PK 11195 in various brain regions was expressed relative to that in the cerebellum and compared to values determined in one normal elderly subject and in clinically and anatomically unaffected hemispheres of seven patients with small unliateral gliomas. **Results:** No increases in peripheral benzodiazepine binding were identified in patients with probable Alzheimer's disease, and binding was lowest in regions that were most hypometabolic. **Conclusion:** The peripheral benzodiazepine binding sites associated with microgliosis and cellular inflammation in Alzheimer's disease at postmortem are undetectable by PET using [^{11}C]PK 11195 in patients with mild-to-moderate dementia.

Key Words: positron emission tomography; Alzheimer's disease; peripheral benzodiazepine binding site; microgliosis; inflammation; PK-11195

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Reactive microgliosis and evidence of cellular inflammatory infiltration are increasingly recognized in Alzheimer's disease, but their significance is uncertain (*1*). Mature amyloid plaques possess a prominent microglial cellular component. Many proteins that potentially could contribute to neuronal death are expressed by microglia such as interleukin-2, tumor necrosis factor and complement receptors (*2,3*), giving rise to the hypothesis that immune-mediated inflammatory effects may produce neurodegeneration by an *innocent bystander lysis* effect (*1*). Other studies have additionally identified elevated levels of a1-antichymotrypsin in the CSF of Alzheimer's disease patients, suggesting increased cellular inflammatory activity (*4*). Limited clinical trials have suggested a role for anti-inflammatory drugs which may suppress reactive microgliosis and cell-mediated inflammation (*5*). Unlike the characteristic neurofibrillary tangles observed predominantly in Alzheimer's disease cortex, however, microgliosis has not been correlated with symptomatic disease severity, and its clinical significance is uncertain.

To explore the potential of emission imaging to elucidate further the role of microgliosis and cellular inflamation in Alzheimer's disease during life, we used PET and [^{11}C]PK 11195, a peripheral benzodiazepine receptor ligand known to bind avidly to mononuclear inflammatory cells (*6,7*). Since activated microglia are closely related to mature neuritic plaques and amyloid deposits at postmortem examination (*8*), we hypothesized that in vivo [^{11}C]PK 11195 binding would be increased in Alzheimer's disease, especially in regions of the brain that exhibit the greatest pathology.

MATERIALS AND METHODS

Patients

Eight patients with a clinical diagnosis of probable Alzheimer's disease by NINCDS-ADRDA criteria (*9*) were recruited for the study. For comparison, we utilized studies of seven patients with small cerebral gliomas, who had previously been imaged with both [^{18}F]FDG and [^{11}C]PK 11195 and one elderly normal volunteer. All patients with brain tumors had unifocal hemispheric tumors. Involvement of the opposite hemisphere was excluded in each case by MRI, including T2-weighted images, which are highly sensitive for detection of tumor involvement. Informed consent was provided by each patient and their next of kin or legal guardian. This study was approved by the university's Institutional Review Board.

There were five women and three men in the Alzheimer's disease group, with an age range of 69–80 yr. All had mild-to-moderate dementia with Mini-Mental State scores (*10*) of 6–23 and Clinical Dementia Ratings (*11*) of 1–2. The range of disease duration was 3–12 yr. The control group's age range was from 42 to 78 yr. None of the control group had clinical evidence of dementia on clinical or psychological testing.

PET

Carbon-11-PK 11195 was synthesized by N-alkylation of its desmethyl precursor using [^{11}C]methyl iodide by a modification of the technique of Camsonne et al. (*12*). Radiochemical purity was greater than 99% and the specific activity was greater than 37 GBq/mmol (1 Ci/mmole).

Each subject underwent PET imaging of the brain after intra-

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venous administration of 888 MBq (24 mCi) [^{11}C]PK 11195 with a whole-body PET scanner that generated 15 cross-sectional slices of brain (8 direct, 7 cross planes) parallel to and beginning 1 cm above the canthomeatal line. The scanner has an intrinsic in-plane resolution of 5.5 mm FWHM and axial resolution of 6.5–8.0 mm FWHM. Sequential scans were acquired over 60 min (0–5, 5–10, 10–20, 20–30, 30–40 and 40–60 min). Thirty to 60 min after completion of [^{11}C]PK 11195 imaging (90 to 120 min following injection of [^{11}C]PK 11195), 370 MBq (10 mCi) [^{18}F]FDG [synthesized by a modification of the technique of Hamacher et al. (*13*)] were administered. The estimated radiation dose from both radiopharmaceuticals was less than 5 cGy (5 rad) in all organs. Arterial blood samples were obtained for quantification of regional cerebral glucose metabolism (*14*). Imaging was performed in two interleaved bed positions after an incorporation time of 30 min. Throughout the study, the patients were lying on the scanning bed in a quiet room with their eyes open. Fiducial markers, using [^{11}C]PK 11195 and [^{18}F]FDG, were placed for the two studies to allow for realignment between scans.

Data Analysis

Following reconstruction of the [^{11}C]PK 11195 and [^{18}F]FDG images, the following anatomically-configured regions of interest (ROIs) were determined visually from the FDG study: most affected cerebral cortex (i.e., most hypometabolic, usually posterior parietal), sensorimotor cortex, average cerebral cortex, average cerebral white matter, thalamus and cerebellum. These ROIs were hand-drawn, encompassing at least 100 voxels for each cortical region and the cerebellum and approximately 70 voxels for the thalamus. The most affected and sensorimotor cortical ROIs were taken from the same image slice. Likewise, the average cortex and white matter ROIs were chosen from a single slice above the level of the ventricles. Thalamic and cerebellar ROIs were also drawn from single brain slices where these structures were most clearly defined.

For the control subjects, identical ROIs were drawn, with the exception that the most affected cerebral cortical region was replaced by an ROI encompassing normal parietal association cortex. Only the cerebral hemisphere contralateral and the cerebellar cortex ipsilateral to the glioma were used to generate values for the ROIs.

These ROIs were then superimposed on the [^{11}C]PK 11195 images (realigned for patient motion if necessary) that were collected from 40 to 60 min after [^{11}C]PK 11195 injection and average counts per voxel were recorded. Late images were used because saturable binding of [^{11}C]PK 11195 in gliomas is best depicted at this time (*15*). Figures 1 and 2 are of a representative Alzheimer's disease subject and the elderly normal volunteer. To control for differences in arterial tracer activities, these ROI values were then normalized to those of the cerebellum, as studies have shown this to be one of the least pathologically affected brain structures in Alzheimer's disease (*16*).

RESULTS

On visual inspection of the [^{11}C]PK 11195 images, there were no cerebral regions of increased binding in Alzheimer's disease patients compared to controls. The only identifiable difference between the images of Alzheimer's disease and those of control subjects was enlargement of the subcortical region of low activity in the vicinity of the lateral and third ventricles. In the control subjects with tumor, increased binding was limited to within the glioma and did not extend into the contralateral hemisphere. Fluorine-18-FDG images in Alzheimer's disease patients showed typical areas of hypometabolism in the association cortices. The control group had normal patterns of [^{18}F]FDG metabolism in the regions of selected ROIs; tumor metabolism was variably increased or decreased.

In quantitative analysis of the [^{11}C]PK 11195 ROIs in the Alzheimer's disease patients, no regions had normalized values higher than in controls (Table 1). The most affected region of the cerebral cortex on the FDG images, in fact,

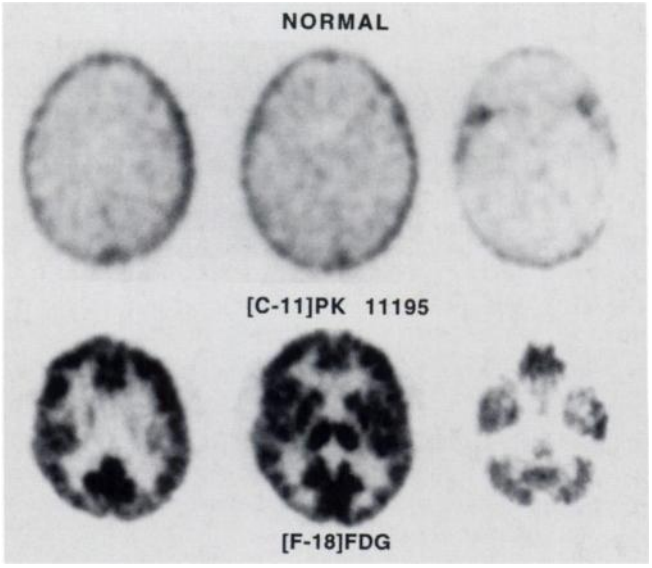

FIGURE 1. Selected slices of [^{11}C]PK 11195 and [^{18}F]FDG studies in a normal elderly volunteer.

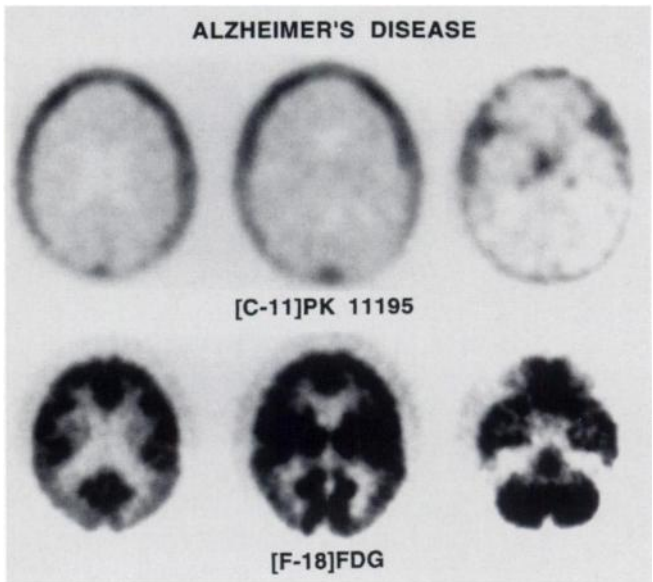

FIGURE 2. Selected slices of [^{11}C]PK 11195 and [^{18}F]FDG studies in a subject with moderate Alzheimer's disease. The activity in the midline in the third [^{11}C]PK 11195 image is physiologic uptake within the paranasal sinuses.

TABLE 1
Normalized Region of Interest Values for Carbon-11-PK 11195 Binding*

	Alzheimer's patients	Controls
Most affected cerebral cortex	0.97 ± 0.13	1.10 ± 0.12
Sensorimotor cortex	1.10 ± 0.15	1.13 ± 0.12
Averaged cerebral cortex	1.08 ± 0.10	1.16 ± 0.10
Averaged cerebral white matter	0.93 ± 0.16†	1.09 ± 0.08†
Thalamus	1.12 ± 0.14†	1.25 ± 0.11†

*Values were normalized to the cerebellum.
†Significant at the $p < 0.05$ level between Alzheimer's disease and control groups. (Student's two-tailed t-test).

had the lowest normalized value of all cortical ROIs analyzed on the [^{11}C]PK 11195 images.

DISCUSSION

Benzodiazepines have been shown to bind with high affinity to saturable sites, both within the brain and in various non-neuronal tissues. The predominant binding sites found within the brain, which are associated with neuronal gamma-amino butyric acid receptors, are designated central benzodiazepine binding sites. Binding sites of the type found predominantly in non-neural tissues are known as peripheral benzodiazepine binding sites (PBBSs or PBBS) (*17*), w_3 (omega-3) receptors (*18*) or peripheral benzodiazepine receptors (PBZDRs) (*19*). The two classes of binding sites are pharmacologically distinct: the ligand PK 11195 recognizes only the PBBS. The highest densities of PBBS are found in the adrenal gland, lung, kidney, heart and skin (*22*). Prior studies have identified increased PBBS binding in a variety of neuropathologic conditions, characterized by the presence of non-neural elements including neoplasms (*15*) and areas of cellular inflammatory infiltration and microgliosis.

Unlike prior in vitro binding studies (*23*), however, our present study fails to identify elevated PBBS in Alzheimer's disease brain. Further, we failed to find the expected inverse correlation between [^{11}C]PK 11195 binding and glucose metabolism. Instead, normalized [^{11}C]PK 11195 uptake tended to be lower in brain regions that had the most impaired glucose metabolism. We have observed a similar discordance between in vivo and in vitro PK 11195 binding in mesial temporal sclerosis of epileptic patients. Although, resected temporal lobe tissues reveal large (up to 100%) increases in [^{3}H]PK 11195 binding as reported by other investigators (*24*), we have observed no elevation of in vivo PK 11195 uptake in patients with the clinical syndrome of medically-refractory mesial temporal lobe epilepsy (Junck L, *unpublished data*, 1994). In contrast to the negative findings in Alzheimer's disease and epilepsy, PET imaging with [^{11}C]PK 11195 has shown that binding in malignant astrocytomas (*15*) and in the inflammatory response to stroke (*27,28*) is increased 2–3-fold in comparison with normal cerebral gray matter. There may be several reasons for the apparent in vivo versus in vitro discrepancies.

A greater increase in PBBS density in tumors and infarcts may account for the successful PET imaging of PBBS in these disorders in contrast with Alzheimer's disease and epilepsy. In human surgical glioma specimens, Ferrarese et al. (*29*) found B_{max} for [^{3}H]PK 11195 in tumor to be increased 4.9-fold in glioblastomas and 4.5-fold in grade 1–2 astrocytomas when compared to remote gray matter and measured per milligram of protein. Similarly, Black et al. (*30*) found specific binding of [^{3}H]PK 11195 in human surgical glioma specimens compared to non-neoplastic brain to be increased 5.7-fold in high-grade gliomas and 3.4-fold for low-grade gliomas. In PET imaging studies of untreated gliomas with [^{11}C]PK 11195, we have successfully imaged tumor PBBS in all of 10 glioblastomas and in 4 of 12 low-grade gliomas (Junck L, *unpublished data*, 1994). Considered together, these results suggest that an ~5-fold increase in PBBS density can be readily imaged with [^{11}C]PK 11195 and PET. In contrast, the in vitro binding of [^{3}H]PK 11195 is increased only ~2-fold in Alzheimer's disease brain and in hippocampal sclerosis. A 2-fold increase may be detectable in vitro, but not in vivo, where the unbound and nonspecifically bound ligand are greater. These factors may account for our negative results in Alzheimer's disease.

In addition to these technical factors, it is also possible that microgliosis is a final manifestation of severe neuronal injury rather than an inciting pathophysiologic factor. Then, cellular inflammation and microgliosis would not be prominent in mild-to-moderate Alzheimer's disease and its observation at postmortem would reflect only the final phases of the disease process. Our results suggest a testable hypothesis that postmortem examinations would demonstrate increased binding of [^{11}C]PK 11195 only in the brains of Alzheimer's disease patients who were severely demented.

CONCLUSION

The mechanism for neuronal loss in Alzheimer's disease is unknown. It has been proposed that this injury may be in part mediated by an inflammatory response that initiates a reactive microgliosis and leads to cell injury (*2*). PET with [^{11}C]PK 11195, however, does not detect the presence of microgliosis or cellular inflammation in living Alzheimer's disease patients who are mildly-to-moderately demented.

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