

Quantitative Analysis of Peripheral Benzodiazepine Receptor in the Human Brain Using PET with ^{11}C -AC-5216

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Peripheral benzodiazepine receptor (PBR) is upregulated in activated glial cells and is therefore a useful biomarker for inflammation in the brain and neurodegenerative disorders. We developed a new PET radioligand, ^{11}C -AC-*N*-benzyl-*N*-ethyl-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9H-purin-9-yl)acetamide (^{11}C -AC-5216), that allows the imaging and quantification of PBRs in monkey and mouse brains. The aim of this study was to evaluate a quantification method of ^{11}C -AC-5216 binding in the human brain. **Methods:** A 90-min dynamic PET scan was obtained for each of 12 healthy men after an intravenous injection of ^{11}C -AC-5216. Regions of interest were drawn on several brain regions. Binding potential, compared with nondisplaceable uptake (BP_{ND}), was calculated by a nonlinear least-squares fitting (NLS) method with the 2-tissue-compartment model, and total volume of distribution (V_T) was estimated by NLS and graphical analysis methods. **Results:** BP_{ND} was highest in the thalamus (4.6 ± 1.0) and lowest in the striatum (3.5 ± 0.7). V_T obtained by NLS or graphical analysis showed regional distribution similar to BP_{ND} . However, there was no correlation between BP_{ND} and V_T because of the interindividual variation of K_1/k_2 . BP_{ND} obtained with data from a scan time of 60 min was in good agreement with that from a scan time of 90 min ($r = 0.87$). **Conclusion:** Regional distribution of ^{11}C -AC-5216 was in good agreement with previous PET studies of PBRs in the human brain. BP_{ND} is more appropriate for estimating ^{11}C -AC-5216 binding than is V_T because of the interindividual variation of K_1/k_2 . ^{11}C -AC-5216 is a promising PET ligand for quantifying PBR in the human brain.

Key Words: ^{11}C -AC-5216; peripheral benzodiazepine receptor; microglia; human brain; positron emission tomography

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Peripheral benzodiazepine receptor (PBR) is pharmacologically distinct from central benzodiazepine receptor (CBR). PBR is primarily found on mitochondrial membranes in various peripheral organs (1–4) and is mainly located in glial cells in the brain; its expression was found to be increased in activated microglia in vivo by brain injury (5,6). The increase in PBR density has thus been used as an indicator of neuronal damage and neurodegenerative disorders, such as Alzheimer disease (7,8). These findings then gave impetus to the development of an imaging agent labeled with a radioisotope, making it possible to visualize the distribution of PBR also in the animal brain in vivo (9–11). Specific radiolabeled ligands for PBR, such as ^{11}C -PK11195 and ^{11}C -DAA1106, have been used in many clinical studies. ^{11}C -PK11195 was first developed to characterize PBR in the human brain (12). ^{11}C -PK11195 binding was reported to have increased in the stroke region (13), plaque of multiple sclerosis (14–16), brain of Alzheimer disease (17,18), and epileptic foci (19). However, the relatively low uptake of ^{11}C -PK11195 in the brain limited its wider application (20,21). Therefore, to characterize PBR precisely using PET with advantages over ^{11}C -PK11195, we developed 2 novel radioligands, ^{11}C -DAA1106 (22,23) and ^{18}F -FEDAA1106 (24). These ligands exhibited more potent in vitro binding affinity for PBR than did PK11195 and displayed high selectivity against other receptors, including CBR (24,25). In vivo studies demonstrated that these ligands had higher uptake and specific binding in rodent and primate brains than did ^{11}C -PK11195 (22–24). We have reported a kinetic analysis method for ^{11}C -DAA1106 and ^{18}F -FEDAA1106 binding in the human brain (26,27) and a broad increase of PBR binding in the Alzheimer disease brain using ^{11}C -DAA1106 (28).

N-benzyl-*N*-ethyl-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9H-purin-9-yl)acetamide (AC-5216) is a new candidate as a PET ligand for PBR (29). Affinity of AC-5216 for PBR

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(inhibition constant, 0.297 nM) was higher than PK11195 (0.602 nM) (29) and lower than DAA1106 (36–108 pM) (30). AC-5216 exhibited negligible affinity for CBR and other receptors, monoamine transporters, and ion channels. The synthesis of ^{11}C -labeled AC-5216 (^{11}C -AC-5216) and its high accumulation in the mouse brain have been reported (31). A PET study in monkeys demonstrated high uptake of ^{11}C -AC-5216 in the brain, and the binding was inhibited by unlabeled AC-5216 and PK11195 (31). ^{11}C -AC-5216 can be used for the clinical investigation of PBR expression and therefore also microglia activation in neurologic diseases, but the quantification method of ^{11}C -AC-5216 binding in the living human brain has not yet been established. In the present study, PET measurements with ^{11}C -AC-5216 were performed on healthy human subjects. We evaluated kinetic analysis methods for the quantification of ^{11}C -AC-5216 binding in the human brain.

MATERIALS AND METHODS

Subjects

Twelve male healthy volunteers (age range, 20–33 y; mean age $\pm$ SD, 24.6 ± 4.5 y) participated in this study. All volunteers were free of any somatic, neurologic, or psychiatric disorders, and they had no history of current or previous drug abuse. This study was approved by the ethics and radiation safety committees of the National Institute of Radiologic Sciences. Written informed consent was obtained from each subject.

Radioligand Preparation

^{11}C -AC-5216 was synthesized as described previously (31). In brief, AC-5216 and its desmethyl precursor were synthesized starting from commercially available compounds. ^{11}C -AC-5216 was radiosynthesized through the reaction of the precursor with ^{11}C - CH_3I in the presence of sodium hydride.

PET

An ECAT EXACT HR+ PET scanner system (CTI-Siemens) was used for all measurements. A head-fixation device was used to minimize head movement. A transmission scan for attenuation correction was obtained using a $^{68}\text{Ge}/^{68}\text{Ga}$ source. Dynamic PET scans were obtained after a 1-min intravenous slow bolus injection of ^{11}C -AC-5216 (354.6–385.1 MBq [372.8 ± 9.7 MBq]). The specific radioactivities were 93.7–224.2 GBq/ μmol (148.2 ± 35.6 GBq/ μmol) at the time of injection. Brain radioactivities were measured from 0 to 90 min (20×9 , 60×5 , 120×4 , 240×11 , and 300×6). MR images of the brain were acquired with a 1.5-T MRI scanner (Gyroscan NT; Philips). T1-weighted images were obtained at 1-mm slices.

Arterial Blood Sampling

To obtain the arterial input function, arterial blood samples were taken manually 32 times (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, and 150 s and 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 25, 30, 40, 50, 60, 70, 80, and 90 min after the radiotracer injection) during the PET scan. Each blood sample was centrifuged to obtain plasma and blood cell fractions, and the concentrations of radioactivity in whole blood and plasma were measured. Plasma protein binding was not determined in this study.

The percentage of unchanged ^{11}C -AC-5216 in plasma was determined by high-performance liquid chromatography (HPLC)

in 10 blood samples (3, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min after injection). Acetonitrile was added to each plasma sample, and the samples were then centrifuged. The supernatant was subjected to radio-high-performance liquid chromatography analysis (column, XBridge Prep C18 [Waters]; mobile phase, 50% acetonitrile). The plasma input function was defined as the radioactivity of plasma multiplied by the percentage of unchanged radioligand.

Regions of Interest (ROIs)

All MR images were coregistered to PET images using PMOD (version 2.8; PMOD Technologies). Eleven ROIs were drawn manually on the PET images with reference to coregistered MR images. ROIs were delineated for the cerebellum, dorsolateral prefrontal cortex, medial prefrontal cortex, parietal cortex, lateral temporal cortex, medial temporal cortex, occipital cortex, anterior cingulate cortex, posterior cingulate cortex, striatum, and thalamus.

Kinetic Model of ^{11}C -AC-5216

To describe the kinetics of ^{11}C -AC-5216 in the brain, the 2-tissue-compartment model with 4 rate constants (K_1 , k_2 , k_3 , and k_4) was used (32). The 3 compartments were defined as follows: C_P , the radioactivity concentration of unchanged radioligand in plasma (arterial input function); C_{ND} , the radioactivity concentration of nondisplaceable radioligand in brain, including nonspecifically bound and free radioligand; and C_S , the radioactivity concentration of radioligand specifically bound to receptors. The rate constants K_1 and k_2 represent the influx and efflux rates for radioligand diffusion through the blood–brain barrier. The rate constants k_3 and k_4 represent the radioligand transfers between the compartments for nondisplaceable and specifically bound radioligand. This model can be described by the following equations:

$$dC_{ND}(t)/dt = K_1 C_P(t) - (k_2 + k_3) C_{ND}(t) + k_4 C_S(t) \quad \text{Eq. 1}$$

$$dC_S(t)/dt = k_3 C_{ND}(t) - k_4 C_S(t) \quad \text{Eq. 2}$$

$$C_T(t) = C_{ND}(t) + C_S(t). \quad \text{Eq. 3}$$

$C_T(t)$ is the total radioactivity concentration in a brain region measured by PET. In this analysis, blood volume (V_b) was fixed to be 0.05 mL/mL (26).

Calculation of ^{11}C -AC-5216 Binding

In the nonlinear least-squares fitting (NLS) method, ^{11}C -AC-5216 bindings were expressed as binding potentials relative to nondisplaceable binding (BP_{ND}). BP_{ND} can be expressed as:

$$BP_{ND} = f_{ND} B_{\max} / K_d = k_3 / k_4. \quad \text{Eq. 4}$$

$B_{\max}$ is the receptor density, $1/K_d$ is the affinity, and f_{ND} is the free fraction of ligand in the nondisplaceable tissue compartment. We also calculated the total distribution volume (V_T), which can be expressed as:

$$V_T = K_1 / k_2 (1 + k_3 / k_4). \quad \text{Eq. 5}$$

In the graphical analysis (GA) method, GA yields V_T from the arterial input function and the tissue time–activity curve (33). Parameters are estimated from the equation:

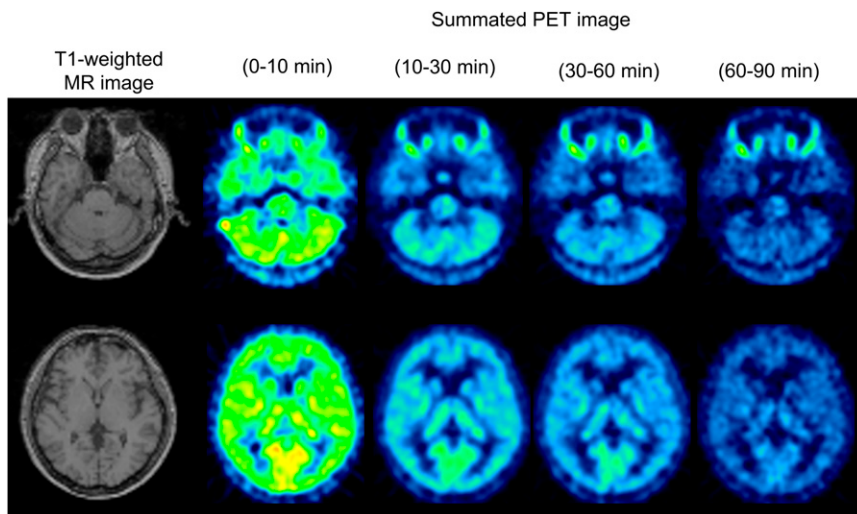


FIGURE 1. Typical summed PET images from 0 to 10, 10 to 30, 30 to 60, and 60 to 90 min of ^{11}C -AC-5216 and T1-weighted MR images.

$$\frac{\int_0^T \text{CT}(t)dt}{\text{CT}(T)} = V \frac{\int_0^T \text{CP}(t)dt}{\text{CT}(T)} + b \quad \text{for } T > t^*, \quad \text{Eq. 6}$$

where V represents V_T after equilibration time t^* . Because b becomes constant only after equilibration time t^* , V and b are estimated as a slope and an intercept, respectively, using the points of $T > t^*$. The starting time for linear regression t^* was set at 30 min.

In these methods, the PET scan data for 90 min and those for 60 min were used. For these analyses, PMOD was used.

RESULTS

Typical summed PET images (0–10, 10–30, 30–60, and 60–90 min) and T1-weighted MR images are shown in [Fig. 1] Figure 1. The distribution of radioactivity was widespread and fairly uniform in the gray matter of the cerebral cortices and cerebellum, striatum, and thalamus. Typical

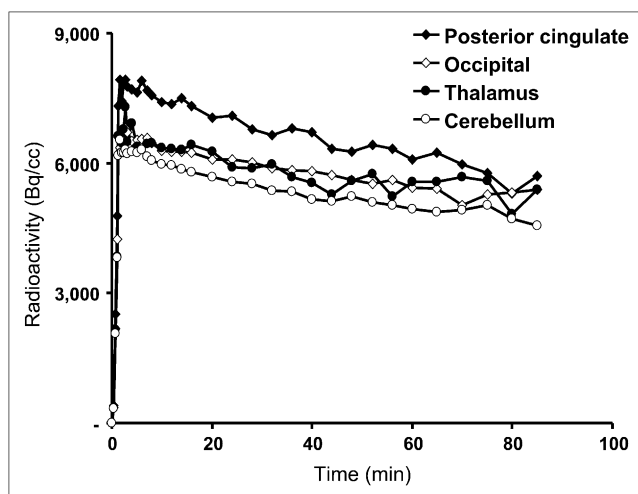


FIGURE 2. Typical time-activity curves of ^{11}C -AC-5216 in occipital cortex, posterior cingulate cortex, thalamus, and cerebellum.

time-activity curves of measured brain regions are shown in Figure 2. After an intervenous injection of ^{11}C -AC-5216, [Fig. 2] radioactivity peaked at about 2–3 min, followed by slow washout. The average percentage of unchanged ^{11}C -AC-5216 in plasma was $99.8\% \pm 0.2\%$ at 3 min, $89.1\% \pm 4.5\%$ at 30 min, and $69.6\% \pm 12.0\%$ at 90 min (mean $\pm$ [Fig. 3] SD) (Fig.3).

The K_1 , K_1/k_2 , k_3 , k_3/k_4 , and V_T values estimated by NLS and V_T estimated by GA are listed in Tables 1 and 2 for [Table 1] scan times of 90 and 60 min, showing the mean and coefficient of variation (COV) (SD/mean [%]) of all ROIs. [Table 2] Although the mean V_T values estimated by GA were larger than those by NLS in all regions, good correlation was observed between them ($r = 0.93$, $P < 0.001$) (Fig. 4). [Fig. 4] However, no correlation was observed between BP_{ND}

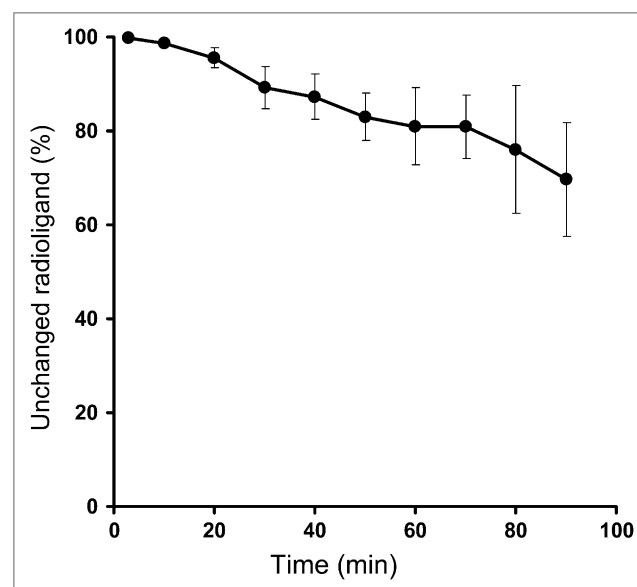


FIGURE 3. Average percentage of unchanged ^{11}C -AC-5216 in plasma. Bars indicate SD.

TABLE 1. Average Values of Parameters Estimated by Each Method with 90-Minute Scan Time ($n = 12$)

Region	Method					
	NLS					GA (V_T)
	K_1	K_1/k_2	k_3	k_3/k_4	V_T	
Cerebellum	0.184 (24.4)	1.40 (28.7)	0.091 (32.8)	4.25 (26.7)	5.70 (25.5)	6.64 (22.9)
Dorsolateral frontal	0.174 (29.8)	1.60 (22.6)	0.072 (25.7)	3.47 (19.4)	5.49 (25.1)	6.40 (22.5)
Medial frontal	0.171 (28.2)	1.46 (19.5)	0.079 (24.4)	3.61 (20.9)	5.21 (26.6)	6.14 (24.0)
Parietal	0.175 (27.6)	1.55 (19.3)	0.076 (23.2)	3.53 (20.5)	5.41 (25.2)	6.42 (22.4)
Lateral temporal	0.170 (27.2)	1.50 (22.5)	0.079 (24.0)	3.75 (23.0)	5.49 (25.8)	6.45 (22.8)
Medial temporal	0.146 (28.1)	1.33 (25.8)	0.077 (31.2)	4.40 (28.6)	5.72 (31.4)	6.08 (27.9)
Occipital	0.197 (23.6)	1.47 (24.7)	0.097 (22.5)	4.18 (19.3)	6.08 (30.0)	7.08 (25.4)
Anterior cingulate	0.172 (30.8)	1.38 (23.8)	0.075 (32.6)	4.11 (26.9)	5.58 (28.9)	6.22 (25.3)
Posterior cingulate	0.222 (23.9)	1.44 (34.4)	0.110 (30.3)	4.37 (24.6)	5.97 (28.0)	7.08 (25.9)
Striatum	0.159 (22.5)	1.43 (17.6)	0.074 (23.1)	3.46 (20.2)	4.94 (30.3)	5.66 (22.4)
Thalamus	0.188 (29.6)	1.51 (24.2)	0.085 (34.9)	4.64 (20.5)	6.86 (26.0)	7.39 (24.7)

Values are mean, with COV in parentheses.

estimated by NLS and V_T estimated by NLS or GA for all regions (V_T by NLS, $r = 0.31$; V_T by GA, $r = 0.19$) (Figs. 5A and 5B). BP_{ND} values estimated by NLS with a scan time of 60 min were in good agreement with those with a scan time of 90 min ($r = 0.87$, $P < 0.001$) (Fig. 6). V_T values estimated by NLS or GA with a scan time of 60 min were in good agreement with those with a scan time of 90 min (V_T by NLS, $r = 0.82$, $P < 0.001$; V_T by GA, $r = 0.92$, $P < 0.001$).

DISCUSSION

This study describes the first, to our knowledge, quantitative analysis of PET measurement with ^{11}C -AC-5216 for PBR binding in the living human brain. Intravenous injection of ^{11}C -AC-5216 showed radioactivity to peak at about 2–3 min, followed by slow washout, similar to the pattern of ^{11}C -DAA1106 in animal (31) and human studies (26). BP_{ND} was highest in the thalamus in this study, consistent with ^{11}C -PK11195 (18) and ^{11}C -DAA1106 (26)

studies. BP_{ND} was relatively higher in the medial temporal cortex and cerebellum and lower in the striatum, which was also consistent with ^{11}C -DAA1106 (26). V_T obtained by NLS or GA showed regional distribution similar to BP_{ND} in this study, whereas V_T was highest in the cerebellum in the ^{11}C -DAA1106 study (26).

Although V_T showed regional distribution similar to BP_{ND} , BP_{ND} did not correlate with V_T obtained by NLS or GA. The COVs of K_1/k_2 (mean, 27.5; range, 17.6–34.4) were larger than those of k_3/k_4 (mean, 22.8; range, 19.3–26.9), indicating that the interindividual variation of V_T is mainly caused by the variation of K_1/k_2 rather than k_3/k_4 . The same observation was documented in the ^{11}C -DAA1106 study, in which we reported that interindividual variation of K_1/k_2 may have been due to errors in plasma input functions, including interindividual variation of plasma protein binding of the radioligand, and that BP_{ND} with the NLS method was the most appropriate for the quantification of ^{11}C -DAA1106 binding (26). The COVs of

TABLE 2. Average Values of Parameters Estimated by Each Method with 60-Minute Scan Time ($n = 12$)

Region	Method					
	NLS					GA (V_T)
	K_1	K_1/k_2	k_3	k_3/k_4	V_T	
Cerebellum	0.183 (25.1)	1.38 (25.5)	0.097 (28.3)	3.89 (26.5)	5.22 (24.5)	6.06 (21.5)
Dorsolateral frontal	0.172 (31.0)	1.63 (18.7)	0.071 (31.3)	3.35 (22.0)	5.37 (22.9)	6.04 (23.9)
Medial frontal	0.171 (28.8)	1.42 (18.1)	0.085 (28.1)	3.48 (21.5)	4.91 (25.1)	5.66 (23.3)
Parietal	0.175 (28.3)	1.53 (17.4)	0.079 (27.0)	3.46 (20.8)	5.25 (23.7)	6.10 (22.8)
Lateral temporal	0.170 (28.1)	1.45 (19.8)	0.087 (28.3)	3.64 (23.8)	5.17 (23.7)	6.02 (22.3)
Medial temporal	0.147 (29.0)	1.27 (25.3)	0.089 (36.8)	4.31 (27.5)	5.32 (29.9)	5.50 (26.9)
Occipital	0.198 (24.5)	1.41 (22.1)	0.107 (26.9)	4.06 (19.5)	5.67 (26.0)	6.68 (22.5)
Anterior cingulate	0.173 (32.0)	1.31 (22.9)	0.089 (42.7)	3.88 (26.6)	4.94 (23.7)	5.47 (22.7)
Posterior cingulate	0.225 (24.4)	1.43 (35.7)	0.139 (35.0)	4.35 (26.2)	5.38 (27.9)	6.36 (25.6)
Striatum	0.159 (23.2)	1.46 (15.2)	0.073 (19.4)	3.39 (19.1)	4.86 (25.5)	5.35 (22.2)
Thalamus	0.189 (30.5)	1.56 (23.9)	0.094 (40.8)	4.49 (22.0)	6.45 (28.0)	6.66 (25.5)

Values are mean, with COV in parentheses.

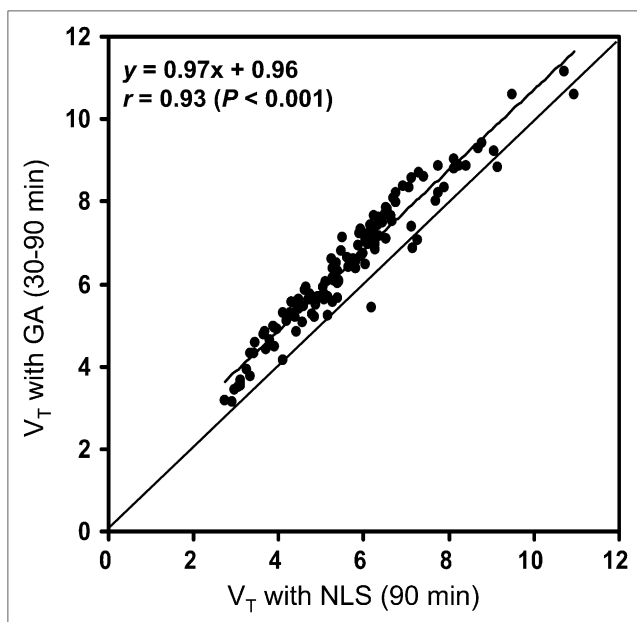


FIGURE 4. Relationship between V_T estimated by NLS method and V_T estimated by GA method in all regions.

K_1/k_2 of ^{11}C -AC-5216 were similar to those of ^{11}C -DAA1106 (mean, 26.2; range, 22.0–30.4). Thus, we concluded that BP_{ND} is more appropriate for the quantification of ^{11}C -AC-5216 binding than V_T .

For clinical research, a short scan time is preferred. In the present study, the BP_{ND} values obtained by the NLS method with a scan time 60 min were in good agreement with those obtained with a scan time of 90 min ($r = 0.87$, $P < 0.001$). V_T values estimated by NLS or GA with a scan time of 60 min were also in good agreement with those with a scan time of 90 min (V_T by NLS, $r = 0.82$, $P < 0.001$; V_T by GA, $r = 0.92$, $P < 0.001$). These results suggest that the scan time of 60 min would be valid for clinical studies.

^{11}C -AC-5216 is suitable for the *in vivo* quantification of PBR in human subjects, because our recent investigation of

animal models has indicated roles of PBR-positive microglia and astrocytes in neurodegenerative processes (34,35). Indeed, the monitoring of PBR levels in living brains could provide mechanistic insights into Alzheimer disease and allied dementias, in consideration of the intimate links between amyloid plaques and reactive astrocytes expressing PBR in mice transgenic for amyloid precursor protein (APP) (35), and between the accumulation of phosphorylated tau proteins and PBR-positive microglia in transgenic mice overexpressing a frontotemporal dementia with parkinsonism-17 (FTDP-17) mutant tau (35,36). Although we have also revealed elevated levels of PBR in multiple regions of Alzheimer disease brains as measured with ^{11}C -DAA1106 (28), detection of earlier changes in this component is required for elucidating how the gliotic reactions extensively spread in the course of the aging–disease continuum. In this consideration, our PET assays of PBR in APP and tau transgenics have shown the superiority of ^{11}C -AC-5216 to other existing radioligands in sensitively capturing glial responses to the hallmark pathologies in these animals (Jun Maeda et al., unpublished data), supporting the potential utility of ^{11}C -AC-5216 for the pursuit of neurodegenerative pathologies in humans from an incipient stage.

Accuracy in the measurements of PBR by means of PET would also be critical for gaining statistical power in assessing the neuroinflammatory status after therapies of neurologic conditions. As demonstrated by our PET scans of APP transgenic mice receiving an antibody against amyloid β peptide using ^{18}F -FEDAA1106 and an amyloid-binding radiotracer, ^{11}C -Pittsburgh compound-B (37), PBR and amyloid ligands may conjunctively allow clarification of relationships between amyloid removal and glial activations yielded by the amyloid β immunization. In addition, the amounts of initially existing amyloid were found to positively correlate with the neuroinflammation provoked by the immunization (37), and this observation highlights the need for rigorous regulation of the glial response within an appropriate range to avoid treatment-induced microglial overactivations, which may accelerate

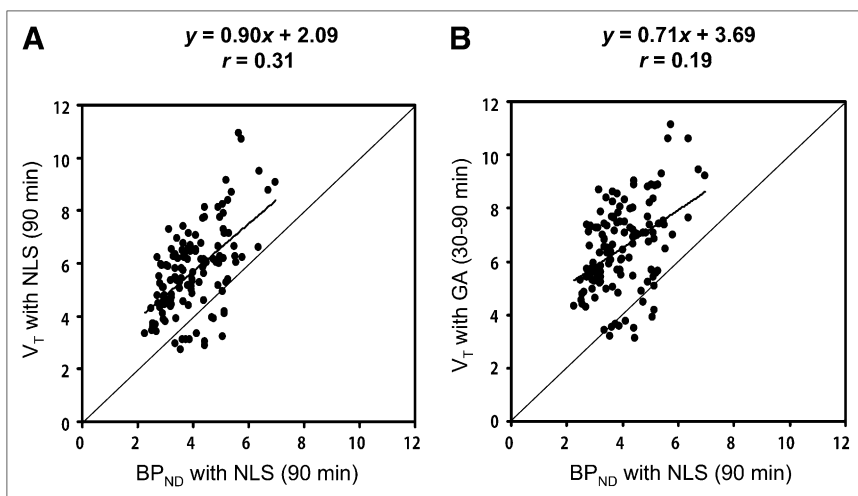


FIGURE 5. Relationship between BP_{ND} estimated by NLS method and V_T estimated by NLS (A) or GA (B) method in all regions.

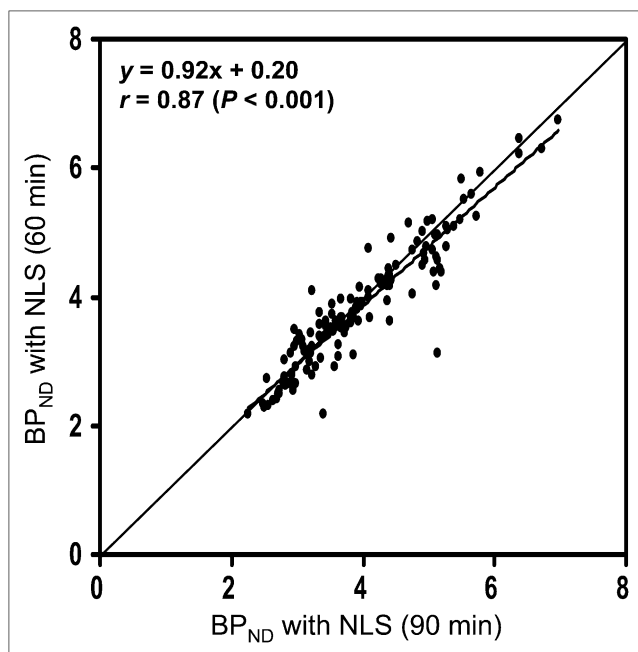


FIGURE 6. Relationship between BP_{ND} estimated by NLS method with scan time of 90 min and that with scan time of 60 min in all regions.

tau-associated neuropathologies as proven by our previous study on FTDP-17 mutant tau transgenic mice (36). For this purpose, the analytic methods for ^{11}C -AC-5216-PET data established here would provide an acceptably precise index of therapeutic and adverse effects.

Longitudinal in vivo PBR imaging with ^{11}C -AC-5216 could be used as a powerful tool for quantitatively estimating the neuroinflammatory pathology and outcomes of disease-modifying treatments in neurodegenerative disorders exemplified by Alzheimer disease.

CONCLUSION

The regional distribution of ^{11}C -AC-5216 was in good agreement with previous PET studies using PBR ligand in the human brain, for example, ^{11}C -PK11195 and ^{11}C -DAA1106. BP_{ND} estimated from NLS did not correlate with V_T estimated from NLS or GA. In this study, the variation of K_1/k_2 between individuals was larger than that of k_3/k_4 . Thus, BP_{ND} is more appropriate than is V_T for the quantitative analysis of ^{11}C -AC-5216. ^{11}C -AC-5216 is a promising PET ligand for quantifying PBR in the human brain.

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