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In vitro and ex vivo autoradiography studies on peripheral-type benzodiazepine receptor binding using [^{11}C]AC-5216 in normal and kainic acid-lesioned rats

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Abstract

AC-5216 was reported as a novel ligand for peripheral-type benzodiazepine receptor (PBR) with a different chemical structure from DAA1106 analogues. This ligand had potent affinity for PBR and selectivity for PBR over other neurotransmitters. We have previously labeled AC-5216 using positron-emitter ^{11}C. The aim of this study was to evaluate [^{11}C]AC-5216 in a rat brain model with neuroinflammation using an autoradiography (ARG) technique. In vitro ARG of normal rat brain showed that [^{11}C]AC-5216 accumulated highly in the olfactory bulb, choroid plexus and cerebellum. The distribution pattern agreed with the localization of PBR in the rodent brain. Infusion of kainic acid (KA: 1, 2.5 and 5 nmol) into the rat striatum resulted in neuroinflammation. In vitro and ex vivo ARG revealed that the radioactivity level of [^{11}C]AC-5216 was increased significantly in the KA-lesioned striatum compared to the non-lesioned striatum. Increasing the amount of KA infused into the striatum augmented radioactivity in the striatum as well as the cerebral cortex and hippocampus of the lesioned side. Treatment with a large amount of non-radioactive AC-5216 or PK11195 inhibited the binding of [^{11}C]AC-5216 and diminished the difference of radioactivity levels between the lesion and non-lesioned sides. These results demonstrated that [^{11}C]AC-5216 had high specific binding to PBR in the KA-lesioned rat brain. Thus, [^{11}C]AC-5216 is a promising PET ligand for imaging PBR in a brain with neuroinflammation.

Keywords: [^{11}C]AC-5216; Peripheral-type benzodiazepine receptor; Neuroinflammation; Autoradiography

Peripheral-type benzodiazepine receptor (PBR) is widely expressed in the peripheral tissues including the adrenal gland, kidney, lung, heart and testis [2,7]. In the central nervous system, PBR is distributed in the olfactory bulb and cerebellum of rodents [4,7,20] and in the occipital cortex of primates [24,26,30], but the normal brain has a lower level of PBR than peripheral tissues. Many studies have documented the use of PBR as a biomarker of brain injury and neuroinflammation in experimental animals and in human neurodegenerative diseases such as Alzheimer's disease [30], Huntington's disease [28], multiple sclerosis [11] and stroke-induced brain injury [27]. In the brain, PBR is predominantly localized in microglia and astrocytes [9,19], and PBR expression is markedly increased following brain injury or neuroinflammation.

Several positron emission tomography (PET) ligands for imaging PBR have been reported, such as [^{11}C]PK11195 [17], [^{11}C]DPA-713 [6] and [^{11}C]PBR28 [16]. Recently, [^{11}C]DAA1106 [31] and [^{18}F]FEDAA1106 [33] have been developed as novel PET ligands for PBR imaging in our laboratory. These ligands had high specific and selective binding to PBR in the rodent and primate brain. Currently, [^{11}C]DAA1106 and [^{18}F]FEDAA1106 are being used to investigate PBR in the human brain in our facility to elucidate the relationship between PBR and brain diseases.

Recently, AC-5216 was reported as a novel PBR ligand with a different chemical structure from DAA1106 analogues [18]. The pharmacological profile of AC-5216 demonstrated that this ligand had higher affinity for PBR in rat brain (Ki: 0.297 nM)

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than PK11195 (Ki: 0.602 nM), and selectivity for PBR over other neurotransmitters [18]. Further, AC-5216 has a more similar binding site in the PBR domain to PK11195 than other PBR ligands such as Ro5-4864 [18]. AC-5216 has a moderate lipophilicity ($c\log P$: 3.5, $\log D$: 3.3, [29]), which is a prerequisite for a favorable PET ligand guaranteeing high uptake and low non-specific binding in the brain.

We have previously labeled this ligand using positron-emitter ^{11}C and evaluated [^{11}C]AC-5216 by determining its intratumoral distribution relative to PBR in fibrosarcoma-bearing mice [1]. In the present study, we continued to evaluate this radioligand in a rat model with neuroinflammation with the expectation that this radioligand could be used to image PBR in the brain. Using the autoradiography (ARG) technique, we determined whether [^{11}C]AC-5216 could be used to image PBR in a kainic acid (KA)-lesioned rat brain. To induce neuroinflammation in the rat brain, KA was infused into the striatum. This is a well-known rat model of neuroinflammation connected to Huntington's disease [10].

Non-radioactive AC-5216 and desmethyl precursor were prepared in our laboratory [1]. [^{11}C]AC-5216 was synthesized by *N*-methylation of desmethyl AC-5216 with [^{11}C]CH_3I. PK11195 and kainic acid (KA) were purchased from Sigma (St. Louis, MO). Male Wistar rats (7–9 weeks old) were maintained and handled in accordance with recommendations by the US National Institutes of Health and our guidelines (National Institute of Radiological Sciences, NIRS, Chiba, Japan). All animal experiments were approved by the Animals Ethics Committee of NIRS.

The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotactic frame. KA (1, 2.5 or 5 nmol in PBS of 2 μL) was infused into the right striatum according to the following stereotaxic coordinates: 0.2 mm anterior to the bregma, 3.0 mm lateral to the midline and 5.0 mm ventral from the skull surface [23]. The infusion was continued for 5 min at 0.4 μL/min, and the infusion needle was left in place for an additional 5 min before being slowly withdrawn. For comparison, PBS (2 μL) was infused into the left striatum. After suturing the skin, the rats were allowed to recover. The ARG study was conducted at the 7th day after KA infusion.

Normal or KA-lesioned rats were sacrificed by decapitation under ether anesthesia, and their brains were quickly removed and frozen on powdered dry ice. Brain sagittal or horizontal sections (20 μm) were cut in a cryostat microtome (HM560, Carl Zeiss, Germany) and thaw-mounted on MAS-coated glass slides (Matsunami Glass Ind., Tokyo), which were then dried and stored at −80 °C until used for experiments. Brain sections were preincubated at 25 °C for 20 min in 50 mM Tris–HCl (pH 7.4) buffer. After preincubation, these sections were incubated at 25 °C for 30 min in buffer containing [^{11}C]AC-5216 (0.4–0.5 nM; 7 MBq; specific activity, 68–106 GBq/μmol). Non-radioactive AC-5216 or PK11195 (10 μM) was used for the blocking study. After incubation, the sections were washed twice for 2 min each time with cold buffer, dipped in cold distilled water, and dried with cold air. These sections were then placed in contact with imaging plates (BAS-MS2025, Fuji Photo Film, Tokyo). Autoradiograms were obtained and quantified using a Bio-Imaging Analyzer System (BAS5000, Fuji Photo Film). Radioactivity levels in the brain regions were measured and expressed as photo-stimulated luminescence (PSL)/area (mm^2).

For ex vivo ARG, [^{11}C]AC-5216 (37–74 MBq) was injected intravenously into KA-lesioned rats either alone or in combination with non-radioactive AC-5216 (1 mg/kg in distilled water containing 10% ethanol and Tween) or PK11195 (10 mg/kg in distilled water containing 10% ethanol and Tween). Rats were sacrificed by decapitation at 30 min after radioligand injection. Brain slices (20 μm) were prepared and contacted with imaging plates. Radioactivity levels in the regions were measured using the same method as described above. Data values were expressed as the mean ± S.D. (in each group) and analyzed using Student's *t*-test.

First, we examined the in vitro distribution of [^{11}C]AC-5216 in normal rat brain as shown in Fig. 1. High radioactivity was found in the olfactory bulb, choroid plexus and cerebellum (Fig. 1A). The distribution pattern was in agreement with PBR localization in the rodent brain [4,7,20,31]. In the presence of non-radioactive AC-5216 (10 μM, Fig. 1B) or PK11195 (10 μM, Fig. 1C), radioactivity was decreased significantly in the corresponding regions. Compared to the radioactivity level of [^{11}C]AC-5216 alone (Fig. 1A), radioactivity was reduced by AC-5216 to 31% in the olfactory bulb, 30% in the choroid plexus, and 58% in the cerebellum, respectively (Fig. 1D). PK11195 also decreased the radioactivity of [^{11}C]AC-5216 in the brain regions to a similar level as that by AC-5216. The in vitro result demonstrated that [^{11}C]AC-5216 is a specific radioligand to PBR,

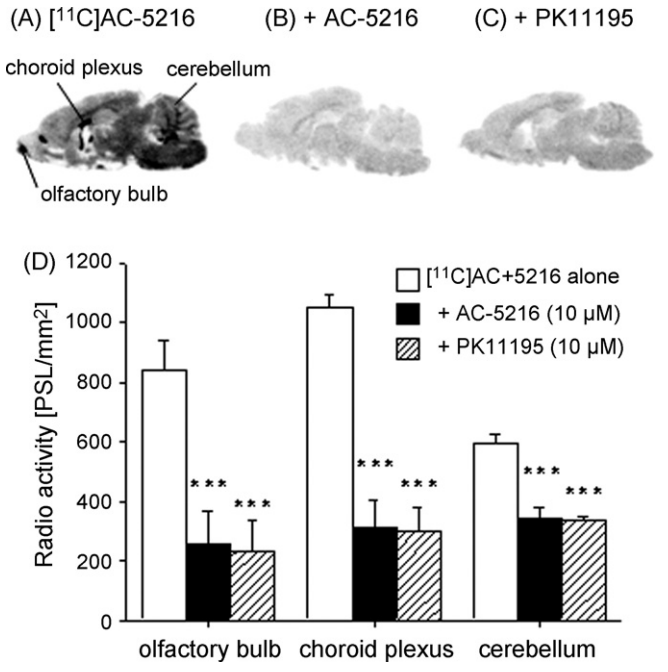

Fig. 1. In vitro autoradiograms in normal rat brain. (A) [^{11}C]AC-5216 alone; (B) [^{11}C]AC-5216 with AC-5216 (10 μM); (C) [^{11}C]AC-5216 with PK11195 (10 μM) and (D) quantitative result. The radioactivity levels determined in these regions are expressed as PSL/mm^2. Values are given as the means ± S.D. for four rats per group. $^{***}P < 0.001$ vs. [^{11}C]AC-5216 alone.