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ScienceDirect
Neuroscience Letters 411 (2007) 200–205
Neuroscience Letters
www.elsevier.com/locate/neulet

PET imaging with [^{11}C]PBR28 can localize and quantify upregulated peripheral benzodiazepine receptors associated with cerebral ischemia in rat

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Received 12 July 2006; received in revised form 2 September 2006; accepted 7 September 2006

Abstract

Peripheral benzodiazepine receptors (PBRs) are upregulated on activated microglia. We recently developed a promising positron emission tomography (PET) ligand, [^{11}C]PBR28, with high affinity and excellent ratio of specific to nonspecific binding. We assessed the ability of [^{11}C]PBR28 PET to localize PBRs in a rat permanent middle cerebral artery occlusion (MCAO) model of neuroinflammation. [^{11}C]PBR28 was intravenously administered to rats at 4 and 7 days after permanent MCAO. In all experiments, arterial blood was sampled for compartmental modeling of regional distribution volumes, and rat brains were sampled after imaging for *in vitro* [^{3}H]PK 11195 autoradiography and histological evaluation. [^{11}C]PBR28 PET and [^{3}H]PK 11195 autoradiography showed similar areas of increased PBRs, especially in the peri-ischemic core. Results from these in vivo and in vitro methods were strongly correlated. In this first study to demonstrate neuroinflammation *in vivo* with small animal PET, [^{11}C]PBR28 had adequate sensitivity to localize and quantify the associated increase in PBRs.
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Keywords: Peripheral benzodiazepine receptor; Neuroinflammation; Small animal PET; Cerebral ischemia; Activated microglia

Although the peripheral benzodiazepine receptor (PBR) was initially discovered in organs such as kidney and lung, it was later identified in the central nervous system [16]. PBR is distinct from the central benzodiazepine allosteric site that is associated with $GABA_A$ receptors. In normal conditions, PBR is expressed in low levels in some neurons and glial cells. PBR can be a clinically useful marker to detect neuroinflammation, because activated microglial cells in inflammatory areas express much greater levels of PBR than those in resting conditions [1].

PBR has been imaged with positron emission tomography (PET) using [*N*-methyl-^{11}C]1-(2-chlorophenyl-*N*-methylpropyl)-3-isoquinoline carboxamide (PK 11195), either as racemate or (*R*)-enantiomer. However, a low ratio of specific to nonspecific binding may limit its sensitivity to detect therapeutic interventions. Its relatively high lipophilicity ($c\log P = 5.3$) may have caused high nonspecific binding in brain [9]. We developed a new radioligand, *N*-acetyl-*N*-(2-[^{11}C]methoxybenzyl)-2-phenoxy-5-pyridinamine ([^{11}C]PBR28) which showed much greater specific signal than [^{11}C]PK 11195 in nonhuman primates [3]. PBR28 is a close analog of [methoxy-^{11}C](*N*-5-fluoro-2-phenoxyphenyl)-*N*-(2,5-dimethoxybenzyl)acetamide (DAA1106) developed by Zhang et al. [19] and that was used to localize neuroinflammation with *in vitro* tissue sections [10]. The purpose of this study was to determine whether increased PBRs in a rat permanent middle cerebral artery occlusion (MCAO) model could be measured *in vivo* with [^{11}C]PBR28 and a small animal PET scanner. If successful in this model, [^{11}C]PBR28 could be used to explore the pathophysiology of neuroinflammation associ-

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0304-3940/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.neulet.2006.09.093

ated with human disorders such as cerebral ischemia, multiple sclerosis, Alzheimer's disease, and Parkinson's disease.

All procedures were performed in compliance with Guide for Care and Use of Laboratory Animals. Details of the surgical procedures for rat permanent MCAO model are described previously [15]. Animals were anesthetized with 3% halothane in a mixture of 30% O_2 and 70% NO_2, and a 4-0 nylon suture with a silicon-coated tip was inserted from the left external carotid artery to the left internal carotid artery and then to the Circle of Willis to occlude the origin of the left middle cerebral artery.

N-Acetyl-*N*-(2-methoxybenzyl)-2-phenoxy-5-pyridinamine [11] has moderate lipophilicity ($c\log P = 2.98$) and high affinity for PBR ($IC_{50} = 0.6$ nM measured *versus* [^{3}H]PK 11195). Selectivity of PBR28 was screened at 10 μM and found to have <50% displacement of the target radioligand for the following receptors: 11 subtypes of serotonin receptor, 8 subtypes of adrenergic receptor, 4 subtypes of dopamine receptor, 4 subtypes of histamine receptor, 2 subtypes of muscarinic cholinergic receptor, and dopamine, norepinephrine, and serotonin transporters. As expected, PBR28 showed $K_i > 10$ μM for several central benzodiazepine receptor subtypes (*i.e.*, $GABA_A$ receptor). PBR28 was labeled by ^{11}C-methylation of the *O-desmethyl* precursor [3]. The specific radioactivity of [^{11}C]PBR28 at the time of injection was 59.7 ± 2.7 GBq/μmol with this and subsequent data expressed as mean ± S.D.

Four male Sprague–Dawley rats with permanent MCAO (239 ± 66 g) were used under 1–1.5% isoflurane anesthesia. At 4 and 7 days after permanent MCAO, two bolus and two bolus plus infusion (B/I) studies were performed with arterial blood sampling. Details of the experimental procedures are described previously [5]. PET data were acquired with the Advanced Technology Laboratory Animal Scanner (ATLAS).

In two bolus studies with 120 min scan, [^{11}C]PBR28 (activity: 47 and 53 MBq) was injected intravenously over 6 min. B/I experiments were acquired with two different bolus to infusion ratios (B/I = 2 and 5 h). The bolus portion of the activity was administered over 3 min, after which the pump administered the remaining activity at a constant rate over 150 min. In total, the animals received 185 and 259 MBq from the bolus and infusion components. PK 11195 (10 and 20 mg/kg, i.v.) was administered at 60 min to measure specific binding.

In all experiments, arterial blood samples were collected in heparin-coated tubes (Thomas Scientific, Swedesboro, NJ, USA) eight times between 0 and 10 min and at 20, 40, 60, 90, 120, and 150 min. The volume was 100 μL for the initial eight samples and 200 μL for the last samples. Body temperature was maintained by a heating pad and monitored with a rectal temperature probe.

PET images were reconstructed with a 3D ordered subset expectation maximization algorithm, achieving 1.7 mm full-width at half maximum resolution [17]. Image data were not corrected for attenuation or scatter. Head movements were corrected using Statistical Parametric Mapping2 (SPM2, Wellcome Department of Cognitive Neurology, London, UK), and an average image was created from all realigned images. Within the resolution of the PET images, we visually identified the approximate level of the coronal section relative to an atlas of the rat brain [13]. We then visually selected the autoradiographic and histological sections corresponding to the PET tomograph. Regions of interest were manually placed over the ischemic core, peri-ischemic core, and contralateral side on the averaged PET images based on morphological changes in stained histological sections. Brain uptake of radioactivity was decay corrected to injection time and expressed as percent standard uptake value (%SUV), which normalizes for injected activity and body weight.

$$\%\mathrm{SUV} = \left[\frac{\text{activity per gram tissue}}{\text{injected activity}}\right] \times \text{gram body weight} \times 100$$

Plasma samples were mixed with acetonitrile containing reference PBR28. Distilled water was added and mixed well. Total radioactivity in this solution was measured with a calibrated gamma counter. Deproteinized plasma samples were centrifuged at $10{,}000 \times g$ for 1 min to remove denatured proteins. The supernatant was then analyzed directly by reversed phase high-performance liquid chromatography (HPLC). The percent recovery of radioactivity in the supernatant was calculated relative to that in the precipitate.

As described previously [5], total distribution volume (V'_T) was measured by obtaining arterial input function with 60 min data before displacement in each experiment and by applying nonlinear least-squares fitting with an unconstrained two-compartment model using PMOD 2.65. The standard errors of nonlinear least-squares fit were determined from the covariance matrix and expressed as a percentage of the value estimation relative to the estimated values (coefficient of variation, %COV).

Rats were decapitated after PET imaging, and the brains were quickly removed, frozen in powdered dry ice, and stored at −70 °C until sectioning. Cryostat sections (20 μm thick) were thaw-mounted onto poly-L-lysine coated glass slides (Sigma–Aldrich, Natick, MA, USA). PBRs were labeled with [^{3}H]PK 11195 (PerkinElmer Life and Analytical Sciences Inc., Boston, MA, USA, specific activity 71.1 Ci/mmol; 1.0 mCi/mL), using a methodology adapted from the literature [7]. Total binding was determined with 1 nM [^{3}H]PK 11195, and nonspecific binding was determined on consecutive sections in the presence of excess (20 μM) unlabeled PK 11195. Tissue sections and calibrated tritium scales (Amersham Biosciences Inc., Piscataway, NJ, USA) were opposed to a ^{3}H imaging plate (TR2025, Fujifilm, Stamford, CT, USA) for 7 days. Images were analyzed with Multi Gauge® (ver. 3.0, Fujifilm).

For a histological evaluation, serial frozen sections (20 μm thick) were stained with 0.5% cresyl violet, dehydrated through graded alcohols (75, 95, and100%), dipped in xylene, and coverslipped.

Correlation was assessed with the Pearson correlation coefficients. All statistical tests were considered significant at $P < 0.05$. SPSS 12.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Histological sections confirmed that the cerebral artery occlusion caused brain ischemia (Fig. 1C). The core of the ischemic lesion showed poor cresyl violet staining and consisted of loose necrotic tissue with partial cavitation (panel 3 in Fig. 1D). The