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In vivo imaging of neuroinflammation in the rodent brain with [^{11}C]SSR180575, a novel indoleacetamide radioligand of the translocator protein (18 kDa)

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Abstract

Purpose Neuroinflammation is involved in neurological disorders through the activation of microglial cells. Imaging of neuroinflammation with radioligands for the translocator protein (18 kDa) (TSPO) could prove to be an attractive biomarker for disease diagnosis and therapeutic evaluation. The indoleacetamide-derived 7-chloro-*N,N*,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide, SSR180575, is a selective high-affinity TSPO ligand in human and rodents with neuroprotective effects.

Methods Here we report the radiolabelling of SSR180575 with ^{11}C and in vitro and in vivo imaging in an acute model of neuroinflammation in rats.

Results The image contrast and the binding of [^{11}C]SSR180575 are higher than that obtained with the isoquinoline-based TSPO radioligand, [^{11}C]PK11195. Competition

studies demonstrate that [^{11}C]SSR180575 has high specific binding for the TSPO.

Conclusion [^{11}C]SSR180575 is the first PET radioligand for the TSPO based on an indoleacetamide scaffold designed for imaging neuroinflammation in animal models and in the clinic.

Keywords Translocator protein (18 kDa) · Peripheral benzodiazepine receptor · Neuroinflammation · ^{11}C -SSR180575 · ^{11}C -PK11195

Introduction

The translocator protein (18 kDa) (TSPO), previously known as the peripheral benzodiazepine receptor, is a mitochondrial membrane protein found in very low amounts in the brain. However, its cerebral expression is dramatically increased

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during microglial activation in response to brain insults [1]. The development of radioligands for the TSPO has therefore attracted considerable interest for nuclear imaging of neuroinflammation [2]. After PK11195 was first labelled with ^{11}C , several new TSPO radioligands were introduced during the last 20 years [3]. SSR180575 (7-chloro-*N,N*,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide) is the lead compound of a new series of pyridazino[4,5-*b*]indoleacetamides with in vitro nanomolar affinity for rodent and human TSPO [4]. Of particular interest is the observation that SSR180575 exhibits in vivo neuroprotective [5] and mitochondrial-rescuing [6] properties. Here we report the first in vivo evaluation of an indoleacetamide-based TSPO ligand, [^{11}C]SSR180575, and its comparison to [^{11}C](*R*)-PK11195 in a well-characterized rodent model of acute neuroinflammation [7, 8].

Materials and methods

Radiochemicals

[^{11}C]SSR180575 (7-chloro-*N,N*-dimethyl-5-[^{11}C]methyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide) and [^{11}C]PK11195 {(*R*)-*N*-[^{11}C]methyl-*N*-(1-methylpropyl)-1-(2-chlorophenyl)-isoquinoline-3-carboxamide, *R*-enantiomer} were synthesized according to reported procedures [9–11].

Log D

The *n*-octanol/buffer at pH 7.4 partition coefficient of [^{11}C]SSR180575 and [^{11}C]PK11195 were determined using the shake-flask method as previously reported [9].

Surgery

The protocol used to induce local acute neuroinflammation in the brain of Wistar rats (body weight 250–350 g, CERJ, Le Genest-Saint-Isle, France) has been reported previously [7, 8, 11, 12]. Briefly, 0.5 μl of (*R,S*)- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA, 15 mM in PBS buffer, Sigma[®]) was stereotaxically injected into the right striatum (bregma +0.7 mm, from sagittal suture 2.7 mm, depth from brain surface 5.5 mm) of anaesthetized normothermic animals. All further experiments were performed 7 days after injection.

Autoradiography

[^{11}C]SSR180575 (18 nM) autoradiography was performed on 20- μm frozen sections from five brains. Nonspecific binding was assessed using an excess of either unlabelled

PK11195 (23 μM) or SSR180575 (22 μM). Specificity for TSPO vs central benzodiazepine binding sites was determined using an excess of unlabelled flumazenil (27 μM). Sections were incubated for 20 min in Tris buffer at 4°C, rinsed twice, unsalted in distilled water, dried and placed on a PhosphorImager screen. Autoradiograms were analysed using ImageQuant[®] software.

Radiolabelled metabolites

AMPA-lesioned rats ($n=3$) were intravenously injected with [^{11}C]SSR180575. Animals were sacrificed 10, 20 or 30 min later. Plasma was isolated by centrifugation, plasma proteins were precipitated with acetonitrile and the supernatant was injected onto the HPLC column (see below). Brain hemispheres were sonicated in acetonitrile. After centrifugation, the supernatant was concentrated and injected onto the HPLC [Hewlett-Packard; Waters Bondapak[™] C18 column (300 \times 7.8 mm; porosity 10 μm); elution 0.1% aq. TFA/CH₃CN 60:40 during 10 min, flow rate 5 ml/min; $\lambda=258$ nm].

PET imaging

PET imaging was performed on a Concorde Focus 220 PET scanner. Radiolabelled compounds ([^{11}C]PK11195 or [^{11}C]SSR180575, bolus at the start of PET acquisition) and unlabelled ligands (PK11195 or SSR180575 at 1 mg/kg, bolus 20 min after radioligands) were injected into the catheterized tail vein of animals anaesthetized with isoflurane. Data were acquired and reconstructed as described elsewhere [11].

Image analysis

Image analysis was performed using ASIPro VM[™] (CTI Concorde Microsystems, Knoxville TN, USA). The hyperintense region on the summed PET images was segmented as a region of interest (ROI) that was then mirrored in the contralateral hemisphere as a control region.

Modelization

Binding potential was calculated using the PMOD software package (PMOD Technologies Ltd., Zurich, Switzerland), the single reference tissue model and the contralateral ROI as the reference region. Three parameters were estimated: the ratio of tracer delivery R_1 (k_1/k'_1), the clearance from the tissue into the vascular compartment k_2 and the binding potential ($\text{BP}=k_3/k_4$).

Statistical analysis

Data are expressed as mean \pm SD. Comparisons were performed using the Mann-Whitney nonparametric test

(PET) or analysis of variance (ANOVA) followed by Bonferroni's *t* test (autoradiography). The significance level was $p < 0.05$.

Results

Radiochemistry

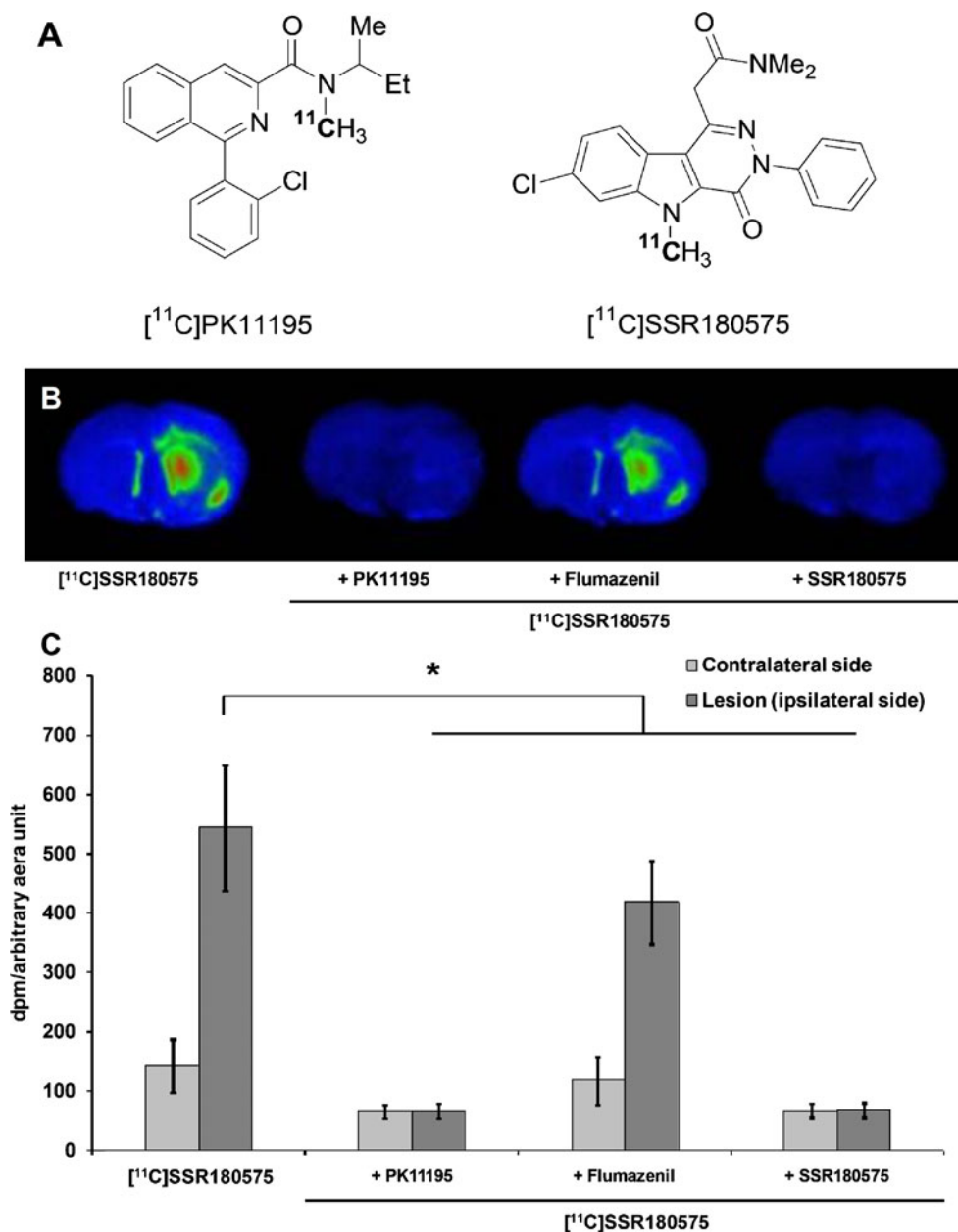
SSR180575 and PK11195 were both isotopically radio-labelled with the short-lived positron emitter ^{11}C (Fig. 1a). Starting from a 55 GBq ^{11}C CO₂ cyclotron production batch, 4.5–5.0 GBq (19–21% decay-corrected radiochemical

yields) of ^{11}C PK11195 and ^{11}C SSR180575 (>98% radiochemically pure) was routinely obtained within 25 min of radiosynthesis with specific radioactivities ranging from 50 to 90 GBq/μmol. Partition coefficients (log D) were 1.9 and 2.8 for ^{11}C SSR180575 and ^{11}C PK11195, respectively.

Autoradiography

Autoradiography of brain sections demonstrated increased ^{11}C SSR180575 binding in the right, AMPA-lesioned striatum compared to the contralateral striatum (Fig. 1b). The ratio of binding in the ipsi- over the contralateral

Fig. 1 **a** Chemical structures of ^{11}C PK11195 and ^{11}C SSR180575. **b** In vitro autoradiography of five AMPA-lesioned rat brain sections: representative autoradiograms depicted in identical colour scales. **c** Relative quantification of autoradiograms in ipsilateral and contralateral striatum. ^{11}C SSR180575 was used at 18 nM and unlabelled PK11195, flumazenil and SSR180575 were used at 23 μM, 27 μM and 22 μM, respectively. *Significant difference relative to the ^{11}C SSR180575 condition on the ipsilateral side (ANOVA followed by Bonferroni's *t* test, $p < 0.05$). Variations on the contralateral side were not significant



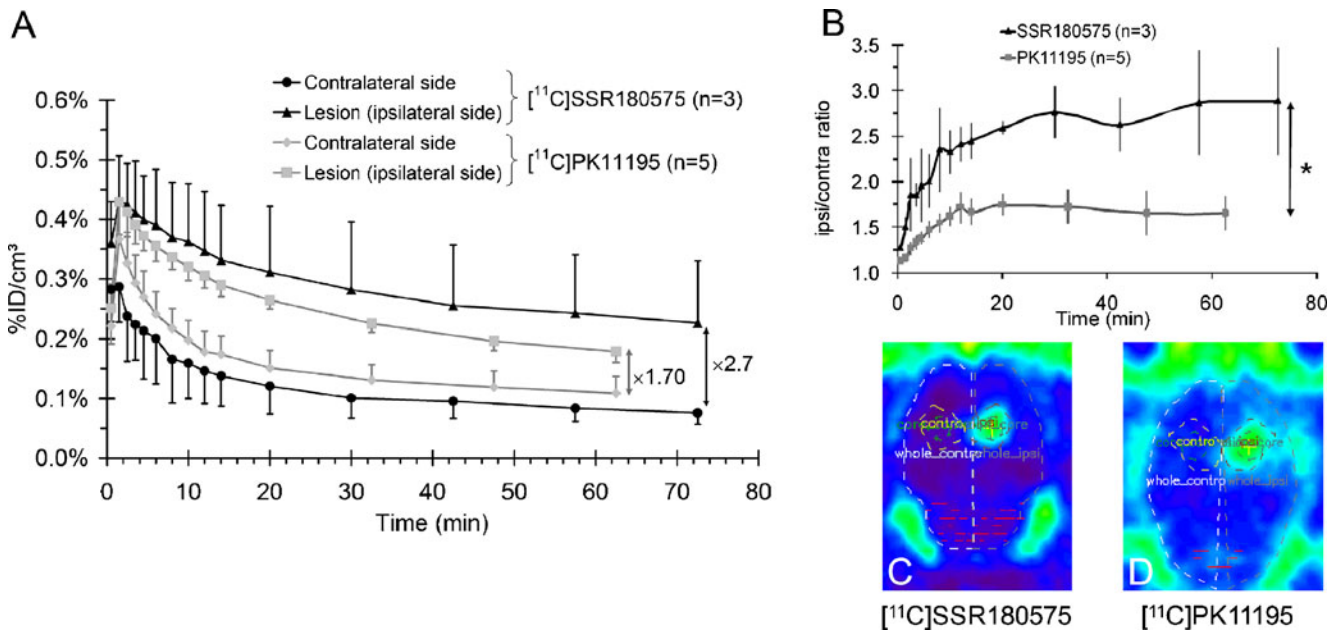


Fig. 2 Time-activity curves of [¹¹C]SSR180575 and [¹¹C]PK11195 (a), resulting ipsilateral to contralateral ratios (b) and representative PET images of [¹¹C]SSR180575 and [¹¹C]PK11195 (summed images

over 70 min). *Significant difference between [¹¹C]SSR180575 and [¹¹C]PK11195 (Mann-Whitney U tests, *p*<0.05)

striatum was 3.8 and returned to 1 in the presence of an excess of PK11195 or SSR180575 (Fig. 1c). When compared with [¹¹C]SSR180575 alone, competition for [¹¹C]SSR180575 binding with an excess of unlabelled PK11195 and SSR180575 resulted in a large reduction of ipsilateral binding (−88% with both PK11195 and SSR180575, *p*<0.05), while a more limited, nonsignificant reduction in contralateral binding was observed (−54% with PK11195, −53% with SSR180575). Competition for [¹¹C]SSR180575 binding with an excess of flumazenil, a central benzodiazepine receptor (CBR) ligand, did not influence the ipsilateral to contralateral ratio (3.5 versus 3.8 in the absence of competitor).

Radiolabelled metabolites

Up to 30 min following injection, the only radioactive peak recovered from plasma and brain tissue corresponded to the retention time of intact [¹¹C]SSR180575 on radio-HPLC (*t_R*=8.0 min).

PET imaging

[¹¹C]SSR180575 uptake was higher in the ROI over the ipsilateral striatum, in which an increase in TSPO expression was observed, than in the contralateral ROI corresponding to intact tissue (Fig. 2). Uptake in the ipsilateral ROI was higher

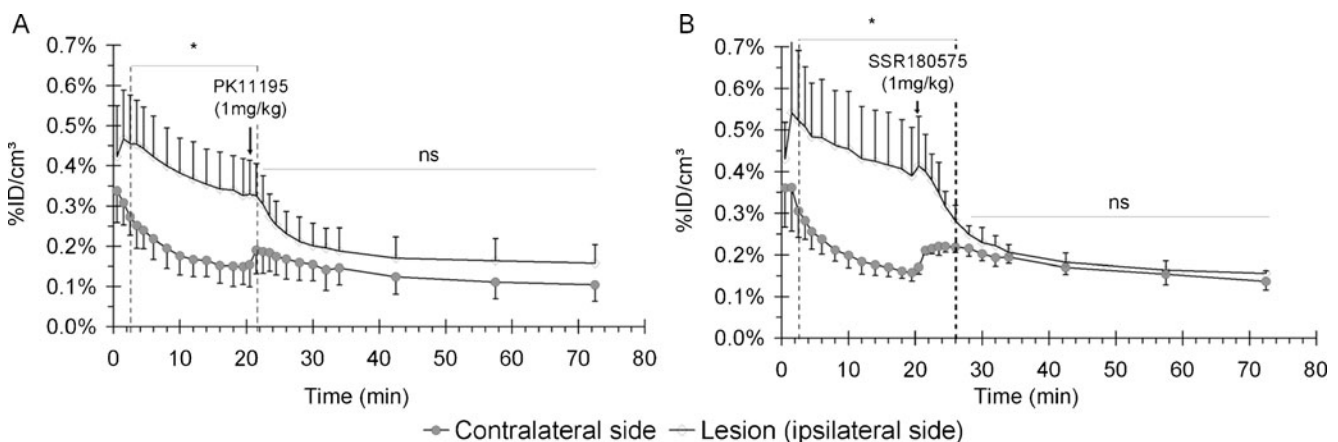


Fig. 3 Time-activity curves of [¹¹C]SSR180575 displaced with unlabelled PK11195 (a) or SSR180575 (b). *Indicates that uptake values are significantly different between the ipsi- and contralateral ROIs (Mann-Whitney U tests, *p*<0.05) and *ns* that they are not significant

for [^{11}C]SSR180575 than for [^{11}C]PK11195, whereas uptake in the contralateral ROI was lower for [^{11}C]SSR180575 than for [^{11}C]PK11195. As a result, the ratio of the ipsilateral to contralateral uptakes, averaged between 20 and 80 min after radioligand administration, was higher for [^{11}C]SSR180575 (2.75 ± 0.13) than for [^{11}C]PK11195 (1.70 ± 0.05) (Fig. 2). Accordingly, the binding potential (BP), as well as the ratio of tracer delivery (R_1), were significantly higher for [^{11}C]SSR180575 than for [^{11}C]PK11195 (BP = 1.65 ± 0.36 vs 0.66 ± 0.15 ; $R_1 = 1.26 \pm 0.08$ vs 1.10 ± 0.05 ; Mann-Whitney U test, $p < 0.05$). Displacement of [^{11}C]SSR180575 binding in rat brain tissue by administering an excess of PK11195 or SSR180575 (1 mg/kg i.v.; 20 min post [^{11}C]SSR180575 injection) significantly reduced most of the tracer binding from the ipsilateral ROI (Fig. 3). Both PK11195 and SSR180575 induced a transient increase in [^{11}C]SSR180575 levels in the contralateral side.

Discussion

To date, no indoleacetamide-derived TSPO ligand has been labelled for PET imaging studies of neuroinflammation. Only *N,N*-diethyl-[5-chloro-2-(4-iodophenyl)indol-3-yl]glyoxylamide, radiolabelled with ^{123}I , has been tested for single photon emission computed tomography (SPECT) imaging in normal rats [13, 14]. Therefore, the value of indoleacetamide derivatives as TSPO radioligands for detection of microglial activation remains unclear, even though this chemical class contains several compounds with attractive pharmacological properties, in particular as antiapoptotic agents [5, 6, 15].

SSR180575 was labelled with ^{11}C to produce radiochemically pure [^{11}C]SSR180575 in high yield. Cerebral binding of [^{11}C]SSR180575 was measured in vitro and in vivo after a focal induction of neuroinflammation. The characterization and reproducibility of TSPO overexpression in the acute neuroinflammation provided by intracerebral AMPA injection has been previously reported in several studies [7, 8, 11, 12].

Autoradiography of brain sections with [^{11}C]SSR180575 clearly delineated the lesion as an enhanced binding area compared to the intact brain tissue. The specificity of [^{11}C]SSR180575 binding was further confirmed by the lack of contrast in the presence of a >1,000-fold excess of PK11195 or SSR180575: both decreased most of the ipsilateral binding (90%), but also part of the contralateral binding (50%), possibly reflecting the detection of basal specific binding in the intact brain. Flumazenil, a CBR ligand with widespread receptors in the brain, did not significantly affect the [^{11}C]SSR180575 ipsilateral to contralateral binding ratio.

In vivo brain PET imaging confirmed the high ipsilateral to contralateral ratio measured in vitro: after the initial distribution phase of the radiotracers, the ipsilateral to contralateral ratio for [^{11}C]SSR180575 was significantly higher than that observed with [^{11}C]PK11195 (2.8 versus 1.7, respectively). In vivo displacement studies demonstrated a rapid but incomplete displacement of [^{11}C]SSR180575 binding in the lesion by an excess of unlabelled PK11195 or SSR180575. In the contralateral region, the transient increase observed shortly after the injection of an excess of PK11195 is probably due to an increase in the blood concentration of [^{11}C]SSR180575 resulting from the release of [^{11}C]SSR180575 from extracerebral binding sites. However, this increase was long lasting after SSR180575 injection. These observations are consistent with previous reports of irreversible binding of SSR180575 to the TSPO in rat tissue [4].

Modelling of PET data confirmed that [^{11}C]SSR180575 has a better capacity than [^{11}C]PK11195 to discriminate between inflammatory cerebral tissue expressing TSPO and intact tissue (BP of 1.7 versus 0.7 for [^{11}C]SSR180575 and [^{11}C]PK11195, respectively). The increased ligand transport rate (R_1) of [^{11}C]SSR180575 when compared with that of [^{11}C]PK11195 may reflect a slight perturbation of the blood-brain barrier (BBB) or differences in the BBB transfer of the tracers related to their different polarity. However, R_1 is not likely to bias the final BP values since it mainly reflects the initial distribution phase. Finally, the absence of plasmatic and cerebral metabolites is noteworthy for future input-derived modelling and treatment monitoring in larger animals or in humans.

In conclusion, the indoleacetamide [^{11}C]SSR180575 is a new PET radioligand for the TSPO with interesting properties for the in vivo imaging of neuroinflammation. Additional studies in acute and chronic models of neurodegeneration are required to further evaluate the potential of this radioligand as a biomarker of neuroinflammation in neuropathological conditions.

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