^{18}F -VUIIS1009B Features a Superior Imaging Performance to ^{18}F -DPA-714 in TSPO Density Characterization for Neuroinflammatory PET Imaging

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Abstract

Purpose

Translocator protein (TSPO), an out-mitochondrial membrane protein, is regarded as a key biomarker for neuroinflammation in a variety of neurodegenerative diseases. In this study, we aim to evaluate two highly specific TSPO radiotracers ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B in a mild cerebral ischemic rat model, and to compare their *in vivo* performance to the well-established TSPO probe ^{18}F -DPA-714 for neuroinflammation imaging. With multiple graphic analytical methods tested and macro parameters determined, we propose to find a suitable and best quantification method to profile neuroinflammation and measure TSPO density with the three TSPO radiotracers.

Methods

Cerebral ischemia rat model was created and imaged using ^{18}F -VUIIS1009A, ^{18}F -VUIIS1009B and ^{18}F -DPA-714. Displacement studies using cold analogs were performed to evaluate the binding specificities of ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B individually. Imaging analysis using arterial plasma input functions (AIFs) was employed to generate Logan plots and parametric images of total distribution volume (V_T) for each radiotracer. Reference Logan model using healthy brain as a reference region was introduced to generate parametric images for binding potential (BP_{ND}).

Results

When compared to ^{18}F -DPA-714, ^{18}F -VUIIS1009B demonstrated higher *in vitro* binding specificity, binding potential (BP_{ND}) and distribution volume ratio (DVR). Parameter images of BP_{ND} and V_T also indicate ^{18}F -VUIIS1009B has a superior imaging profile when compared with other two radiotracers in TSPO imaging. Correlation analysis between BP_{ND} for ^{18}F -VUIIS1009B and ^{18}F -DPA-714 also indicates ^{18}F -VUIIS1009B is more sensitive than ^{18}F -DPA-714 in TSPO density measurement.

Conclusions

This study demonstrates the superiority of ^{18}F -VUIIS1009B to ^{18}F -VUIIS1009A and ^{18}F -DPA-714 in the neuroinflammation imaging. It also demonstrates that ^{18}F -VUIIS1009B PET imaging coupled with parameter mapping (V_T and BP_{ND}) and graphic analysis holds great promise for neuroinflammation characterization and TSPO density measurement.

Introduction

Neuroinflammation, as a response to local insult or the distally existing pathological events in central nervous system (CNS), often occurs in a variety of neurological disease states, including Alzheimer's diseases (AD), Parkinson's disease (PD), multiple sclerosis (MS), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) [1–4]. Neuroinflammation often plays an important role in various neuropathologies, and neuroinflammation targeted diagnosis and therapy are often regarded to be crucial for disease progress monitoring and novel therapy evaluation [5]. Among those studies, neuroimaging provides a non-invasive tool to characterize and monitor *in vivo* neuroinflammation. As one of the neuroimaging modalities, positron emission tomography (PET) can be employed to visualize and quantify the molecular target at nanomolar level, with both high sensitivity and specificity.

Translocator protein (TSPO, 18 kDa), one of the most popular PET imaging biomarkers for neuroinflammation, has been studied in neuroinflammation for over 20 years[6]. Formally referred to as the peripheral benzodiazepine receptor, TSPO is an out-mitochondrial membrane protein primely located in the peripheral organs including the lungs, heart, kidneys, and nasal epithelium[7]. In the healthy brain, TSPO has an extremely low level expression[8], but it will be overexpressed when microglia are activated in response to an injury in neuroinflammation[5]. The overexpression of TSPO is considered as a biomarker for neuroinflammation in numerous neurological diseases including stroke, AD, PD, MS, HD, and ALS[8]. In previous studies, TSPO imaging has been employed as a powerful tool to elucidate the roles of neuroinflammation and assess the novel therapeutic effects for brain diseases in both the clinical and preclinical studies[5].

During last decades, many TSPO PET radiotracers were developed to study neuroinflammation in a variety of diseases[5]. Of these radiotracers, the most common one used to date is ^{11}C -PK11195[6], which nonetheless has several limitations, including high non-specific binding, low brain penetrance, low signal-to-noise ratio (SNR), low sensitivity, as well as poor amenability to quantification[9]. Consequently, many studies have been conducted to develop novel TSPO radiotracers, including phenoxyarylacetamides derivatives labeled with ^{11}C (^{11}C -DAA1106, ^{11}C -PBR28) or ^{18}F (^{18}F -FEDAA1106, ^{18}F -FEPPA, ^{18}F -PBR06), imidazopyridines derivatives (^{11}C -CLINME), and pyrazolopyrimidines derivatives (^{18}F -DPA-714) [10]. Among these, ^{18}F -DPA-714 is a pyrazolopyrimidinal radiotracer featuring a high TSPO binding affinity (Fig. 1) [11] [12]. Labeled with ^{18}F , ^{18}F -DPA-714 has a longer half-time (110 min) than ^{11}C -PK11195 (20 min). It is widely used in TSPO PET imaging to dynamically monitor the brain inflammatory response and disease progression, and showed improved bioavailability, lower nonspecific binding, and higher non-displaceable binding potential (BP_{ND}) [13, 14]. Clinical studies using ^{18}F -DPA-714 have been performed on patients with ALS[15] [16], AD [16] [17], as well as in post-stroke studies[18], with great promise demonstrated in neuroimaging studies.

Based on the scaffold of ^{18}F -DPA-714, many novel radiotracers have been developed and introduced in both clinical and preclinical TSPO PET imaging, including ^{18}F -DPA-C5yne[19], ^{18}F -VUIIS1008[20, 21], ^{18}F -VUIIS1009A/B[22], ^{18}F -FDPA[23], ^{18}F -VUIIS1018A[24, 25]. Of these radiotracers, ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B demonstrate over 500-fold higher binding affinities when compared to the parent radiotracer

^{18}F -DPA-714. Imaging with these two radiotracers in C6 glioma model also demonstrates the superiority of ^{18}F -VUIIS1009B to ^{18}F -VUIIS1009A and ^{18}F -DPA-714, including higher BP_{ND} , higher SNR and higher total distribution volume (V_{T})[22].

Although TSPO PET imaging has been performed in a variety of neuroinflammatory diseases, it still has some drawbacks, including relatively low neuroinflammatory uptake, low SNR and insensitivity to the small variance of TSPO expression when quantified with the semi-quantitative parameters (%ID/cc or SUV) [26]. In order to overcome these limitations, macro parameters like V_{T} , BP_{ND} and DVR were often introduced to TSPO imaging, with the aim to fully characterize radiotracer pharmacokinetics, TSPO expression level, as well as to increase SNR and to improve imaging visual effects [13] [26–29]. In practice, radiotracers featuring higher binding specificity often demonstrate higher macro parameter values (like BP_{ND}), higher SNR and thus better visual effects in PET imaging as well as the higher sensitivity to TSPO density variance. Considering this, in this study, for the first time we evaluated the performance of the highly specific TSPO radiotracers ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B in a mild neuroinflammation model, with directly comparison to the performance of the well-known TSPO radiotracer ^{18}F -DPA-714 in the same neuroinflammatory animal model. As shown in Fig. 1, VUIIS1009A (IC_{50} : 14.4 pM) features a 750-fold higher *in vitro* TSPO binding affinity than DPA-714 (IC_{50} : 10.9 nM)^[30]. VUIIS1009B (IC_{50} : 19.4 pM) features a 560-fold higher *in vitro* TSPO binding affinity than DPA-714. With *in vivo* PET dynamic scans, we evaluated their semi-quantitative parameters (%ID/cc or SUV), as well as macro parameters including binding potential (BP_{ND}), total distribution volumes (V_{T}) and distribution volume ratio (DVR) using graphic analysis. Specific parametric images (BP_{ND} , V_{T}) were also generated and compared to identify the suitable quantitative methods and parameters to profile neuroinflammation in the future studies.

Materials And Methods

All chemicals were purchased from commercial sources. Unlabeled DPA-714, VUIIS1009A/B and their precursor for radiosynthesis were synthesized in-house, and ^{18}F was produced using an IBA cyclotron (Belgium). Effluent radioactivity was monitored using a NaI (TI) scintillation detector system. All other synthesis reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification, unless noted otherwise.

Radiosynthesis. Radiosynthesis of probe ^{18}F -DPA-714, ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B was performed according to our previously published methods^[22, 38]. In detail, precursors underwent nucleophilic fluorination with fluorine-18. Purification of ^{18}F -labeled radiotracers was performed with preparative HPLC. The retention time of all the radiotracers according to gamma detection was double checked to make sure it was in agreement with the UV retention time. The radiochemical purity measured using HPLC was consistently greater than 99%, with specific activity consistently greater than 4203 Ci/mmol (156 TBq/mmol).

Animals. Animals were maintained and handled in accordance with the recommendations of the National Institute of Health in China. Animal studies were approved by the local University and Hospital Ethics Committee. All experiments conducted at the Shanghai Jiao Tong University School of Medicine were approved by the Animal Ethics Committee. Male Wistar rats (7 weeks old, 230–250 g) were purchased from Vital River (Beijing, China) and housed under a 12-h/12-h dark/light cycle under optimal conditions.

Ischemia Rat Models. Mild focal ischemia was induced by intraluminal occlusion of the middle cerebral artery for 30 min based on the intraluminal thread model^[39]. After ischemic surgery, the rats were used for PET imaging, and metabolite analysis at 5–7 days. Rats were affixed with arterial catheters prior to the MRI and PET/CT studies.

In Vivo Dynamic PET Imaging Study. PET imaging was carried out using a small-animal PET scanner (Siemens Medical Solutions USA, Knoxville, TN, USA) according to the protocols reported previously^[11]. Rats (n = 6) were first anesthetised with isoflurane (3.5% for induction, 1.5–2% for maintenance). Immediately after rats were intravenously injected *via* the tail vein with specific radiotracers, a 45-min list-mode emission scan was conducted immediately. The study was performed using ¹⁸F-VUIIS1009A (46.0 ± 8.0 MBq), ¹⁸F-VUIIS1009B (48.8 ± 10.7 MBq) and ¹⁸F-DPA-714 (47.2 ± 9.9 MBq) in consecutive days. During the scans, blood samples were drawn according to the following schedule: 15 µl every 10 s for the first 90 s and at 5, 8, 20, and 45 min while 200 µl were drawn at 2, 12, 30, and 60 min for metabolite corrections. The time frame reconstruction for PET was as follows: 10 s × 12 frames, 1 min × 3 frames, 5 min × 8 frames. In addition, the metabolite-corrected parent plasma input function (AIF) was measured according to our previous published protocol^[21, 22].

For the displacement experiments, unlabeled TSPO compounds VUIIS1009A (10.0 mg/kg) or VUIIS1009B (10.0 mg/kg) were dissolved in 1 mL of saline containing 10% ethanol and 5% Tween-80, and injected 20 min after the PET scans were initiated. Three independent experiments (triplicates) were performed for each group. PET scan data modeling was conducted using three-dimensional sinograms, which were transformed into two-dimensional sinograms by Fourier rebinning. Dynamic image reconstruction was achieved by filtered back-projection using Hanning's filter with a Nyquist cutoff frequency of 0.5 cycles/pixel.

PET Image Co-registration

In order to analyze the voxel values of the regions of interest (ROI) and calculate the parametric maps, PET images of the same rat with different radiotracers were co-registered. In detail, using Inveon Research Workplace 4.0 (Siemens Medical Solutions USA, Knoxville, TN, USA), corresponding CT images were first co-registered to make the special alignment of brain regions. The transformation file for this co-registration was generated and then treated as an input to register the corresponding PET images. With the registered PET images, ROIs were drawn and values of voxels was analyzed.

Dynamic PET Data Analysis

The PET images were analyzed using PMOD version 3.4 image analysis software (PMOD Technologies, Zurich, Switzerland) and Inveon Research Workplace 4.0 (Siemens Medical Solutions USA, Knoxville, TN, USA). The ROIs were manually drawn on ipsilateral and contralateral sides of the striatum and cortex.

In this study, parametric V_T images were generated using plasma input-based Logan analysis^[40], with the plasma contribution factor v_p set to 0.05 for all the analysis. With the same animals, parametric BP_{ND} images were also generated using Logan Reference Tissue models with contralateral brain TACs as an input^[40]. For DVR or BP_{ND} determination and parametric image generation, k_{2_REF} was calculated using SRTM methods with contralateral brain used as the reference tissue^[41]. DVR_{Logan} from the **Table 1** was generated directly from the ratio of distribution volume of inflammation and brain generated *via* the plasma input-based Logan analysis.

Correlation of %ID/cc, BP_{ND} and V_T was analyzed using parameters determined from the same voxels in the normal and parametric PET image for each radiotracer. Correlation was then analyzed with Pearson correlation coefficient (r) and p values generated using the GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA).

Statistical Analyses. All quantitative data are expressed as the mean $\pm$ standard deviation (SD). The relationship between the regional outcome parameters under test and retest conditions were estimated using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA).

Results

In Vivo 45-min Dynamic Scan. Performance of ^{18}F -DPA-714, ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B was evaluated during the 45-min dynamic PET scan using a focal cerebral ischemic rat model, in which TSPO expression was up-regulated with activated microglia, whereas the blood-brain barrier (BBB) was intact or not seriously disrupted^[31]. As expected, the ipsilateral side (ischemia side) showed a higher uptake of the radiotracer than the contralateral side of the brain in the PET imaging for all of the three radiotracers, as shown in Fig. 2a, 2b for ^{18}F -DPA-714, Fig. 2c, 2d for ^{18}F -VUIIS1009A and Fig. 2e, 2f for ^{18}F -VUIIS1009B. A fast uptake of the radiotracers was demonstrated according to the analysis of time-activity curves (TACs) on the ipsilateral side (Fig. 2g for ^{18}F -DPA-714, Fig. 2h for ^{18}F -VUIIS1009A and Fig. 2i for ^{18}F -VUIIS1009B). Moreover, consistent levels of radiotracer uptake at the ipsilateral side were also achieved 2 min after the radiotracer injection as indicated by TACs (Fig. 2g for ^{18}F -DPA-714, Fig. 2h for ^{18}F -VUIIS1009A and Fig. 2i for ^{18}F -VUIIS1009B). Compared to ^{18}F -DPA-714 (%ID/cc = 0.73 at 45 min), ^{18}F -VUIIS1009B (%ID/cc = 0.70 at 45 min) has a comparable %ID/cc in the neuroinflammation region, while ^{18}F -VUIIS1009A (%ID/cc = 0.50 at 45 min) has a significantly lower uptake in the same region as shown in Fig. 2c, 2d and 2 h.

The contralateral side had a comparatively low uptake, and a consistent %ID/cc level was achieved at 10 min after the radiotracer injection for all three radiotracers (Fig. 2g, Fig. 2h and Fig. 2i). When compared to ^{18}F -VUIIS1009A, ^{18}F -VUIIS1009B and ^{18}F -DPA-714 demonstrate a higher contralateral healthy brain uptake as indicated in the 45 min uptake (0.20 vs 0.15, ID%/cc at 45 min). For ^{18}F -DPA-714, the %ID/cc ratio at 45 min between the ipsilateral side and contralateral side in this study was 3.50 as shown in the TACs (0.73 vs. 0.20%ID/cc at 60 min; Fig. 2g), 3.33 for ^{18}F -VUIIS1009A (0.50 vs. 0.15%ID/cc at 45 min) and 3.50 for ^{18}F -VUIIS1009B (0.70 vs. 0.2%ID/cc at 45 min), indicating a high and comparable signal-to-noise ratio of all the three TSPO radiotracers in this study.

In Vivo Displacement Study. The *in vivo* displacement study was also performed to evaluate TSPO binding specificity for both ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B in the same rats. ^{19}F -VUIIS1009A and ^{19}F -VUIIS1009B were used for the displacement assay and were injected at 20 min in the course of a 45-min dynamic scan. Based on the imaging results, both TSPO compounds could displace the radioactivity uptake on the ipsilateral side of the brain. As shown in Fig. 3a(^{18}F -VUIIS1009A) and 3c(^{18}F -VUIIS1009B), the uptake on the ipsilateral side was significantly higher than the uptake on the contralateral side for both radiotracers before the injection of their cold analogs. However, after the displacement, radiotracer uptake of the ipsilateral side dropped dramatically (Fig. 3b and 3d), which accounts to more than 66% decrease of the tracer uptake as shown in the TACs for both radiotracers (as shown in Fig. 3e and 3f). After displaced with their cold analogs, the uptake level of ipsilateral side was almost comparable to the contralateral side, both demonstrated by the imaging profiles and TACs in Fig. 3. All these indicate the significantly high binding specificity for both radiotracers in neuroinflammation region.

Macro Parameters Determined Using Graphic Analysis

Although both ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B have significantly higher *in vitro* binding affinities when compared to ^{18}F -DPA-714, they did not demonstrate a more promising imaging characterized by the semi-quantitative uptakes (like %ID/cc) as shown in Fig. 2. In order to further evaluate their *in vivo* performances, macro parameters like V_T , DVR and BP_{ND} were determined using graphic analysis with AIFs or reference tissue TACs input. According to our previous studies, TSPO PET imaging can be analyzed using a two-tissue, four-parameter model as shown in Fig. 4a. Based on this model, graphic analysis using AIFs can also be employed to determine the V_T for both neuroinflammatory region and the normal brain region. As shown in Table 1, V_T determined for inflammation are higher than the corresponding values for normal brain for all the three radiotracers, indicating all these radiotracers have the tendency to be uptake by inflammation region instead of the healthy brain. Direct comparison of the inflammation V_T from three radiotracers indicates ^{18}F -VUIIS1009B has a higher V_T than the other two radiotracers as shown in Table 1. Moreover, for the contralateral healthy brain, ^{18}F -VUIIS1009B also demonstrates a slightly higher V_T than the other two radiotracers. The ratios of the V_T values (noted as DVR) between inflammation region and the contralateral healthy brain indicate a higher DVR for ^{18}F -

VUIIS1009B (8.53 ± 1.06) when compared with ^{18}F -DPA-714 (6.00 ± 0.41) and ^{18}F -VUIIS1009A (4.37 ± 0.82). Further study using contralateral healthy brain as a reference region was also performed to determine DVRs and BP_{ND} for all three radiotracers (as shown in Fig. 4 and Table 1). Logan plots using contralateral brain as a reference region was plotted with BP_{ND} and DVR determined (Fig. 4b, 4c and 4d), demonstrating a good fit and linearity for all three radiotracers with $r > 0.96$. As shown in Table 1, ^{18}F -VUIIS1009B (7.55 ± 0.65) demonstrating a much higher DVR than ^{18}F -DPA-714 (5.37 ± 0.36) and ^{18}F -VUIIS1009A (3.91 ± 0.50). Similarly, as for the relationship between DVR and BP_{ND} (noted as $\text{DVR} = BP_{\text{ND}} + 1$), ^{18}F -VUIIS1009B also demonstrates a much higher BP_{ND} value than other two radiotracers as shown in Table 1. In sum, the macro parameter analysis revealed that ^{18}F -VUIIS1009B has a superior imaging potential than the other two radiotracers on V_T , DVR and BP_{ND} .

Parametric Image Analysis

Macro parameter analysis using Logan plot revealed ^{18}F -VUIIS1009B is superior to ^{18}F -DPA-714 and ^{18}F -VUIIS1009A as shown in dynamic PET imaging. In this study, we further compared the performance of the three radiotracers with parameter images (V_T and BP_{ND}) generated (Fig. 5). In detail, voxel-wise V_T images were generated *via* a Logan graphic method with AIFs input. Voxel-wise BP_{ND} images were generated using a Logan reference model with the contralateral healthy brain input as the reference region. As expected, compared to ^{18}F -DPA-714, ^{18}F -VUIIS1009B has similar biodistribution profile for neuroinflammation region in both V_T and BP_{ND} parameter images (Fig. 5), but with higher V_T , BP_{ND} in the neuroinflammatory region, demonstrating its promising characteristics for TSPO imaging. Interestingly, although ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B feature the comparably high *in vitro* binding affinity, ^{18}F -VUIIS1009A did not show a similar imaging profile as ^{18}F -VUIIS1009B in both V_T and BP_{ND} parametric images (Fig. 5). Compared to the parametric image of ^{18}F -VUIIS1009B, ^{18}F -VUIIS1009A imaging analysis generated a noisier parametric image as shown in Fig. 3a and Fig. 3b.

Correlation between %ID/cc, BP_{ND} and V_T at the Voxel Level

This study also determined and evaluated %ID/cc, BP_{ND} and V_T at the voxel level, with the aim to better elucidate the relationship among these parameters derived from the dynamic PET images. As shown in Fig. 6, with %ID/cc, BP_{ND} and V_T determined for the voxels in the neuroinflammatory region, we made different plots for the three probes and measured the correlation coefficient r and p value for each data set. As shown in Fig. 6a and 6c, a strong correlation was elucidated for ^{18}F -DPA-714, ^{18}F -VUIIS1009B among all three parameters (%ID/cc, BP_{ND} and V_T). Compared to ^{18}F -DPA-714, ^{18}F -VUIIS1009B demonstrates a higher r value (0.99 vs. 0.78) and thus a more positive linear relationship between V_T and BP_{ND} as shown in Fig. 6a and 6c. Furthermore, ^{18}F -VUIIS1009B also demonstrates a stronger correlation between semi-quantitative parameter %ID/cc and BP_{ND} or V_T , as shown by the plots in Fig. 6a and 6c, which is probably due to the lower non-specific binding profile for ^{18}F -VUIIS1009B instead of ^{18}F -DPA-

714. While for ^{18}F -VUIIS1009A, a weaker correlation is found between %ID/cc and the other two parameters (BP_{ND} and V_T) as shown in Fig. 6b.

Correlation Analysis between BP_{ND} for ^{18}F -VUIIS1009B and ^{18}F -DPA-714

In this study, both ^{18}F -VUIIS1009B and ^{18}F -DPA-714 PET imaging were performed using the same rats with the aim to more accurately reflect their BP_{ND} and *in vivo* performance. In order to compare the performance of ^{18}F -VUIIS1009B and ^{18}F -DPA-714, we co-registered the PET images from the same rats and calculated BP_{ND} for ^{18}F -VUIIS1009B and ^{18}F -DPA-714 both at the regional level and voxel level with the same ROIs. As shown in **Fig. 7a**, ^{18}F -VUIIS1009B demonstrates a strong positive correlation with ^{18}F -DPA-714 ($r = 0.88$) at the voxel level in the same rat. The slope of the fitting curve is 1.35, which indicate the BP_{ND} for ^{18}F -VUIIS1009B is more sensitive to TSPO expression when compared to ^{18}F -DPA-714. Regional BP_{ND} was also obtained and analyzed for both ^{18}F -VUIIS1009B and ^{18}F -DPA-714 for the same rats ($n = 6$). As shown in **Fig. 7b**, ^{18}F -VUIIS1009B also demonstrates a stronger correlation of ^{18}F -DPA-714 in the regional BP_{ND} ($r = 0.89$) for a cohort of rats ($n = 6$). The slope for the fitting curve is 1.45, which also demonstrates the higher sensitivity of ^{18}F -VUIIS1009B to profile TSPO expression when compared to ^{18}F -DPA-714.

Discussion

With a low expression in normal brain, TSPO is normally overexpressed in neuropathological conditions, such as stroke, brain trauma, AD, and PD. TSPO PET imaging is now becoming a useful tool in neuroinflammation evaluation, as well as in diagnosis and therapy evaluation for many neurological diseases. In the longitudinal studies of neurological diseases, TSPO density evaluation with different radiotracers and analytical approaches have been performed to increase the sensitivity of TSPO expression characterization, and thus to elucidate the relationship between the disease progression and TSPO density [32–35]. In these studies, semi-quantitative parameters like %ID/cc and SUV are widely evaluated in both clinical and preclinical studies. Compared to the semi-quantitative parameters, macro parameters (like BP_{ND} , DVR, V_T) can provide more detailed information on radiotracer pharmacokinetics and TSPO expression, which is more meaningful in longitudinal monitoring of neuroinflammatory diseases [36]. Moreover, TSPO radiotracers with higher binding affinities tend to have higher binding potential (BP_{ND}), which can be more sensitive to measure the variance of TSPO densities in the longitudinal analysis of disease progression. During last 20 years, many studies have been performed to develop novel TSPO probes with higher binding affinity, specificity, binding potential as well as good pharmacokinetics. To date, ^{11}C -PK11195 is the most widely used probe specific for TSPO imaging. However, it still has many limitations in brain imaging as its low brain penetrance, high non-specific binding, low sensitivity, low signal-to-noise ratio, and poor amenability to quantification. In order to overcome these limitations, a variety of novel radiotracers have been developed. ^{18}F -DPA-714, a second-generation radiotracer developed by James *et al.*[12], demonstrated its superiority in neuroinflammation

imaging, with higher binding potential, binding specificity and SNR. Golla *et al.* used multiple methods to generate the quantitative images for ^{18}F -DPA-714 in a clinic study with healthy subjects and AD patients participated [37]. In this study, they concluded that both Logan analysis and spectral analysis are suitable plasma input-based methods to generate quantitatively accurate parametric V_T images. Meanwhile, in reference tissue approaches, reference Logan analysis or SRTM2 can be used to generate parametric BP_{ND} images [37].

In our previous studies, we further modified ^{18}F -DPA-714 and discovered several novel TSPO imaging radiotracers, including ^{18}F -VUIIS1008 [20, 21], ^{18}F -VUIIS1018A [24, 25] and ^{18}F -VUIIS1009A/B [22]. These novel radiotracers feature higher *in vitro* TSPO binding affinities and suitable lipophilicities for brain imaging, when compared to the parent radiotracer ^{18}F -DPA-714. Moreover, they also demonstrated great potential in TSPO PET imaging, including high SNR, higher binding potential, and enhanced visual effects [20–22]. In this study, we further evaluated the performance of ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B in a preclinical model of neuroinflammation. PET imaging shows both radiotracers can be employed to detect the neuroinflammation region with a high SNR, as well as a fast distribution and equilibration in both contralateral and neuroinflammatory regions. In order to confirm further the binding specificity of ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B for TSPO, we performed *in vivo* displacement assays by treating animals with unlabeled VUIIS1009A and VUIIS1009B during the 45-min dynamic scan. The result showed a high displacement ratio (up to 66%) for both radiotracers, reflecting high TSPO binding specificity for both of these two radiotracers.

Although ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B feature more than 500-fold enhancement of binding affinity when compared to ^{18}F -DPA-714, they did not demonstrate a superior semi-quantitative imaging profiles (%ID/cc) when compared with ^{18}F -DPA-714. Graphic analysis was also performed to determine the macro parameters (BP_{ND} , V_T and DVR) for these radiotracers in this study. The results demonstrate ^{18}F -VUIIS1009B has a higher V_T for both inflammation region and normal brain region, as well as higher BP_{ND} , V_T and DVR value when compared to ^{18}F -DPA-714, highlighting a superior performance to ^{18}F -DPA-714. Parameter images (V_T and BP_{ND}) generated using Logan methods also demonstrate an enhanced visual effect for ^{18}F -VUIIS1009B when compared to ^{18}F -DPA-714. In pharmacokinetics, BP_{ND} is a combined measure of the density of "available" neuroreceptors and the affinity of a drug or radiotracer to that neuroreceptor. PET imaging performed in this study using the same rats with an equal TSPO density for each radiotracer. Consequently, BP_{ND} determined herein can reflect the binding affinities of the radiotracers and the sensitivity for TSPO density measurement. As expected, ^{18}F -VUIIS1009B demonstrates a much higher BP_{ND} than ^{18}F -DPA-714, as well as a strong correlation with BP_{ND} of ^{18}F -DPA-714 for both regional ROIs or at the voxel level. Moreover, slope of linearity is greater than one in Fig. 7, reflecting ^{18}F -VUIIS1009B has the superior imaging potential and greater sensitivity to determine the TSPO expression in PET imaging when compared to ^{18}F -DPA-714. Correlation analysis was also performed for %ID/cc, BP_{ND} and V_T at the voxel level for each radiotracer, showing that all these three

parameters has a strong correlation with each other for both ^{18}F -DPA-714 and ^{18}F -VUIIS1009B. Furthermore, ^{18}F -VUIIS1009B demonstrates a stronger positive linear relationship between V_T and BP_{ND} when compared to ^{18}F -DPA-714. This indicates ^{18}F -VUIIS1009B can be more accurately profiled than ^{18}F -DPA-714 using the AIFs based Logan and Logan reference tissue methods in this study. While for ^{18}F -VUIIS1009A, %ID/cc has a weaker correlation with both BP_{ND} and V_T as demonstrated in this study. Furthermore, when compared to ^{18}F -DPA-714 and ^{18}F -VUIIS1009B, ^{18}F -VUIIS1009A does not demonstrate a higher BP_{ND} or DVR, which is also observed in our previous studies on C6 glioma imaging[21, 22]. We believe this behavior is due to the higher plasma binding affinity of ^{18}F -VUIIS1009A when compared to ^{18}F -DPA-714 and ^{18}F -VUIIS1009B which was also noticed in our previous studies[22]. For ^{18}F -VUIIS1009A, the higher plasma binding limits the partitioning of the radiotracer from blood to the tissues, and thus decreases its tissue uptake and image SNR, which then impacts the following macro parameter determination.

In this study, by evaluating two highly specific TSPO radiotracers ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B in a neuroinflammatory model, we aim to test and find a more sensitive radiotracer with superior macro parameters to reflect TSPO expression. Our present findings suggest that ^{18}F -VUIIS1009B will be a suitable PET radiotracer to fulfill this aim. We envision that ^{18}F -VUIIS1009B PET imaging coupled with parameter images (V_T and BP_{ND}) and graphic/kinetic analysis holds great promise for longitudinal neuroinflammation characterization. Further studies using the same neuroinflammation model are still warranted for the comparison of ^{18}F -VUIIS1009B with other TSPO radiotracers, including ^{18}F -DPA-714. Moreover, the pharmacokinetics and *in vivo* specific binding of ^{18}F -VUIIS1009A/B with TSPO, as well as the macro parameter-based analysis and images still need to be determined in PET studies on primate brains.

Conclusion

This study focused on the radiosynthesis and evaluation of the novel TSPO probe ^{18}F -VUIIS1009A/B for neuroinflammation imaging in ischemic rats, using a series of *in vivo* assays and graphic analysis. The results of this study confirm that ^{18}F -VUIIS1009B PET imaging coupled with parameter images (V_T and BP_{ND}) and graphic analysis holds great promise for neuroinflammation characterization. We envision that ^{18}F -VUIIS1009B will be a suitable PET radiotracer for neuroinflammation imaging in many diseases.

Declarations

Ethics approval and consent to participate.

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Consent for publication.

Not applicable.

Availability of data and material.

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests.

The authors have declared that no competing interests exist.

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Authors' contributions

Chen Huang: Experimental conception and design, data acquisition and analysis, manuscript drafting and revision.

Fan Ding: Data acquisition and analysis, manuscript drafting.

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Mengxin Wang: Data acquisition and analysis.

Wenxian Peng: Data acquisition and analysis, manuscript revision.

Wei Li: Data acquisition and analysis.

Cheng Wang: Experimental conception, data acquisition and analysis.

Dewei Tang: Experimental design, manuscript revision, approval of final content of manuscript.

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Table

Table 1. Macro Parameters determined for ¹⁸F-DPA-714, ¹⁸F-VUIIS1009A and ¹⁸F-VUIIS1009B with the same cerebral ischemia rats (n = 6) (data = mean ± SD).

Probe	Inflammation (V _T)	Brain (V _T)	DVR _{Logan}	DVR (Ref. Logan)	BP _{ND} (Ref. Logan)
¹⁸ F-DPA-714	4.19 ± 0.79	0.69 ± 0.09	6.00 ± 0.41	5.37 ± 0.36	4.19 ± 0.10
¹⁸ F-VUIIS1009A	2.89 ± 0.81	0.67 ± 0.21	4.37 ± 0.82	3.91 ± 0.50	2.90 ± 0.50
¹⁸ F-VUIIS1009B	7.55 ± 0.65	0.92 ± 0.09	8.53 ± 1.06	7.55 ± 0.65	6.55 ± 0.65

Figures

Figure 1

Figure 2

45-min PET dynamic scan for ^{18}F -DPA-714, ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B. (a) Coronal and (b) transverse PET image of a cerebral ischemia rat from dynamic scan with ^{18}F -DPA-714. (c) Coronal and (d) transverse PET image of the same cerebral ischemia rat from dynamic scan with ^{18}F -VUIIS1009A. (e) Coronal and (f) transverse PET image of the same cerebral ischemia rat from dynamic scan with ^{18}F -VUIIS1009B. Time activity curves (TACs) for the inflammation (red) and contralateral brain (blue) in the 45-min dynamic scan ($n = 6$) for ^{18}F -DPA-714 (g), ^{18}F -VUIIS1009A (h) and ^{18}F -VUIIS1009A (f). %ID/cm³ = percentage injected dose per cubic centimeter. In TACs, data = mean $\pm$ SD. Inflammation region is marked by the white arrow on the image.

Figure 3

Displacement analysis for rats imaged by ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B with the injection of their corresponding cold analog (10 mg/kg) at 20 min. (a) Summation of the first 20 min PET imaging (coronal) with ^{18}F -VUIIS1009A. (b) Summation of the last 25 min of PET imaging (coronal) with ^{18}F -VUIIS1009A. (c) TACs for brain and tumor in ^{18}F -VUIIS1009A displacement analysis. (d) Summation of the first 20 min of PET imaging (coronal) with ^{18}F -VUIIS1009B ($n = 3$). (e) Summation of the last 25 min of PET imaging (coronal) with ^{18}F -VUIIS1009B. (f) TACs for brain and tumor in ^{18}F -VUIIS1009B displacement analysis ($n = 3$). Inflammation region is marked with the white arrow. Data = mean $\pm$ SD.

Figure 4a

Figure 4b

Figure 4c

Figure 4d

Figure 4

(a) The 2-tissue, 4-kinetic-parameter model used for describing 18F-DPA-714, 18F-VUIIS1009A and 18F-VUIIS1009B kinetics in reference tissues, including the inflammation region and the healthy contralateral brain. The four parameters of K_1 , k_2 , k_3 , and k_4 reflect the transport or binding rates of the radiotracer. In this specific study, K_1 indicates the perfusion rate of the probe from plasma to tissue, while k_2 reflects the clearance rate for the tracer from tissue to plasma. k_3 and k_4 represent the specific binding rates and dissociation rates of the tracer, respectively. Graphic plot of the Logan Reference Tissue for (b) 18F-DPA-714, (c) 18F-VUIIS1009A and (d) 18F-VUIIS1009B.

Figure 5

(a) Parametric images for ^{18}F -DPA-714, ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B using Logan method with AIFs input. (b) Parametric images for ^{18}F -DPA-714, ^{18}F -VUIIS1009A and ^{18}F -VUIIS1009B using Logan reference tissue method with TACs of the contralateral brain input as a reference region.

Figure 6

Correlation of %ID/cc, BPND and VT determined in this study for (a) ^{18}F -DPA-714, (b) ^{18}F -VUIIS1009A and (c) ^{18}F -VUIIS1009B at the voxel level.

Figure 7

(a) Correlation of BPND determined from ^{18}F -VUIIS1009B and ^{18}F -DPA-714 dynamic PET scan in the same rat at the voxel level. (b) Correlation of regional BPND determined from ^{18}F -VUIIS1009B and ^{18}F -DPA-714 dynamic PET scans in a cohort of rats ($n = 6$).