[18F]VUIIS1009B Features a Superior Imaging Performance to [18F]DPA-714 in TSPO Density Characterization for Neuroinflammatory PET Imaging

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Abstract

Purpose: Translocator protein (TSPO), an outer 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 $[^{18}F]$VU115S1009A and $[^{18}F]$VU115S1009B 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 $[^{18}F]$VU115S1009A, $[^{18}F]$VU115S1009B and $[^{18}F]$DPA-714. Displacement studies using non-radioactive analogs were performed to evaluate the binding specificities of $[^{18}F]$VU115S1009A and $[^{18}F]$VU115S1009B 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 contralateral brain as a reference region was introduced to generate parametric images for binding potential ($BP_{ND}$).

Results: When compared to $[^{18}F]$DPA-714, $[^{18}F]$VU115S1009B demonstrated higher binding potential ($BP_{ND}$) and distribution volume ratio (DVR). Parameter images of $BP_{ND}$ and $V_T$ also indicate $[^{18}F]$VU115S1009B has a superior imaging profile with higher $BP_{ND}$ and DVR when compared with other two radiotracers in TSPO imaging. Correlation analysis between $BP_{ND}$ for $[^{18}F]$VU115S1009B and $[^{18}F]$DPA-714 also indicates $[^{18}F]$VU115S1009B is more sensitive than $[^{18}F]$DPA-714 in TSPO density measurement.

Conclusions: This study demonstrates the superiority of $[^{18}F]$VU115S1009B to $[^{18}F]$VU115S1009A and $[^{18}F]$DPA-714 in the neuroinflammation imaging. It also demonstrates that $[^{18}F]$VU115S1009B PET imaging coupled with parameter mapping ($V_T$ and $BP_{ND}$) and graphic analysis using Logan analysis and reference Logan 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. 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 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\[6\]. As an outer mitochondrial membrane protein, TSPO has an extremely low expression level in healthy brain\[7\], but an overexpression when microglia are activated in neuroinflammation in response to brain injury\[5\]. Overexpression of TSPO is considered as a biomarker for neuroinflammation in numerous neurological diseases including stroke, AD, PD, MS, HD, and ALS\[7\]. Development of TSPO-PET imaging was characterized by the discovery of many novel TSPO radiotracers\[5\], including the most common radiotracer $[^{11}\text{C}]PK11195$\[6\], phenoxyacetamides derivatives ($[^{11}\text{C}]DAA1106, [^{11}\text{C}]PBR28$, $[^{18}\text{F}]FEEDAA1106, [^{18}\text{F}]FEPPA, [^{18}\text{F}]PBR06$), imidazopyridines derivatives ($[^{11}\text{C}]CLINME$), and pyrazolopyrimidines derivatives ($[^{18}\text{F}]DPA-714$)\[8\]. Among these, $[^{18}\text{F}]DPA-714$ is a pyrazolopyrimidinal radiotracer featuring a high TSPO binding affinity (Figure 1)\[9\][10]. Labeled with $^{18}\text{F}$, $[^{18}\text{F}]DPA-714$ also has a longer half-time (110 min) than $[^{11}\text{C}]PK11195$ (20 min). Clinical studies using $[^{18}\text{F}]DPA-714$ have been performed on patients with ALS\[11\][12], AD\[12][13], as well as in post-stroke studies\[14\], with great promise demonstrated in neuroimaging.

Based on the scaffold of $[^{18}\text{F}]DPA-714$, many novel radiotracers have been developed and introduced in both clinical and preclinical studies, including $[^{18}\text{F}]DPA-C5yne$\[15\], $[^{18}\text{F}]VUIIS1008$\[16-17\], $[^{18}\text{F}]VUIIS1009A/B$\[18\], $[^{18}\text{F}]FDPA$\[19\], $[^{18}\text{F}]VUIIS1018A$\[20-21\]. Of these radiotracers, $[^{18}\text{F}]VUIIS1009A$ and $[^{18}\text{F}]VUIIS1009B$ demonstrate over 500-fold higher binding affinities when compared to the parent radiotracer $[^{18}\text{F}]DPA-714$\[18\]. Imaging with these two radiotracers in C6 glioma model also demonstrates the superiority of $[^{18}\text{F}]VUIIS1009B$ to $[^{18}\text{F}]VUIIS1009A$ and $[^{18}\text{F}]DPA-714$, including higher $BP_{ND}$, higher SNR and higher distribution volume ratio (DVR)\[18\].

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)\[22\]. 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\[23\][22,24-26]. 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 $[^{18}\text{F}]VUIIS1009A$ and $[^{18}\text{F}]VUIIS1009B$ with directly comparison to the performance of $[^{18}\text{F}]DPA-714$ in a mild neuroinflammation model. As shown in Figure 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)\[27\]. 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). Specific parametric images ($BP_{ND}$, $V_t$) were also generated and compared to identify the suitable quantitative methods and parameters to profile neuroinflammation in this study.

**Materials And Methods**

II 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 (Tl) 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 $[^{18}\text{F}]$DPA-714, $[^{18}\text{F}]$VUIIS1009A and $[^{18}\text{F}]$VUIIS1009B was performed according to our previously published methods $^{18, 28}$. 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, which was determined using the corresponding non-radioactive analogs under the same condition for HPLC analysis. 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 ($n = 9$, 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$^{29}$. After ischemic surgery, the rats were used for PET imaging, and metabolite analysis at 5 - 7 days. Rats ($n = 9$) were affixed with arterial catheters prior to the PET/CT studies. Of the 9 animals, 6 rats were used repeatedly for PET imaging using the three individual radiotracers, 3 for repeatable displacement studies with $[^{18}\text{F}]$VUIIS1009A and $[^{18}\text{F}]$VUIIS1009B, with a total of 24 PET scans performed in this study. We chose 5-7 days for PET imaging with the aim to image the early stage of neuroinflammation instead of the advanced stage with maximal uptake. This early stage imaging is meaningful to early detection of neuroinflammation. Moreover, in the early stage of neuroinflammation, contralateral brain should have little or tiny expression of TSPO, which will benefit the following graphic analysis using it as the reference region.

**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$^9$. 
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 $[^{18}\text{F}]\text{VUIIS1009A (46.0 ± 8.0 MBq)}$, $[^{18}\text{F}]\text{VUIIS1009B (48.8 ± 10.7 MBq)}$ and $[^{18}\text{F}]\text{DPA-714 (47.2 ± 9.9 MBq)}$ 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 2, 5, 8, 12, 20, and 45 min. The time frame reconstruction for PET was as follows: 10s × 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[17-18].

For the displacement experiments, unlabeled TSPO compounds VUIIS1009A (10.0 mg/kg) or VUIIS1009B (10.0 mg/kg) were dissolved in 1.0 mL of saline containing 10% ethanol and 5% Tween-80, and injected 20 min after the PET scans were initiated. In this study, the same cohort of rats (n = 3) were imaged and evaluated for each radiotracer individually. 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 spatial 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 of ipsilateral and contralateral brain 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). Regions of interest (ROIs) were manually defined for each rat on the region of increased radiotracer binding in the ipsilateral hemisphere. The ROIs were manually drawn on ipsilateral and contralateral brain as shown in **Figure 2** and **Figure 3**.

In this study, parametric $V_T$ images were generated using plasma input-based Logan analysis[30], with the plasma contribution factor $\nu_p$ set to 0.05 for all the analysis. With the same animals, parametric $BP_{ND}$ images were also generated using Logan Reference Tissue model with contralateral brain TACs as an input[30]. For $DVR_{LoganRef}$ or $BP_{ND}$ determination and parametric image generation, $k_{2\text{, REF}}$ was calculated using SRTM methods with contralateral brain TACs input as the reference tissue [31]. $DVR_{Logan}$ from the **Table 1** was generated directly from the ratio of distribution volume of ipsilateral and contralateral brain generated via the plasma input-based Logan analysis.
**Statistical Analyses.** All quantitative data are expressed as the mean ± standard deviation (SD).

Pearson's correlation analysis was applied to investigate the relationship between different parameters (\(V_T, BP_{ND}, \%ID/cc\)) both at the voxel level and regional level, 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. As expected, the ipsilateral side (ischemia side) showed a higher uptake of the radiotracer than the contralateral brain in the PET imaging for all of the three radiotracers, as shown in Figure 2a, 2b for \([^{18}F]DPA-714\), Figure 2c, 2d for \([^{18}F]VUIIS1009A\) and Figure 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 (Figure 2g for \([^{18}F]DPA-714\), Figure 2h for \([^{18}F]VUIIS1009A\) and Figure 2i for \([^{18}F]VUIIS1009B\)). Moreover, consistent levels of radiotracer uptake on the ipsilateral side were achieved 2 min after the radiotracer injection as indicated by TACs (Figure 2g for \([^{18}F]DPA-714\), Figure 2h for \([^{18}F]VUIIS1009A\) and Figure 2i for \([^{18}F]VUIIS1009\)). TACs for corrected AIFs was also shown in Supplemental Figure 1.

Compared to \([^{18}F]DPA-714\) (%ID/cc = 0.73 ± 0.08 at 45 min), \([^{18}F]VUIIS1009B\) (%ID/cc = 0.70 ± 0.05 at 45 min) has a comparable %ID/cc in the ipsilateral region, while \([^{18}F]VUIIS1009A\) (%ID/cc = 0.50 ± 0.05 at 45 min) has a significantly lower uptake in the same region as shown in Figure 2c, 2d and 2h.

The contralateral brain had a comparatively low uptake, and a consistent %ID/cc level was achieved at 10 min after the radiotracer injection for all three radiotracers (Figure 2g, Figure 2h and Figure 2i). When compared to \([^{18}F]VUIIS1009A\), \([^{18}F]VUIIS1009B\) and \([^{18}F]DPA-714\) demonstrate a higher contralateral brain uptake as indicated in the 45 min uptake (0.20 ± 0.03 vs 0.15 ± 0.01, ID%/cc at 45 min). For \([^{18}F]DPA-714\), the %ID/cc ratio at 45 min between the ipsilateral brain and contralateral brain in this study was 3.50 as shown in the TACs (0.73 ± 0.08 vs. 0.20 ± 0.03 %ID/cc at 45 min; Figure 2g), 3.33 for \([^{18}F]VUIIS1009A\) (0.50 ± 0.05 vs. 0.15 ± 0.01 %ID/cc at 45 min) and 3.50 for \([^{18}F]VUIIS1009B\) (0.70 ± 0.05 vs. 0.2 ± 0.03 %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.** \([^{19}F]VUIIS1009A\) 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 Figure 3a(\([^{18}F]VUIIS1009A\)) and 3c(\([^{18}F]VUIIS1009B\)), the uptake on the ipsilateral side was significantly higher than the uptake on the contralateral brain for both radiotracers before the injection of their non-radioactive analogs. However, after the displacement, radiotracer uptake of the ipsilateral brain dropped
dramatically (Figure 3b and 3d), which accounts to more than 66% decrease of the tracer uptake when compared to the normal uptake in the same intervals as shown in the TACs for both radiotracers (as shown in Figure 2g, 2h, 3e and 3f). This is similar to the displacement ratio reported by Martin et al on $^{[18F]}$DPA-714 displacement study with the same ischemia animal model$^{[33]}$. After displaced with their non-radioactive analogs, the uptake level of ipsilateral brain was almost comparable to the contralateral brain, both demonstrated by the imaging profiles and TACs in Figure 3. All these indicate the significantly high binding specificity for both radiotracers in ipsilateral brain.

**Macro Parameters Determined using Graphic Analysis**

Although both $^{[18F]}$VUIIS1009A and $^{[18F]}$VUIIS1009B have significantly higher *in vitro* binding affinities when compared to $^{[18F]}$DPA-714, they did not demonstrate a more promising imaging characterized by the semi-quantitative uptakes (like %ID/cc) as shown in Figure 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$^{[9, 18, 20]}$ (model fit as shown in Supplemental Figure 2). In this study, graphic analysis using AIFs can also be employed to determine the $V_T$ for both ipsilateral and contralateral brain region. As shown in Table 1, $V_T$ determined for ipsilateral region are higher than the corresponding values for contralateral brain for all the three radiotracers, indicating all these radiotracers tend to accumulate in ipsilateral region instead of the contralateral brain. Direct comparison of the ipsilateral $V_T$ from three radiotracers indicates $^{[18F]}$VUIIS1009B has a higher $V_T$ than the other two radiotracers as shown in Table 1. Moreover, for the contralateral brain, $^{[18F]}$VUIIS1009B also demonstrates a slightly higher $V_T$ than the other two radiotracers. The ratios of the $V_T$ values (noted as $DVR_{Logan}$) between ipsilateral brain and contralateral brain indicate a higher $DVR_{Logan}$ for $^{[18F]}$VUIIS1009B (8.53 ± 1.06) when compared with $^{[18F]}$DPA-714 (6.00 ± 0.41) and $^{[18F]}$VUIIS1009A (4.37 ± 0.82). Further study using contralateral brain as a reference region was also performed to determine $DVR_{LoganRef}$ and $BP_{ND}$ for all three radiotracers (as shown in Figure 4 and Table 1). Logan plots using contralateral brain as a reference region was plotted with $BP_{ND}$ and $DVR_{LoganRef}$ determined (Figure 4a, 4b and 4c), demonstrating a good fit and linearity for all three radiotracers with $r > 0.96$. As shown in Table 1, $^{[18F]}$VUIIS1009B (7.55 ± 0.65) demonstrating a much higher $DVR_{LoganRef}$ than $^{[18F]}$DPA-714 (5.37 ± 0.36) and $^{[18F]}$VUIIS1009A (3.91 ± 0.50). Similarly, $^{[18F]}$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 $^{[18F]}$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 $^{[18F]}$VUIIS1009B is superior to $^{[18F]}$DPA-714 and $^{[18F]}$VUIIS1009A as shown in dynamic PET imaging. In this study, we further compared the performance
of the three radiotracers using parameter images ($V_T$ and $BP_{ND}$) generated (Figure 5). In detail, voxel-wise $V_T$ images were generated via a Logan graphic method using AIFs. Voxel-wise $BP_{ND}$ images were generated using a Logan reference model with the contralateral brain input as the reference region. As expected, compared to $[^{18}F]$DPA-714, $[^{18}F]$VUIIS1009B has similar biodistribution profile for both $V_T$ and $BP_{ND}$ parameter images (Figure 5), but features higher DVR, $BP_{ND}$ in the ipsilateral 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 (Figure 5). Compared to the parametric image of $[^{18}F]$VUIIS1009B, $[^{18}F]$VUIIS1009A imaging analysis generated a noisier parametric image as shown in Figure 3a and Figure 3b. The distribution for $[^{18}F]$VUIIS1009A $V_T$ image does not match the %ID/cc image of $[^{18}F]$VUIIS1009A as shown in Figure 2C, as well as the other images for $[^{18}F]$DPA-714 and $[^{18}F]$VUIIS1009B as shown in Figure 2 and Figure 5, indicating a poor visual quality for $[^{18}F]$VUIIS1009A imaging when compared to other two radiotracers.

**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 Figure 6, with %ID/cc, $BP_{ND}$ and $V_T$ determined for the voxels in the ipsilateral brain, we made different plots for the three probes and measured the correlation coefficient $r$ and $p$ value for each data set. As shown in Figure 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 Figure 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 Figure 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 Figure 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 Figure 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-
Regional $BP_{ND}$ was also obtained and analyzed for both $[^{18}\text{F}]\text{VUIIS1009B}$ and $[^{18}\text{F}]\text{DPA-714}$ for the same rats ($n = 6$). As shown in Figure 7b, $[^{18}\text{F}]\text{VUIIS1009B}$ also demonstrates a stronger correlation of $[^{18}\text{F}]\text{DPA-714}$ 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 $[^{18}\text{F}]\text{VUIIS1009B}$ to profile TSPO expression when compared to $[^{18}\text{F}]\text{DPA-714}$.

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 $^{34-37}$. In these studies, semi-quantitative parameters like %ID/cc and SUV are widely evaluated in both clinical and preclinical studies$^{33, 38}$. 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 $^{39}$. 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. $[^{18}\text{F}]\text{DPA-714}$, a second-generation radiotracer developed by James et al.$^{[10]}$, demonstrated its superiority in neuroinflammation imaging, with higher binding potential, binding specificity and SNR. Martin et al performed the $[^{18}\text{F}]\text{DPA-714}$ PET imaging to determine the time course of TSPO expression over several days in a focal cerebral ischemia model of rat. In this study, the in vivo PET imaging and in vitro autoradiographic results confirmed increase in $[^{18}\text{F}]\text{DPA-714}$ binding at 4 and 7 days after cerebral ischemia, reaching a maximal value at 11 days, followed by slow return to normal values at 30 days. This study also demonstrated that $[^{18}\text{F}]\text{DPA-714}$ provides accurate informative information of the expression and distribution of TSPO activity after cerebral ischemia$^{33}$. Similarly, Pulagam et al also demonstrated the feasibility to use $[^{18}\text{F}]\text{VUIIS1008}$ to monitor TSPO expression longitudinally in a rat model of cerebral ischemia, with the results suggesting $^{18}\text{F}$-VUIIS1008 could become a valuable tool for the diagnosis and treatment evaluation of neuroinflammation following ischemic stroke$^{38}$. Furthermore, Golla et al. used multiple methods to generate the quantitative images for $[^{18}\text{F}]\text{DPA-714}$ in a clinic study with healthy subjects and AD patients participated $^{40}$. 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 $^{40}$. 
In our previous studies, we further modified $^{[18}\text{F}]$DPA-714 and discovered several novel TSPO imaging radiotracers, including $^{[18}\text{F}]$VUIIS1008 $^{[16-17]}$, $^{[18}\text{F}]$VUIIS1018A $^{[20-21]}$ and $^{[18}\text{F}]$VUIIS1009A/B $^{[18]}$. These novel radiotracers feature higher *in vitro* TSPO binding affinities and suitable lipophilicities for brain imaging, when compared to the parent radiotracer $^{[18}\text{F}]$DPA-714. Moreover, they also demonstrated great potential in TSPO PET imaging, including high SNR, higher binding potential, and enhanced visual effects $^{[16-18]}$. In this study, we further evaluated the performance of $^{[18}\text{F}]$VUIIS1009A and $^{[18}\text{F}]$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 ipsilateral regions. In order to confirm further the binding specificity of $^{[18}\text{F}]$VUIIS1009A and $^{[18}\text{F}]$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, which is almost the same to the ratio (up to 70%) reported by Martin *et al* for $^{[18}\text{F}]$DPA-714 displacement study using the same animal model $^{[33]}$, reflecting the high TSPO binding specificity for $^{[18}\text{F}]$VUIIS1009A/B.

Although $^{[18}\text{F}]$VUIIS1009A and $^{[18}\text{F}]$VUIIS1009B feature more than 500-fold enhancement of binding affinity when compared to $^{[18}\text{F}]$DPA-714, they did not demonstrate a superior semi-quantitative imaging profiles (%ID/cc) when compared with $^{[18}\text{F}]$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 $^{[18}\text{F}]$VUIIS1009B has a higher $V_T$ for both ipsilateral region and contralateral region, as well as higher $BP_{ND}$, $V_T$ and DVR value when compared to $^{[18}\text{F}]$DPA-714, highlighting a superior performance to $^{[18}\text{F}]$DPA714. Parameter images ($V_T$ and $BP_{ND}$) generated using Logan methods also demonstrate an enhanced visual effect for $^{[18}\text{F}]$VUIIS1009B when compared to $^{[18}\text{F}]$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, $^{[18}\text{F}]$VUIIS1009B demonstrates a much higher $BP_{ND}$ than $^{[18}\text{F}]$DPA-714, as well as a strong correlation with $BP_{ND}$ of $^{[18}\text{F}]$DPA-714 for both regional ROIs or at the voxel level. Moreover, slope of linearity is greater than one in *Figure 7*, reflecting $^{[18}\text{F}]$VUIIS1009B has the superior imaging potential and greater sensitivity to determine the TSPO expression in PET imaging when compared to $^{[18}\text{F}]$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 $^{[18}\text{F}]$DPA-714 and $^{[18}\text{F}]$VUIIS1009B. Furthermore, $^{[18}\text{F}]$VUIIS1009B demonstrates a stronger positive linear relationship between $V_T$ and $BP_{ND}$ when compared to $^{[18}\text{F}]$DPA-714. This indicates $^{[18}\text{F}]$VUIIS1009B can be more accurately profiled than $^{[18}\text{F}]$DPA-714 using the AIFs based Logan and Logan reference tissue methods in this study. While for $^{[18}\text{F}]$VUIIS1009A, %ID/cc has a weaker correlation with both $BP_{ND}$ and $V_T$ as demonstrated in this study.
Furthermore, when compared to $[^{18}\text{F}]$DPA-714 and $[^{18}\text{F}]$VUIIS1009B, $[^{18}\text{F}]$VUIIS1009A does not demonstrate a higher $BP_{ND}$ or DVR, which is also observed in our previous studies on C6 glioma imaging$^{[17-18]}$. We believe this behavior is due to the higher plasma protein binding affinity of $[^{18}\text{F}]$VUIIS1009A when compared to $[^{18}\text{F}]$DPA-714 and $[^{18}\text{F}]$VUIIS1009B which was also noticed in our previous studies$^{[18]}$. For $[^{18}\text{F}]$VUIIS1009A, the higher plasma protein binding affinity limits the partitioning of the radiotracer from blood to the tissues, and thus decreases its tissue uptake and image SNR, as well as the uptake of specific binding compartment, which will then impact the following macro parameter determination.

In this study, by evaluating two highly specific TSPO radiotracers $[^{18}\text{F}]$VUIIS1009A and $[^{18}\text{F}]$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 $[^{18}\text{F}]$VUIIS1009B will be a suitable PET radiotracer to fulfill this aim. We envision that $[^{18}\text{F}]$VUIIS1009B PET imaging coupled with parameter images ($V_T$ and $BP_{ND}$) and graphic analysis (including Logan analysis and Reference Logan analysis) holds great promise for longitudinal neuroinflammation characterization. Although both $[^{18}\text{F}]$VUIIS1009B and $[^{18}\text{F}]$DPA-714 demonstrated a strong correlation between the semi quantitative parameter (%ID/cc) and the macro parameters ($V_T$, $BP_{ND}$) derived from the graphic analysis, further study still need to verify this correlation with the aim to decide which parameter should be employed for TSPO density evaluation under specific conditions. Besides that, further studies still need to be performed to clarify the binding specificities of $[^{18}\text{F}]$VUIIS1009A/B to different TSPO binders, which are produced in human as a result of $rs6971$ single nucleotide polymorphism. Moreover, the pharmacokinetics and in vivo specific binding of $[^{18}\text{F}]$VUIIS1009A/B with TSPO, as well as the macro parameter-based analysis and images still need to be determined in the following primate studies.

**Conclusion**

This study focused on the radiosynthesis and evaluation of the novel TSPO probe $[^{18}\text{F}]$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 $[^{18}\text{F}]$VUIIS1009B PET imaging coupled with parameter images ($V_T$ and $BP_{ND}$) and graphic analysis (both Logan and Reference Logan analysis) holds great promise for neuroinflammation characterization. We envision that $[^{18}\text{F}]$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 analyzed 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.

Yong Hao: Data acquisition, manuscript revision and data analysis.

Zhoumi Hu: Experimental conception, data acquisition and analysis.

Cheng Wang: Experimental conception, data acquisition and analysis.

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Mengxin Wang: Data acquisition and analysis.

Wenxian Peng: Data acquisition and analysis, manuscript revision.

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

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Table

Table 1. Macro Parameters determined for $[^{18}\text{F}]$DPA-714, $[^{18}\text{F}]$VUIIS1009A and $[^{18}\text{F}]$VUIIS1009B with the same cerebral ischemia rats (n = 6) (data = mean ± SD).

| Probe          | Ipsilateral ($V_T$) | Contralateral ($V_T$) | DVR$_{Logan}$ | DVR$_{LoganRef}$ | BP$_{ND}$ (Ref. Logan) |
|----------------|---------------------|-----------------------|---------------|------------------|------------------------|
| $[^{18}\text{F}]$DPA-714 | 41.92 ± 7.91        | 6.94 ± 0.92           | 6.00 ± 0.41   | 5.37 ± 0.36      | 4.19 ± 0.10            |
| $[^{18}\text{F}]$VUIIS1009A | 28.93 ± 8.10        | 6.73 ± 2.14           | 4.37 ± 0.82   | 3.91 ± 0.50      | 2.90 ± 0.50            |
| $[^{18}\text{F}]$VUIIS1009B | 75.52 ± 6.53        | 9.23 ± 0.92           | 8.53 ± 1.06   | 7.55 ± 0.65      | 6.55 ± 0.65            |

Figures

Figure 1

Structures of DPA-714, VUIIS1009A, VUIIS1009B and their corresponding IC50.
Figure 2

45-min PET dynamic scan for [18F]DPA-714, [18F]VUIIS1009A and [18F]VUIIS1009B. (a) Coronal and (b) transverse PET image of a cerebral ischemia rat from dynamic scan with [18F]DPA-714. (c) Coronal and (d) transverse PET image of the same cerebral ischemia rat from dynamic scan with [18F]VUIIS1009A. (e) Coronal and (f) transverse PET image of the same cerebral ischemia rat from dynamic scan with [18F]VUIIS1009B. Time activity curves (TACs) for the ipsilateral (red) and contralateral brain (blue) in the 45-min dynamic scan (n = 6) for [18F]DPA-714 (g), [18F]VUIIS1009A (h) and [18F]VUIIS1009A (f). %ID/cm3 = percentage injected dose per cubic centimeter. In TACs, data = mean ± SD. Ipsilateral ROI is marked by the red circle and contralateral ROI was marked by the yellow circle in the image.
Figure 3

Displacement analysis for rats imaged by [18F]VUIIS1009A and [18F]VUIIS1009B with the injection of their corresponding non-radioactive analog (10 mg/kg) at 20 min. (a) Summation of the first 20 min PET imaging (coronal) with [18F]VUIIS1009A. (b) Summation of the last 25 min of PET imaging (coronal) with [18F]VUIIS1009A. (c) Ipsilateral and contralateral TACs for [18F]VUIIS1009A displacement analysis (n = 3). (d) Summation of the first 20 min of PET imaging (coronal) with [18F]VUIIS1009B (n = 3). (e) Summation of the last 25 min of PET imaging (coronal) with [18F]VUIIS1009B. (f) Ipsilateral and contralateral brain TACs for [18F]VUIIS1009B displacement analysis (n = 3). Ipsilateral ROI is marked with the red circle and contralateral ROI with yellow circle. Data = mean ± SD.
Figure 4

Graphic plot of the Logan Reference Tissue for (a) [18F]DPA-714, (b) [18F]VUIIS1009A and (c) [18F]VUIIS1009B.
Figure 5

(a) Parametric images for [18F]DPA-714, [18F]VUIIS1009A and [18F]VUIIS1009B using Logan method with AIFs input. (b) Parametric images for [18F]DPA-714, [18F]VUIIS1009A and [18F]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)[18F]DPA-714, (b)[18F]VUIIS1009A and (c)[18F]VUIIS1009B at voxel level. Dots (n = 60 to 80) indicates the voxels randomly collected from the ipsilateral brain in a cohort of rats (n = 6).
Figure 7

(a) Correlation of BPND determined from [18F]VUIIS1009B and [18F]DPA-714 dynamic PET scan in the same rats at the voxel level. Dots (n = 60) indicates the voxels randomly collected from the ipsilateral brain in a cohort of rats (n = 6). (b) Correlation of regional BPND determined from [18F]VUIIS1009B and [18F]DPA-714 dynamic PET scans in a cohort of rats (n = 6).

Supplementary Files

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