Preclinical evaluation and quantification of [^{18}F]MK-9470 as a radioligand for PET imaging of the type 1 cannabinoid receptor in rat brain

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Abstract

Purpose [^{18}F]MK-9470 is an inverse agonist for the type 1 cannabinoid (CB1) receptor allowing its use in PET imaging. We characterized the kinetics of [^{18}F]MK-9470 and evaluated its ability to quantify CB1 receptor availability in the rat brain.

Methods Dynamic small-animal PET scans with [^{18}F]MK-9470 were performed in Wistar rats on a FOCUS-220 system for up to 10 h. Both plasma and perfused brain homogenates were analysed using HPLC to quantify radiometabolites. Displacement and blocking experiments were done using cold MK-9470 and another inverse agonist, SR141716A. The distribution volume (V_T) of [^{18}F]MK-9470 was used as a quantitative measure and compared to the use of brain uptake, expressed as SUV, a simplified method of quantification.

Results The percentage of intact [^{18}F]MK-9470 in arterial plasma samples was 80 ± 23 % at 10 min, 38 ± 30 % at 40 min and 13 ± 14 % at 210 min. A polar radiometabolite

fraction was detected in plasma and brain tissue. The brain radiometabolite concentration was uniform across the whole brain. Displacement and pretreatment studies showed that 56 % of the tracer binding was specific and reversible. V_T values obtained with a one-tissue compartment model plus constrained radiometabolite input had good identifiability (≤ 10 %). Ignoring the radiometabolite contribution using a one-tissue compartment model alone, i.e. without constrained radiometabolite input, overestimated the [^{18}F]MK-9470 V_T , but was correlated. A correlation between [^{18}F]MK-9470 V_T and SUV in the brain was also found ($R^2 = 0.26$ – 0.33 ; $p \leq 0.03$). **Conclusion** While the presence of a brain-penetrating radiometabolite fraction complicates the quantification of [^{18}F]MK-9470 in the rat brain, its tracer kinetics can be modelled using a one-tissue compartment model with and without constrained radiometabolite input.

Keywords Type 1 cannabinoid receptor · Small-animal PET · [^{18}F]MK-9470 · Kinetic analysis · Rat

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Introduction

The type 1 cannabinoid (CB1) receptor is one of the most abundant G-protein-coupled receptors discovered in the human brain, with levels of expression up to 1 pmol/mg tissue or greater [1]. The brain CB1 receptor plays an important role in homeostatic mechanisms [2] and is involved in pathophysiological conditions such as epilepsy, addiction, stroke, eating disorders, schizophrenia and movement disorders (for reviews see [3, 4]). The broad-spectrum action of the CB1 receptor occurs through modulating the release, uptake and actions of excitatory and inhibitory transmitters such as γ -aminobutyric acid, glutamate or dopamine [5], and has the potential for use in pharmacological treatments

[6]. Effective PET radioligands for the CB1 receptor act as biomarkers and can guide dosing of therapeutic agents. Since the pioneering work on ^{18}F -labelled Δ^9 -THC in 1991 [7], several PET radioligands have successfully imaged CB1 receptors in the rat, monkey and/or human brain: [^{11}C]JHU75528 and [^{11}C]OMAR [8], [^{11}C]MePPEP [9–11], [^{11}C]FMPEP [12, 13], [^{18}F]FEPEP [13], [^{18}F]FMPEP- d_2 [12, 13] and [^{18}F]MK-9470 [14]. Full kinetic modelling and quantification using concurrent brain and plasma data have only been published in rodents for [^{11}C]MePPEP [9], and in humans for [^{11}C]MePPEP [11], [^{11}C]OMAR [15] and [^{18}F]MK-9470 [14]. For [^{11}C]MePPEP, species differences in the amount of specific binding, in the plasma radiometabolite concentration and subsequently in its quantification have been reported [9, 11].

[^{18}F]MK-9470 is an inverse agonist with high affinity ($K_i=0.7$ nM) and selectivity for the CB1 receptor. Despite its moderately high lipophilicity ($\log D_{7.4}=4.7$), [^{18}F]MK-9470 shows good brain uptake and a high percentage of specific binding in monkey brain [14]. [^{18}F]MK-9470 has been used in the clinical setting to support CB1 receptor drug development for measuring receptor occupancy [16], to investigate CB1 receptor variability with gender and normal ageing [17, 18] and in studying pathophysiological conditions [19–22].

Since interspecies differences in the distribution, metabolism and kinetics of [^{18}F]MK-9470 are probably also present, we report here the preclinical characterization and quantification of [^{18}F]MK-9470 in the rat brain. As the primary objective, we assessed the pharmacological specificity of brain uptake and the binding characteristics of [^{18}F]MK-9470. As the second objective, we quantified imaging data by compartmental modelling and serial arterial measurements of parent radiotracer separated from radiometabolites. The outcome measure was the total distribution volume (V_T) [23], which is the ratio at equilibrium of radioactivity in the brain to the concentration of parent radioligand in plasma. Finally, we evaluated simplified methods of analysis that may help eliminate the need for arterial sampling in this species.

Material and methods

[^{18}F]MK-9470 characteristics and preparation

The precursor for the synthesis of [^{18}F]MK-9470 (*N*-[2-(3-cyano-phenyl)-3-(4-(2-[^{18}F]fluoroethoxy)phenyl)-1-methylpropyl]-2-(5-methyl-2-pyridyloxy)-2-methylpropanamide) was obtained from Merck Research Laboratories (West Point, PA) and labelling was performed on-site using 2-[^{18}F]fluoroethylbromide, as described previously [14]. The final product was obtained after high-performance liquid

chromatography (HPLC) purification and had a radiochemical purity of >95 %. Specific radioactivity was always >90 GBq/ μmol . The tracer was administered in a sterile solution of 5 mM sodium acetate buffer (pH 5.5) containing 6 % ethanol.

Animals

Experiments were conducted in 39 female Wistar rats (Charles River, L'Arbresle, France) weighing 266 ± 37 g at the start of the experiments. Of the 39 rats, 21 were randomly selected for use in *in vivo* imaging, and the others were used for *in vitro* and *ex vivo* measurements. All animals were housed three to a cage at an average room temperature of 22 °C and a 12-h light/dark cycle. Food and water were given *ad libitum*. The research protocol was approved by the local Animal Ethics Committees of the University of Leuven, and was in accordance with European Ethics Committee guidelines (decree 86/609/EEC).

Small-animal PET imaging

Imaging experiments were performed on a lutetium oxy-orthosilicate detector-based tomograph (microPET FOCUS-220; Siemens Medical Solutions USA, Knoxville, TN), which had a transaxial resolution of 1.35 mm in full-width at half-maximum. Data were acquired in a $128\times 128\times 95$ matrix with a pixel width of 0.475 mm and a slice thickness of 0.796 mm. The coincidence window width was set at 6 ns. During PET imaging, rats were kept under gas anaesthesia (2.5 % isoflurane in oxygen at a flow rate of 1 l/min), and their body temperature was maintained between 36.5 and 37 °C with a heating pad. About 44–63 MBq of [^{18}F]MK-9470 (specific activity range 95–253 GBq/ μmol) was injected into the tail vein using an infusion needle set after overnight fasting. Upon injection, residual activity in the tail was monitored in a subset of animals and was assumed to be negligible following quantitative assessment (%ID 2.11 ± 0.33 %). Dynamic 120-min and 240-min acquisitions were started immediately after [^{18}F]MK-9470 injection for pretreatment (three animals per pretreatment) and displacement evaluation (three animals), respectively. Longer scans over 600 min were acquired in seven rats to assess the kinetics of [^{18}F]MK-9470 using compartmental modelling.

For pretreatment experiments, SR141716A/rimonabant (30 mg/kg and 50 mg/kg, Acomplia; Sanofi-Aventis) was diluted in distilled water (final concentration: 20 mg/ml), and administered orally 60 min before radioligand injection. Cold MK-9470 (1 mg/kg) was used for displacement evaluation. Cold MK-9470 was dissolved in a saline solution containing 10 % dimethylsulphoxide and 50 % polyethylene glycol, and was administered by intravenous injection at 2 h after tracer injection. The dose of 30 mg/kg and time of

pretreatment were chosen to completely block CB1 receptors, as previously described by Need et al. [24], and further validated on-site using a much higher dose of SR141617A, i.e. 50 mg/kg.

Acquisition data were Fourier-rebinned in 27, 39 and 57 frames (4×15 , 4×60 , 5×180 , 8×300 , 24×600 , $9 \times 1,200$ and $3 \times 2,400$ s), depending on the total scan duration. Sinograms were reconstructed using filtered back-projection to avoid possible positive bias due to iterative methods [25]. No corrections were made for attenuation or scatter, due to the much smaller size of the rodent head and the lower thickness and density of the skull as compared to primates [26].

Measurement of [^{18}F]MK-9470 in plasma

To measure the concentration of [^{18}F]MK-9470 in rat plasma, an arterial cannula was placed in the femoral artery. After slow bolus injection of [^{18}F]MK-9470, arterial blood was continuously collected for 1 min (12 samples, approximately one sample every 5 s), followed by 100- μl samples at 90, 120 and 150 s and 3, 5, 10, 40, 60 and 120 min. Extra 200- μl samples were drawn at 210, 300, 420 and 540 min for the 240- and 600-min scans. All blood samples were immediately stored on ice to stop tracer metabolism. The plasma time-activity curves were corrected for the fraction of unchanged radioligand using reversed-phase HPLC (RP-HPLC) separation (LaChrom Elite HPLC system, Hitachi; Chromolith C18, 3×100 mm, Merck). Plasma samples were isolated from whole blood by centrifugation at 3,000 rpm for 10 min and placed in CH_3CN (25 μl) spiked with standard MK-9470 (25 μg) prior to HPLC analysis. The HPLC separation was achieved with gradient mixtures of 0.05 M sodium acetate (pH 5.5) (A) and CH_3CN (B) (1 % B, flow rate 0.7 ml/min, from 0 to 6 min; 80 % B, flow rate 0.7 ml/min, from 6 to 9 min; and 1 % B, flow rate 0.7 mL/min, from 9.1 to 15 min). The HPLC eluate was collected as 0.7-ml fractions after passing through an inline ultraviolet detector (280 nm). Radioactivity in all fractions was measured using an automated γ -counter equipped with a 7.62-cm NaI(Tl) well crystal coupled to a multichannel analyser (1480 Wizard; Wallac). Results were corrected for background radiation and physical decay during counting. The recovery of the radioactivity injected onto the HPLC column was calculated as the radioactivity in the supernatant relative to that in the precipitate.

The free fraction of [^{18}F]MK-9470 in plasma was measured by ultrafiltration through Centrifee membrane filters in three rats 2 min after tracer injection, as previously described [27]. Nonspecific binding to the membrane was assayed by adding protein-free plasma (PFP, obtained by centrifugal filtration using the same centrifugal device) spiked with 18.5 MBq of [^{18}F]MK-9470. Measurements were performed in triplicate and the percentage free fraction (f_p) was calculated as:

$$100 \times (\text{cps/g plasma filtrate 2 min after injection}) / [\text{cps/g plasma} \times (\text{cps PFP filtrate/cps filter} + \text{cps PFP filtrate})]$$

Measurement of [^{18}F]MK-9470 radiometabolites in brain

For each time point, two rats were infused with about 37 MBq of [^{18}F]MK-9470 under isoflurane anaesthesia (2.5 % in oxygen at a flow rate of 1 l/min). At 10, 30, 60, 120 and 240 min after injection, the rats were killed by sodium pentobarbital (Nembutal, Ceva Sante Animale, Brussels, Belgium) overdose and perfused transcardially with saline. Their brain was carefully removed from the skull, and the cerebrum and cerebellum were separated and homogenized in 3 and 2 ml of CH_3CN , respectively, for 2 min. The homogenates were processed as previously described by Celen et al. [28]. About 0.5 ml of homogenate extract was injected onto an HPLC system consisting of an analytical XBridge column (C₁₈, 5 μM , 3 mm $\times$ 100 mm; Waters) eluted with a mixture of 0.05 M sodium acetate (pH 5.5) and CH_3CN (50/50 v/v) at a flow rate of 0.8 ml/min. All HPLC eluates were collected as 0.8-ml fractions after passing through the UV detector (280 nm). Radioactivity in the fractions was measured using an automated γ -counter. The recovery of total radioactivity from brain tissue was determined prior to study set-up and was >81 %.

In vitro rat brain and blood stability studies

Fresh rat blood, plasma and homogenized perfused brain were incubated with about 18 MBq of [^{18}F]MK-9470 at 37 °C. After 60 min of incubation, samples were cooled on ice. Blood and brain homogenate samples were processed and analysed by RP-HPLC as described above.

Small-animal PET analysis

PET images were normalized to a custom-made rat brain template in Paxinos stereotactic space, allowing the use of a predefined volume of interest map. The procedure for spatial normalization and its validation have been described previously [29]. Time-activity curves were generated for the bilateral caudate-putamen, cerebral cortex and cerebellum of each individual scan using PMOD software (version 3.1; PMOD Technologies, Zurich, Switzerland). The macrostructures were used based on the spatial resolution of the FOCUS-220 system [30]. The radioactivity concentration in each brain region was normalized to the injected dose and body weight, and expressed as standardized uptake value (SUV).

Compartmental modelling of [^{18}F]MK-9470 in the rat brain

All [^{18}F]MK-9470 input curves were obtained after radiometabolite correction and correction for ^{18}F decay. One- and

two-tissue compartment models were considered to describe [^{18}F]MK-9470 kinetics in the rat brain using the metabolite-corrected plasma input function. Brain radiometabolites were modelled as a separate brain compartment using a radiometabolite input function derived from the entire polar radiometabolite fraction. We first estimated the rate constants for a grouped brain volume of interest comprising all three brain regions, and then used these estimates as the starting values for obtaining the final fitting parameters of each brain region separately. Consensus nomenclature was used [23], where V_T is the total distribution volume, including specific and nondisplaceable uptake. The kinetic analyses were performed using PMOD 3.1.

To determine the minimum scanning time necessary to obtain stable fitting values for the volume of distribution, we analysed PET data from each rat after removing variable durations of the terminal portion of the scans. We analysed data of all animals for the periods 0–60, 0–120, 0–300 and 0–450 min. Estimates of the volume of distribution were compared with the reference value obtained with the complete 600-min dataset. The solution was considered stable after time t if the result was within 10 % of that from the analysis of the entire 600-min dataset.

Simplification of [^{18}F]MK-9470 quantification

As arterial blood sampling in rodents is technically demanding and hampers serial small-animal PET studies over time in this species, we also explored the possibility of simplifying [^{18}F]MK-9470 quantification in rat studies. We evaluated the use of tracer concentration in tissue at the end of the scan, expressed as SUV [31] and modified (mSUV) [32], as an index of tracer binding to CB1 receptors. The ability of SUV and mSUV to quantify tracer binding was assessed by comparing with the gold standard parameter from tracer kinetic analysis, V_T , obtained from the 600-min dataset. Brain uptake not corrected for plasma measurements has been used previously with [^{18}F]MK-9470 to measure CB1 receptor availability in the human brain [27], and has recently been evaluated for [^{11}C]MePPEP and [^{18}F]FMPEP- d_2 [11, 13].

The reproducibility of SUV and mSUV was assessed in five extra rats and expressed as: $|R_1 - R_2| * 2 / (R_1 + R_2)$, where the indices 1 and 2 refer to the two different scans of the same animal.

Statistical analysis

The reported values are means $\pm$ SD. Conventional statistics were carried out using GraphPad Prism v5.0 (San Diego, CA). Significance was defined at the 95 % probability level. Goodness-of-fit by nonlinear least-squares analysis was evaluated using the Akaike information criteria (AIC) and

the model selection criteria (MSC). The most appropriate model is that with the smallest AIC and the largest MSC values. The coefficient of variation was obtained from the covariance matrix resulting from the model sensitivity and is expressed as percentage of the parameter value (%COV).

Results

Brain kinetics

After injection of [^{18}F]MK-9470, all rats showed concentrations of radioactivity in the brain in a pattern consistent with the known CB1 receptor distribution, i.e. the highest uptake in the cerebellum and caudate-putamen, and a good fairly uniform uptake in the cortex (Fig. 1) [33]. [^{18}F]MK-9470 peak uptake values ranged from 0.6 to 1.6 SUV (Fig. 2a). [^{18}F]MK-9470 uptake remained relatively constant from 300 min after tracer injection.

Plasma kinetics

The percentage of intact [^{18}F]MK-9470 in arterial plasma over time is shown in Fig. 2b for the rats scanned over 600 min. At 10 min after injection, 80 ± 23 % of the total radioactivity in arterial plasma corresponded to [^{18}F]MK-9470. This fraction declined to 38 ± 30 % at 40 min, 19 ± 19 % at 120 min and 13 ± 14 % at 210 min after tracer injection. The intact fraction was about 10 % from 300 min to 540 min after injection. The free fraction of [^{18}F]MK-9470 in plasma was very low. The average f_p was 0.0034 ± 0.0004 .

Fig. 1 Mean [^{18}F]MK-9470 in the rat brain. Average cross-sectional small-animal PET images of [^{18}F]MK-9470 uptake in the rat brain during the period 60–80 min after injection ($n=7$). The coloured bar represents SUV. The intersection point of the three planes has been set to the mid-striatal level, i.e. $(x,y,z)=(-2.9, -0.3, -5.6)$ Paxinos coordinates, which corresponds to the right hemisphere

Fig. 2 Brain and plasma kinetics of $[^{18}\text{F}]$ MK-9470 in rats. **a** Time-activity curves of $[^{18}\text{F}]$ MK-9470 in brain from seven different rats scanned for 600 min. Concentrations are expressed as SUV, which normalizes for injected radioactivity and body weight. **b** Average percentage of intact $[^{18}\text{F}]$ MK-9470 in arterial plasma samples from

seven rats scanned for 600 min. Data are expressed as means $\pm$ SEM. **c** Reconstructed radiochromatograms from the analysis of plasma from one rat after $[^{18}\text{F}]$ MK-9470 injection. The retention time of $[^{18}\text{F}]$ MK-9470 is approximately 10 min. A polar radiometabolite eluted at about 2 min (*no* number)

Rat plasma samples contained one radiometabolite fraction, which eluted earlier than $[^{18}\text{F}]$ MK-9470 on HPLC, and was therefore more hydrophilic (polar) than the parent radioligand (Fig. 2c). No radiometabolites with a higher lipophilicity, i.e. less polar, than $[^{18}\text{F}]$ MK-9470 were detected.

Ex vivo and in vitro radiometabolite analysis in rats

To assess whether $[^{18}\text{F}]$ MK-9470 radiometabolite(s) are present in the brain over time, we killed two rats at 10, 30, 60, 120 and 240 min after $[^{18}\text{F}]$ MK-9470 injection. Polar radiometabolite(s) of $[^{18}\text{F}]$ MK-9470 were present in the brain (Table 1). The polar radiometabolite fraction in the cerebrum was comparable to that in the cerebellum at all studied time points. The percentages of radiometabolite(s) in the rat cerebellum were 19.0 ± 3.0 % at 10 min, 13.3 ± 7.5 % at 30 min, 28.2 ± 0.3 % at 60 min, 31.0 ± 8.1 % at 120 min and 14.7 ± 2.5 % at 240 min after injection. The recovery of the HPLC and XBridge column-injected radioactivity was 96.3 ± 2.1 % ($n=3$).

$[^{18}\text{F}]$ MK-9470 was shown to be metabolically stable in rat whole blood, rat plasma and rat brain homogenate in vitro. The fraction of polar radiometabolite(s) observed after 60 min incubation at 37 °C was negligible (<1 %).

Specificity and binding characteristics of $[^{18}\text{F}]$ MK-9470 in rats

$[^{18}\text{F}]$ MK-9470 was selective for the CB1 receptor in rats when assessed by pharmacological pretreatment with a highly selective CB1 inverse agonist. Rimonabant (30 mg/kg) administered orally 1 h prior to $[^{18}\text{F}]$ MK-9470 injection had blocked more than half of the brain activity by 40 min after tracer injection compared to the average baseline scan in all rats (Fig. 3a). The ratio of the areas under the curve of pretreated in relation to baseline animals from 0 to 120 min was

0.44, indicating that about 56 % of $[^{18}\text{F}]$ MK-9470 was specifically bound to CB1 receptors. A much higher oral dose of SR141716A than necessary to completely block brain CB1 receptors, i.e. 50 mg/kg, resulted in a comparable finding with a ratio of the areas under the curve of 0.46.

Displacing the brain activity with cold MK-9470 yielded residual activity similar to seen in the pretreatment study (Fig. 3b). Cold MK-9470 (1 mg/kg) administered 120 min after tracer injection decreased brain uptake significantly from a peak concentration of 1.4 ± 0.5 SUV at 120 min to about 0.6 ± 0.4 SUV at 240 min, indicating reversible binding.

Compartmental modelling of $[^{18}\text{F}]$ MK-9470 in the rat brain

As our data showed that a radiometabolite fraction crosses the blood–brain barrier, we included an additional one-tissue compartment to the brain compartment for modelling the kinetics of the radiometabolite(s). Since $[^{18}\text{F}]$ MK-9470 is metabolized peripherally, no crosstalk between this radiometabolite brain compartment and the intact ligand brain compartment was modelled. In this way, we used two parallel input curves, one for the intact radioligand and one for the radiometabolite(s), to describe $[^{18}\text{F}]$ MK-9470 brain kinetics [34].

We used V_T as the quantitative measure and only focused on the one-tissue compartment for modelling $[^{18}\text{F}]$ MK-9470 brain kinetics as the two-tissue compartment did not provide stable estimates (2–53 %COV) for parameters describing specific binding. In more detail, using the two-tissue compartment model, k_4 tended towards a very small value in four out of the seven rats. On the other hand, setting k_4 to zero, i.e. fitting an irreversible model, did not provide acceptable fit for all the data. Fitting the results using the two-tissue compartment model was also strongly dependent on the initial parameter settings.

When we used the one-tissue compartment model for describing $[^{18}\text{F}]$ MK-9470 brain kinetics, we also assessed the utility of constraining radiometabolite(s) uptake

Table 1 Relative percentages of intact tracer and radiometabolites in perfused rat cerebrum and cerebellum at 10, 30, 60, 120 and 240 min after injection of [^{18}F]MK-9470. The values presented are means $\pm$ SD ($n=2$)

	10 min		30 min		60 min		120 min		240 min	
	Cerebrum	Cerebellum	Cerebrum	Cerebellum	Cerebrum	Cerebellum	Cerebrum	Cerebellum	Cerebrum	Cerebellum
Polar radiometabolite	12.9 $\pm$ 5.1	19.0 $\pm$ 3.0	12.4 $\pm$ 11.0	13.3 $\pm$ 7.5	25.6 $\pm$ 6.3	28.2 $\pm$ 0.3	30.2 $\pm$ 9.7	31.0 $\pm$ 8.1	10.5 $\pm$ 3.1	14.7 $\pm$ 2.5
Intact	87.1 $\pm$ 5.1	81.0 $\pm$ 3.0	87.6 $\pm$ 11.0	86.7 $\pm$ 7.5	74.4 $\pm$ 6.3	71.8 $\pm$ 0.4	69.8 $\pm$ 9.7	69.0 $\pm$ 8.1	89.5 $\pm$ 3.2	85.3 $\pm$ 2.5

(radiometabolite $V_T=K_{1m}/k_{2m}$) to a single value determined from all regions, in line with our brain radiometabolite(s) concentration which displayed a homogeneous distribution throughout the brain (Fig. 4). When comparing AIC and MSC scores, the constrained model fitted the data better than did the unconstrained model in all regions. Constrained [^{18}F]MK-9470 V_T values were 35.5 $\pm$ 11.6 in the cerebellum, 39.5 $\pm$ 8.8 in the caudate-putamen and 46.4 $\pm$ 13.8 in the cortex. The whole brain radiometabolite V_T was 1.1 $\pm$ 0.8 (Table 2).

We also calculated [^{18}F]MK-9470 V_T values with a one-tissue compartment model, ignoring the radiometabolite(s) contribution (Fig. 4). Although V_T values derived from this model overestimated the [^{18}F]MK-9470 V_T values derived with a radiometabolite input by an average of 9 % (Table. 2), the values were correlated ($R^2=0.51$; $p<0.001$; Fig. 5a).

To assess the minimal imaging time required for stable identification of V_T , we increasingly truncated the brain data, from 0–600 to 0–60, at terminal times of 450, 300, 120 and 60 min. We assessed the stability of V_T over time using the one-tissue compartment model with and without constrained radiometabolite input (Fig. 6). The calculated V_T values increased asymptotically from 60 to 600 min, and reached 90 % of the terminal value within 450 min and 500 min for the one-tissue model with and without constrained radiometabolite input, respectively (Fig. 6). This indicates that for full kinetic modelling, impractically long scans would be required for at least 300 min, hence necessitating the use of simplified quantitative measures.

Simplification of [^{18}F]MK-9470 quantification

To assess the ability of SUV and mSUV to quantify tracer binding to CB1 receptors in rats, we calculated brain uptake for four time periods: 60–80, 120–150, 180–220 and 300–360 min. These periods were chosen for their ease of use in future experiments. Brain uptake expressed as SUV and mSUV did not significantly differ over time for all brain regions investigated. Brain uptake between the different periods increased by 6–8 %, 1–4 % and 0.1–3 %, respectively. For these periods, between-subject variability was slightly higher than that of V_T (about 30 %) derived from a one-tissue model with constrained radiometabolite input. The between-subject variability of SUV₆₀₋₈₀, for example, ranged from 36 % in the cerebellum to 39 % in the caudate-putamen, and the equivalent values using mSUV were 35 % and 39 %, respectively.

The SUV values for the different time periods correlated with the [^{18}F]MK-9470 V_T derived from a one-tissue model with constrained radiometabolite input (R^2 0.26–0.33; $p\leq 0.03$; Fig. 5b). No significant correlations between SUV and the radiometabolite V_T were found, or between SUV and V_T when ignoring the radiometabolite(s) contribution. mSUV

Fig. 3 Preblocking and displacement of brain activity in rats. Small-animal PET time-activity curves for $[^{18}\text{F}]$ MK-9470 in the caudate-putamen (triangles), cortex (circles) and cerebellum (squares). **a** Pre-blocking experiment: rimonabant, 30 mg/kg and 50 mg/kg, was administered orally 60 min before $[^{18}\text{F}]$ MK-9470 injection. *Open symbols*

baseline condition ($n=7$); *full symbols* preblocked conditions with 30 mg/kg ($n=3$); *half symbols* preblocked conditions with 50 mg/kg ($n=3$). **b** Displacement experiment: authentic MK-9470, 1 mg/kg, was administered intravenously 120 min after $[^{18}\text{F}]$ MK-9470 injection ($n=3$)

was not significantly correlated with the kinetic modelling outcomes. Regional SUV test–retest variabilities were below 10 % when using data between 60 and 80 min to estimate brain uptake.

Discussion

The evaluation of $[^{18}\text{F}]$ MK-9470 in rats demonstrated uptake in the brain in a pattern consistent with the known CB1 receptor distribution [33]. Specific binding was about 56 % of the total brain uptake and labelling of CB1 receptors with $[^{18}\text{F}]$ MK-9470 was pharmacologically reversible. Using RP-HPLC, we showed that a polar radiometabolite fraction crossed the blood–brain barrier, but displayed a homogeneous

distribution throughout the brain (about 10–30 %). Quantification of $[^{18}\text{F}]$ MK-9470 binding to the CB1 receptor was possible using a model with either a combination of ligand and radiometabolite input functions or ligand input function alone. Values of V_T , as the quantitative measure, had good identifiability (≤ 10 %), but high between-subject variation (about 30 %).

The pattern of uptake in the rat brain was similar to the previously reported ex vivo pattern [33]. The uptake, expressed in SUV, of $[^{18}\text{F}]$ MK-9470 in the rat brain was also comparable in magnitude to the uptake seen in humans [14], ranging from 0.6 to 1.5 SUV. We estimate that these peak concentrations of radioligand correspond to less than 1.5 % receptor occupancy in the rat brain, based on published values of receptor density [35], and are thus well

Fig. 4 One-tissue compartment analysis with and without constrained radiometabolite input. Representative fits of the brain $[^{18}\text{F}]$ MK-9470 time-activity curve using a one-tissue compartment model (dashed lines) and a one-tissue compartment model with constrained radiometabolite input (solid lines). The radiometabolite-corrected plasma input is plotted against the y-axis on the right side

Table 2 Regional V_T estimates obtained from the rats scanned over 600 min using a one-tissue compartment model with and without constrained radiometabolite input. Data are expressed as means $\pm$ SD or mean (range); $n=7$

Region	One-tissue compartment model						One-tissue compartment model with constrained radiometabolite input ^a					
	K_1 (ml cm ⁻³ min ⁻¹)		k_2 (min ⁻¹)		V_T (%COV)		K_1 [¹⁸ F]MK-9470 (ml cm ⁻³ min ⁻¹)		k_2 [¹⁸ F]MK-9470 (min ⁻¹)		V_T [¹⁸ F]MK-9470 (%COV)	V_T metabolite, whole brain (%COV)
	Mean	Range	Mean	Range			Mean	Range	Mean	Range		
Cortex	0.05	0.013–0.155	0.001	0.001–0.002	49.3 $\pm$ 22.8 (8.4 $\pm$ 4.8)		0.05	0.017–0.151	0.001	0.0004–0.002	46.4 $\pm$ 13.8 (8.6 $\pm$ 4.7)	1.1 $\pm$ 0.8 (6.4 $\pm$ 2.7)
Caudate- putamen	0.06	0.015–0.168	0.001	0.001–0.003	42.8 $\pm$ 14.9 (6.4 $\pm$ 3.5)		0.05	0.010–0.166	0.001	0.0002–0.003	39.5 $\pm$ 8.8 (10.1 $\pm$ 8.0)	
Cerebellum	0.06	0.016–0.166	0.001	0.001–0.003	40.3 $\pm$ 18.7 (6.6 $\pm$ 3.7)		0.05	0.010–0.163	0.001	0.0005–0.003	35.5 $\pm$ 11.6 (7.8 $\pm$ 4.1)	

K_1 , uptake rate constant; k_2 , clearance rate constant

^a V_T metabolite (K_{1m}/k_{2m})=c^{te}

below the pharmacological threshold and in the range of microdosing. Comparison of the areas under the curves from both baseline and blocked scans showed that about 56 % of this [¹⁸F]MK-9470 uptake was specific for CB1 receptors. In the same species, Terry et al. reported a similar finding for [¹¹C]MePPEP, showing that 3 mg/kg rimonabant intravenously displaced about 65 % of brain radioactivity [9]. Also, Horti et al. observed an average reduction of about 50 % in the regional binding potential values of [¹¹C]OMAR in mice [8]. However, using [¹¹C]MePPEP and its analogues in monkey brain, rimonabant pretreatment (3 mg/kg intravenously) consistently resulted in a higher specific binding than in rats, ranging from about 60 % for [¹⁸F]-FEPEP to about 90 % for [¹¹C]MePPEP, [¹⁸F]FMPEP and [¹⁸F]FMPEP-d₂ [11, 13]. It is important to note that in comparing the brain activity curves in this study we did not correct for changes in arterial input function, which is required for accurate quantification of the percentage specific signal in the brain.

One polar radiometabolite fraction of [¹⁸F]MK-9470 was detected in plasma. Although this radiometabolite fraction had lower lipophilicity than [¹⁸F]MK-9470, it did cross the blood–brain barrier. Its brain concentration could be considered uniform across the whole brain (radiometabolite V_T 1.1 $\pm$

0.8). Because [¹⁸F]MK-9470 was stable in vitro in rat brain homogenates, its metabolism presumably occurs in the periphery. Incubation of rat liver microsomes with [¹⁸F]MK-9470 has demonstrated that the major fluorine-containing metabolite is the compound where the right hand site methyl group on the pyridine ring is oxidized to the carboxylic acid. This compound has negligible affinity for the CB1 receptor (K_i hCB1 220 nM vs. 0.7 nM for [¹⁸F]-MK9470; unpublished data), and will therefore not be a confounder even if it does penetrate the brain. In addition, the in vitro microsome assay also showed loss of the fluoromoiety via *O*-dealkylation, resulting in [¹⁸F]-fluoroethanol or its oxidized forms. [¹⁸F]-fluoroethanol or its products [¹⁸F]-fluoroacetaldehyde and [¹⁸F]-fluoroacetic acid have previously been suggested to enter the brain, but to the best of our knowledge are not known to be active at the CB1 receptor [28, 36].

While the presence of this brain-penetrating radiometabolite fraction complicates the modelling of [¹⁸F]MK-9470 in the rat brain, we modelled its tracer kinetics using a one-tissue compartment model with and without radiometabolite input. When, in general, the metabolite input function is omitted and no separate brain compartment is used for modelling metabolite kinetics, Fujita et al. demonstrated that the distribution

Fig. 5 Relationship between V_T derived from a parent plus constrained radiometabolite input function and V_T using parent input only (a) and SUV₆₀₋₈₀ (b) (solid lines linear regression, dotted lines 95 % probability level)

Fig. 6 Time stability of V_T in rats. Increasingly truncated intervals of brain time–activity data were analysed from 0 to 450 min. V_T was calculated for one-tissue compartment model with (squares) and without (circles) constrained radiometabolite input and expressed as a ratio to that determined with the complete scanning from 0 to 600 min. The error bars represent the SDs of the ratios ($n=7$ animals)

volume for specific binding remains unchanged for a two-tissue compartment model [34]. However, in the present study, the distribution volume for specific binding could not be considered as an outcome measure since estimation of k_4 was problematic due to slow tracer kinetics. For this reason, only the total distribution volume (V_T) was chosen as the quantitative measure and estimation of V_T was done by including and excluding a radiometabolite input function.

Quantification of [^{18}F]MK-9470 binding as V_T , however, did not provide stable values after about 300 min of scanning. The estimates for V_T continued to increase using both models in the later portion of the scans (by about 25–30 % from 300 to 600 min). Nevertheless, for both models, Fig. 6 shows that 10 hours of scanning very good approximates the V_T that could be obtained with even longer scanning times. Since the increasing estimate of V_T was independent of the model used—whether we ignored the radiometabolite contribution or not—this instability is inconsistent with the accumulation of radiometabolites in the brain over time. The instability of V_T with time could conceivably be related to the slow washout of [^{18}F]MK-9470 from the rat brain while even at later time points intact ligand is still entering the brain from the plasma compartment. This slow equilibrium may be caused by the high affinity of the radioligand ($K_i=0.7$ nM) and by the high density of CB1 receptors in the brain. We estimate that the density of CB1 receptors, if evenly distributed in the entire brain, would be more than seven times that of dopamine D_2 receptors in the striatum [37] and five times that of 5-HT $_{1A}$ receptors in the hippocampal region [38]. The high density of CB1 receptors may allow rapid rebinding of [^{18}F]MK-9470, creating a synaptic barrier that impedes exit of the radioligand from brain to plasma [11]. The concern about slow brain washout of [^{18}F]MK-9470 is more limited in humans than in rats. Sanabria-Bohorquez et al. showed that the peripheral

clearance of [^{18}F]MK-9470 in humans occurs more quickly than in rats with about 5 % of intact [^{18}F]MK-9470 left at 270 min after injection, resulting in more acceptable scan times, i.e. 60-min scans starting 90 to 120 min after injection [27].

While, in this study, our V_T values showed good identifiability (≤ 10 %), the reported between-subject variabilities were high (about 30 %). Similar between-subject variability values have been reported previously. Using [^{18}F]MK-9470 in humans, we reported variabilities of up to 55 % using irreversible density indexes [27]. V_T measurements of [^{11}C]MeP-PEP and [^{18}F]FMPEP- d_2 in humans have also yielded similar findings [11], i.e. regional intersubject variabilities of about 30 % and 26 %, respectively. Interestingly, the free fraction of [^{18}F]MK-9470 in rat plasma (f_p) was relatively small, i.e. <1 %. Since brain uptake is proportional to f_p , as protein-bound ligand cannot cross the blood–brain barrier, subtle changes in the protein binding between rats induce significant effects on the total brain uptake and the V_T values of [^{18}F]MK-9470. For example, if protein binding decreases from 99.9 % to 99.8 %, then f_p and brain uptake would increase 100 %, from 0.1 % to 0.2 %. As a consequence, the high intersubject variability of V_T in the present study might in part also reflect biological variability in f_p , and not only actual differences in receptor density.

Brain uptake during the chosen time periods was correlated with [^{18}F]MK-9470 V_T , showing acceptable test–retest variability (<10 %) when using data between 60 and 80 min after [^{18}F]MK-9470 injection. However, from the current study, we can conclude that compartment modelling using V_T as outcome measure is superior to brain uptake alone. Nevertheless, since SUV correlates with the V_T for the intact radioligand, SUV using data preferably measured between 60 and 80 min after [^{18}F]MK-9470 injection may provide specific information on CB1 receptor availability and may be useful for serial measurements over time if no intervention is needed. This suggestion is also partly in line with recently published work on the kinetic analysis of [^{18}F]MK-9470 in humans, showing that brain uptake is an index for receptor availability at the expense of some potential bias due to between-subject differences in tracer plasma kinetics [27].

In the design of the present study, we explicitly set the aim to investigate [^{18}F]MK-9470 kinetics in regions of high CB1 receptor density only. Although the resolution of the FOCUS-220 system is two to four times better than that of primate PET, the distance between brain structures is about ten times less and thus substructures in rodent brain cannot be measured as reliably as in the human brain. Due to this resolution limitation, we restricted our analysis to macrostructures such as the cerebellum, cortex and caudate-putamen, which all show a relatively high uptake of [^{18}F]MK-9470 [33]. Substructures in the rat brain may be measured more reliably using state-of-the-art small-animal

PET systems with improved sensitivity and spatial resolution properties [39].

In conclusion, we successfully characterized the kinetics of [^{18}F]MK-9470 in the rat brain. Using pharmacological displacement and pretreatment studies, we showed that [^{18}F]MK-9470 specifically and reversibly binds representing about 56 % of total brain radioactivity. A radiometabolite fraction crossed the blood–brain barrier, but displayed a low and homogeneous distribution throughout the brain. Quantification of the tracer binding to the CB1 receptor was possible using a model with the ligand and the radiometabolite input or the ligand input function alone.

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Conflicts of interest None.

References

- Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, et al. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev.* 2002;54:161–202.
- Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov.* 2004;3:771–84.
- Parolaro D, Realini N, Vigano D, Guidali C, Rubino T. The endocannabinoid system and psychiatric disorders. *Exp Neurol.* 2010;224:3–14.
- Scotter EL, Abood ME, Glass M. The endocannabinoid system as a target for the treatment of neurodegenerative disease. *Br J Pharmacol.* 2010;160:480–98.
- Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat Med.* 2008;14:923–30.
- Bahr BA, Karanian DA, Makanji SS, Makriyannis A. Targeting the endocannabinoid system in treating brain disorders. *Expert Opin Investig Drugs.* 2006;15:351–65.
- Charalambous A, Marciniak G, Shiue CY, Dewey SL, Schlyer DJ, Wolf AP, et al. PET studies in the primate brain and biodistribution in mice using (-)-5'-18F-delta 8-THC. *Pharmacol Biochem Behav.* 1991;40:503–7.
- Horti AG, Fan H, Kuwabara H, Hilton J, Ravert HT, Holt DP, et al. 11C-JHU75528: a radiotracer for PET imaging of CB1 cannabinoid receptors. *J Nucl Med.* 2006;47:1689–96.
- Terry G, Liow JS, Chernet E, Zoghbi SS, Phebus L, Felder CC, et al. Positron emission tomography imaging using an inverse agonist radioligand to assess cannabinoid CB1 receptors in rodents. *Neuroimage.* 2008;41:690–8.
- Yasuno F, Brown AK, Zoghbi SS, Krushinski JH, Chernet E, Tauscher J, et al. The PET radioligand [^{11}C]MePPEP binds reversibly and with high specific signal to cannabinoid CB1 receptors in nonhuman primate brain. *Neuropsychopharmacology.* 2008;33:259–69.
- Terry GE, Liow JS, Zoghbi SS, Hirvonen J, Farris AG, Lerner A, et al. Quantitation of cannabinoid CB1 receptors in healthy human brain using positron emission tomography and an inverse agonist radioligand. *Neuroimage.* 2009;48:362–70.
- Terry GE, Hirvonen J, Liow JS, Seneca N, Tauscher JT, Schaus JM, et al. Biodistribution and dosimetry in humans of two inverse agonists to image cannabinoid CB1 receptors using positron emission tomography. *Eur J Nucl Med Mol Imaging.* 2010;37:1499–506.
- Terry GE, Hirvonen J, Liow JS, Zoghbi SS, Gladding R, Tauscher JT, et al. Imaging and quantitation of cannabinoid CB1 receptors in human and monkey brains using (18F)-labeled inverse agonist radioligands. *J Nucl Med.* 2010;51:112–20.
- Burns HD, Van Laere K, Sanabria-Bohorquez S, Hamill TG, Bormans G, Eng WS, et al. [^{18}F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. *Proc Natl Acad Sci U S A.* 2007;104:9800–5.
- Wong DF, Kuwabara H, Horti AG, Rayment V, Brasic J, Guevara M, et al. Quantification of cerebral cannabinoid receptors subtype 1 (CB1) in healthy subjects and schizophrenia by the novel PET radioligand [^{11}C]OMAR. *Neuroimage.* 2010;52:1505–13.
- Addy C, Wright H, Van Laere K, Gantz I, Erond N, Musser BJ, et al. The acyclic CB1R inverse agonist taranabant mediates weight loss by increasing energy expenditure and decreasing caloric intake. *Cell Metab.* 2008;7:68–78.
- Van Laere K, Goffin K, Casteels C, Dupont P, Mortelmans L, de Hoon J, et al. Gender-dependent increases with healthy aging of the human cerebral cannabinoid-type 1 receptor binding using [^{18}F]MK-9470 PET. *Neuroimage.* 2008;39:1533–41.
- Van Laere K, Goffin K, Bormans G, Casteels C, Mortelmans L, de Hoon J, et al. Relationship of type 1 cannabinoid receptor availability in the human brain to novelty-seeking temperament. *Arch Gen Psychiatry.* 2009;66:196–204.
- Van Laere K, Casteels C, Dhollander I, Goffin K, Grachev I, Bormans G, et al. Widespread decrease of type 1 cannabinoid receptor availability in Huntington disease in vivo. *J Nucl Med.* 2010;51:1413–7.
- Gérard N, Pieters G, Goffin K, Bormans G, Van Laere K. Brain type 1 cannabinoid receptor availability in patients with anorexia and bulimia nervosa. *Biol Psychiatry.* 2011;70:777–84.
- Van Laere K, Casteels C, Lunsens S, Goffin K, Grachev ID, Bormans G, et al. Regional changes in type 1 cannabinoid receptor availability in Parkinson's disease in vivo. *Neurobiol Aging.* 2012;33:620.e1–8.
- Goffin K, Van Paesschen W, Van Laere K. In vivo activation of endocannabinoid system in temporal lobe epilepsy with hippocampal sclerosis. *Brain.* 2011;134:1033–40.
- Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab.* 2007;27:1533–9.
- Need AB, Davis RJ, Alexander-Chacko JT, Eastwood B, Chernet E, Phebus LA, et al. The relationship of in vivo central CB1 receptor occupancy to changes in cortical monoamine release and feeding elicited by CB1 receptor antagonists in rats. *Psychopharmacology (Berl).* 2006;184:26–35.
- Boellaard R, van Lingen A, Lammertsma AA. Experimental and clinical evaluation of iterative reconstruction (OSEM) in dynamic PET: quantitative characteristics and effects on kinetic modeling. *J Nucl Med.* 2001;42:808–17.
- Hoekzema E, Rojas S, Herance R, Pareto D, Abad S, Jimenez X, et al. [(11C)]-DASB microPET imaging in the aged rat: frontal and meso-thalamic increases in serotonin transporter binding. *Exp Gerontol.* 2011;46:1020–5.
- Sanabria-Bohorquez SM, Hamill TG, Goffin K, De Lepeleire I, Bormans G, Burns HD, et al. Kinetic analysis of the cannabinoid-1

- receptor PET tracer [(18F)MK-9470 in human brain. *Eur J Nucl Med Mol Imaging*. 2010;37:920–33.
28. Celen S, Koole M, De Angelis M, Sannen I, Chitneni SK, Alcazar J, et al. Preclinical evaluation of 18F-JNJ41510417 as a radioligand for PET imaging of phosphodiesterase-10A in the brain. *J Nucl Med*. 2010;51:1584–91.
29. Casteels C, Vermaelen P, Nuyts J, Van Der Linden A, Baekelandt V, Mortelmans L, et al. Construction and evaluation of multitracer small-animal PET probabilistic atlases for voxel-based functional mapping of the rat brain. *J Nucl Med*. 2006;47:1858–66.
30. Tai YC, Ruangma A, Rowland D, Siegel S, Newport DF, Chow PL, et al. Performance evaluation of the microPET focus: a third-generation microPET scanner dedicated to animal imaging. *J Nucl Med*. 2005;46:455–63.
31. Thie JA. Understanding the standardized uptake value, its methods, and implications for usage. *J Nucl Med*. 2004;45:1431–4.
32. Thie JA, Hubner KF, Isidoro FP, Smith GT. A weight index for the standardized uptake value in 2-deoxy-2-[F-18]fluoro-D-glucose-positron emission tomography. *Mol Imaging Biol*. 2007;9:91–8.
33. Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, et al. Cannabinoid receptor localization in brain. *Proc Natl Acad Sci U S A*. 1990;87:1932–6.
34. Fujita M, Seibyl JP, Verhoeff NP, Ichise M, Baldwin RM, Zoghbi SS, et al. Kinetic and equilibrium analyses of [(123)I]epidepride binding to striatal and extrastriatal dopamine D(2) receptors. *Synapse*. 1999;34:290–304.
35. Hirst RA, Almond SL, Lambert DG. Characterisation of the rat cerebella CB1 receptor using SR141716A, a central cannabinoid receptor antagonist. *Neurosci Lett*. 1996;220:101–4.
36. Zoghbi SS, Shetty HU, Ichise M, Fujita M, Imaizumi M, Liow JS, et al. PET imaging of the dopamine transporter with 18F-FECNT: a polar radiometabolite confounds brain radioligand measurements. *J Nucl Med*. 2006;47:520–7.
37. Dewar KM, Montreuil B, Grondin L, Reader TA. Dopamine D2 receptors labeled with [3H]raclopride in rat and rabbit brains. Equilibrium binding, kinetics, distribution and selectivity. *J Pharmacol Exp Ther*. 1989;250:696–706.
38. Khawaja X, Evans N, Reilly Y, Ennis C, Minchin MC. Characterisation of the binding of [3H]WAY-100635, a novel 5-hydroxytryptamine1A receptor antagonist, to rat brain. *J Neurochem*. 1995;64:2716–26.
39. Vandenbroucke A, Foudray AM, Olcott PD, Levin CS. Performance characterization of a new high resolution PET scintillation detector. *Phys Med Biol*. 2010;55:5895–911.