Quantification of (*R*)-[¹¹C]PK11195 binding in rheumatoid arthritis

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Abstract

Purpose Rheumatoid arthritis (RA) involves migration of macrophages into inflamed areas. (*R*)-[¹¹C]PK11195 binds to peripheral benzodiazepine receptors, expressed on macrophages, and may be used to quantify inflammation using positron emission tomography (PET). This study evaluated methods for the quantification of (*R*)-[¹¹C]PK11195 binding in the knee joints of RA patients.

Methods Data from six patients with RA were analysed. Dynamic PET scans were acquired in 3-D mode following (*R*)-[¹¹C]PK11195 injection. During scanning arterial radioactivity concentrations were measured to determine the plasma (*R*)-[¹¹C]PK11195 concentrations. Data were analysed using irreversible and reversible one-tissue and two-tissue compartment models and input functions with

various types of metabolite correction. Model preferences according to the Akaike information criterion (AIC) and correlations between measures were evaluated. Correlations between distribution volume (V_d) and standardized uptake values (SUV) were evaluated.

Results AIC indicated optimal performance for a one-tissue reversible compartment model including blood volume. High correlations were observed between V_d obtained using different input functions ($R^2=0.80$ – 1.00) and between V_d obtained with one- and two-tissue reversible compartment models ($R^2=0.75$ – 0.94). A high correlation was observed between optimal V_d and SUV after injection ($R^2=0.73$).

Conclusion (*R*)-[¹¹C]PK11195 kinetics in the knee were best described by a reversible single-tissue compartment model including blood volume. Applying metabolite corrections did not increase sensitivity. Due to the high correlation with V_d , SUV is a practical alternative for clinical use.

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Introduction

Rheumatoid arthritis (RA) is an inflammatory autoimmune disorder of the joints. It involves loss of bone and cartilage and inflammation of the synovium. Various studies have been performed to investigate the usefulness of PET using [¹⁸F]FDG for diagnosis of RA [1–4]. As RA is an inflammatory disease, tracers that bind to the peripheral benzodiazepine receptor (PBR), also called omega-3 receptor, which is expressed on macrophages, may be of even more interest.

(*R*)-PK11195 [(*R*)-1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide] is a ligand that binds to the PBR receptor and can therefore be used as a marker of inflammation. The latter has been demonstrated in both in vitro [5] and in vivo [6] studies. When labelled with ^{11}C , (*R*)-[^{11}C]PK11195 concentrations in the body can be quantified using positron emission tomography (PET). In the brain, (*R*)-[^{11}C]PK11195 binds to activated microglia [7] which are part of the brain's immune system. (*R*)-[^{11}C]PK11195 has been used extensively to image brain inflammation using PET [8–16]. Methods have been published regarding the quantification of (*R*)-[^{11}C]PK11195 binding in the brain [17–19]. A limited number of studies have been performed in other organs, such as the lung [6, 20–22] and the heart [23].

To use PET with the tracer (*R*)-[^{11}C]PK11195 as a research tool for RA inflammation, a methodology for quantification of (*R*)-[^{11}C]PK11195 binding in inflamed knee joints in RA using PET has to be available. The purpose of this study was to develop such a methodology.

Materials and methods

Patients

Nine patients (three male, age 54 ± 8 years) were included in the study. Patients were diagnosed with RA according to the criteria of the American College of Rheumatology [24, 25]. For each patient, inflammation was present in at least one knee. The study was approved by the medical ethics committee of the VU University Medical Centre. All patients gave informed consent prior to inclusion.

Scanning protocol

Scans were performed in 3-D acquisition mode using an ECAT EXACT HR+ (CTI/Siemens, Knoxville, TN) PET scanner [26, 27]. All patients underwent a 1-hr dynamic (*R*)-[^{11}C]PK11195 scan of the knee region with progressively increasing frame durations (one 15 s, one 5 s, one 10 s, two 15 s, two 30 s, three 60 s, four 150 s, five 300 s and two 600 s). A bolus of 490 ± 120 MBq (*R*)-[^{11}C]PK11195 with specific activity higher than $18.5 \text{ GBq}/\mu\text{mol}$ at the end of synthesis was injected at the start of the scan. During the scan, arterial radioactivity concentration was monitored continuously using a blood sampler device described previously [28]. In addition, at set times (3, 5, 10, 20, 30, 40 and 60 min after injection), continuous withdrawal was interrupted briefly to collect discrete arterial samples. These samples were used to determine plasma/whole-blood radioactivity concentration ratios and radioactivity concentrations of (*R*)-[^{11}C]PK11195 and

metabolites. Briefly, plasma was processed using solid-phase extraction to separate (*R*)-[^{11}C]PK11195 and its nonpolar metabolites from polar metabolites. (*R*)-[^{11}C]PK11195 and its nonpolar metabolites were then processed using high-performance liquid chromatography to determine radioactivity concentrations [29].

Image reconstructions

All emission scans were normalized and corrected for randoms, dead time, scatter [30], attenuation and decay. All frames of the dynamic emission scans were reconstructed using filtered back projection and a Hanning filter with a cut-off of 0.5 times the Nyquist frequency. In addition, for region of interest (ROI) definition, a summed sinogram from 10 to 60 min after the start of the scan was reconstructed using an ordered subset expectation-maximization (OSEM) algorithm with two iterations and 16 subsets. For both reconstructions, the full-width at half-maximum of the point spread function was approximately 7 mm and the reconstructed voxel size was $1.2 \times 1.2 \times 2.4$ mm.

Kinetic analysis

Areas with visually increased uptake in the synovium were delineated using 50% isocontours on the OSEM reconstructed scans. For five patients four to seven areas and for one patient two areas with increased uptake were identified. The ROI volumes were between 1.1 and 2.5 cm^3 . Time-activity curves (TAC) were created for each ROI. A tracer kinetic model for (*R*)-[^{11}C]PK11195 has been validated for the brain, where the blood–brain barrier prevents passage of labelled metabolites into the brain. There is, however, no blood–tissue barrier in the knee-cap and it is unknown whether metabolites reach the target region. Therefore, three different input functions were generated: (1) the (time-dependent) parent (*R*)-[^{11}C]PK11195 concentration in plasma (C_p^{pk}), (2) the plasma concentration of parent (*R*)-[^{11}C]PK11195 and its nonpolar metabolites together ($C_p^{\text{pk+np}}$), and (3) the total ^{11}C plasma concentration, i.e. (*R*)-[^{11}C]PK11195 with all its metabolites (C_p^{total}). These input functions were generated in the following way. The plasma/whole-blood radioactivity concentration ratio $r(t)$ of the discrete blood samples was fitted to an exponential function. The fraction of (*R*)-[^{11}C]PK11195 in plasma of the discrete blood samples was fitted to the function $f(t) = a_1 * \exp(b_1 * t) + (1 - a_1) * \exp(b_2 * t)$, where $f(t)$ is the fraction of radioactivity in plasma originating from (*R*)-[^{11}C]PK11195 at time t . C_p^{pk} was then estimated by multiplying the concentration in whole blood by both $r(t)$ and $f(t)$. $C_p^{\text{pk+np}}$ was obtained in a similar way, except that the sum of parent compound and nonpolar metabolites was

fitted to the function $f(t)$. For C_p^{total} only a multiplication of the whole-blood concentration by $r(t)$ was required. Delay between arrival of the (R) - $[^{11}\text{C}]$ PK11195 bolus at the external detector and at the place of injection was estimated by fitting a reversible two-tissue compartment model (including a delay parameter) to the sum of all ROIs in the knee using the appropriate input concentration. This (fitted) delay value was then fixed for individual ROIs.

Four compartment models were used to fit the ROI data. These were an irreversible one-tissue model (M1T1K, i.e. one-tissue compartment with one kinetic rate constant), a reversible one-tissue model (M1T2K, one-tissue compartment with two kinetic rate constants), an irreversible two-tissue model (M2T3K, two-tissue compartments with three kinetic rate constants), and a reversible two-tissue model (M2T4K, two-tissue compartments with four kinetic rate constants) [31]. Variants of these models with and without a blood volume (V_b) component were evaluated. If the model contained a correction for blood volume, VB was added to the model name, i.e. M1T2KVB instead of M1T2K. For the blood volume term, the (total) radioactivity concentration in blood instead of in plasma was used. Parameters of interest evaluated were the influx rate constant K_i for M1T1K and M2T3K, the volume of distribution (V_d) for M1T2K and M2T4K, and additionally binding potential (BP) for M2T4K. For M1T1K, K_i is equal to K_1 , while it is equal to $K_1 k_3 / (k_2 + k_3)$ for M2T3K. V_d is defined as K_1 / k_2 for M1T2K and as $K_1 / k_2 (1 + \text{BP})$ for M2T4K, with BP equal to k_3 / k_4 .

For potential use in future clinical applications, where dynamic scanning with arterial blood sampling might be problematic, the performance of the standardized uptake value (SUV) was also evaluated. SUV is the average radioactivity concentration in an ROI, divided by the total injected dose and multiplied by the weight of the subject. It is a semiquantitative measure and is commonly used in oncology for the analysis of FDG scans [32]. SUV was calculated using the average concentrations between 20 and 40 min, 40 and 60 min and 20 and 60 min after injection, respectively.

Due to noise in the data, unrealistic results, such as very high BP or zero K_i , were observed. These results were excluded from comparison. For K_i M1T1K and K_i M1T1KVB, all K_i values were in the same range and none had to be rejected. For V_d M1T2K, V_d M1T2KVB, V_d M2T4K and V_d M2T4KVB, most values of V_d were in the range of 0.5–3 and values were rejected when V_d was greater than 3. When K_i M2T3K and K_i M2T3KVB were used, several K_i values were rejected because they became zero, the lower boundary of the fitting algorithm. BP values were rejected when they were greater than 30. To allow a fair comparison between different input types, results were rejected for all input types when they were rejected for any input type.

The optimal model was selected based on a high preference of the Akaike information criterion (AIC) [33], a low number of unrealistic parameter values and ease of data acquisition. AIC selects the model with the optimal fit to the data, taking into account the number of fitted data points and the number of fitted parameters. When comparing eight models at the same time using AIC, it can be hard to identify the optimal model. Therefore models with and without blood volume were first compared separately. The optimal model was then determined by comparing the optimal models of these comparisons without the lower performing models taken into account. The optimal timing for the calculation of the SUV was determined by a high correlation with the optimal compartment model and preferably short scan duration.

Results

Examples of whole-blood and tissue TAC are shown in Fig. 1. The ratio of plasma over whole-blood concentration was nearly constant over all samples and patients with a value of 1.55 ± 0.07 . It was fitted using a sum of decaying exponents where the number of exponents was based on the AIC. Plasma/whole-blood concentration ratio fits showed little deviation from the measured data. An example is

Fig. 1 Example of (R) - $[^{11}\text{C}]$ PK11195 concentration as function of time for (a) whole blood and (b) inflamed synovium. The curve in b is a fit of a one-tissue reversible model including blood volume using the parent fraction as input function

shown in Fig. 2a. The fraction of radioactivity in plasma originating from (R)-[^{11}C]PK11195, measured over all patients, changed from $90\pm 3\%$ at 3 min to $54\pm 7\%$ at 60 min after injection. The summed fraction of radioactivity originating from both (R)-[^{11}C]PK11195 and nonpolar metabolites was $94\pm 3\%$ at 3 min and $91\pm 3\%$ at 60 min after injection. Three nonpolar metabolites were observed with concentration fractions of 8, 10 and 18% at 60 min after injection. The total polar metabolite fraction was $9\pm 3\%$ at 60 min after injection. Both the fits to the fraction of radioactivity originating from (R)-[^{11}C]PK11195 in plasma and the fits to the fraction of radioactivity originating from (R)-[^{11}C]PK11195 and nonpolar metabolites were fitted using the function $f = 1 - a(2 - \exp(-bt) - \exp(-ct))$, where f is parent fraction and a , b , and c are positive fitted parameters. The fits showed little deviation from the measured data (Fig. 2b and c). All (dynamic) scans were checked for movement artefacts, and for this reason two patients were excluded from further analysis. In one patient, SUV could not be obtained due to a methodological error and only kinetic modelling was performed. Thus, the results from this study are based on the kinetic modelling results from six subjects and the SUV results from five patients.

The number of parameter values that were rejected because they fell outside the physiological range, as defined

in the **Materials and methods** section, varied. The total number of fitted TAC was 33. The highest number of rejected values were observed for K_i obtained with M2T3KVB (six for C_p^{pk} , five for $C_p^{\text{pk+np}}$ and five for C_p^{total} , respectively) and for BP obtained with M2T4K when C_p^{pk} was used as the input function (five). For all other combinations of models and input functions, the numbers of rejected values were two or fewer, i.e. 6% or less of the total number of fits.

The results of various model comparisons using AIC are shown in Tables 1, 2 and 3. AIC trends were consistent over different input functions. For the models without blood volume correction, M2T4K was preferred in most cases, followed by M1T2K. For the models with blood volume correction, the order of the one- and two-tissue reversible models was reversed. As in both cases a reversible model was optimal, no further results from irreversible compartment models are presented. In general, irreversible models resulted in higher numbers of rejected values than reversible models (data not shown). When M1T2KVB was compared with M2T4K (Table 3), M1T2KVB was preferred in most cases for all input functions.

A scatter plot of V_d M1T2KVB calculated using C_p^{pk} and C_p^{total} is shown in Fig. 3. Good correlations were observed between V_d M1T2KVB calculated with different

Fig. 2 Example of a plasma/whole-blood radioactivity concentration ratio fit (a), a plasma parent fraction fit (b), and a plasma parent and nonpolar metabolite fraction fit (c)

Table 1 Number of times a model resulted in the best/second/third/worst fit for the models M1T1K, M1T2K, M2T3K and M2T4K according to AIC. The preference of the models was analysed separately for each input function used

	M1T1K	M1T2K	M2T3K	M2T4K
C_p^{pk}	4/2/1/26	7/14/12/0	0/16/16/1	22/1/4/6
C_p^{pk+np}	0/2/3/28	10/9/14/0	0/18/12/3	23/4/4/2
C_p^{total}	0/2/3/28	10/9/14/0	0/18/12/3	23/4/4/2

input functions ($R^2=0.88$ between C_p^{pk} and C_p^{total} , $R^2=0.89$ between C_p^{pk} and C_p^{pk+np} , and $R^2=1.00$ between C_p^{pk+np} and C_p^{total}).

Scatter plots between V_d M1T2KVB and V_d M2T4KVB and between V_d M1T2K and V_d M1T2KVB using either C_p^{pk} or C_p^{total} as input function are shown in Fig. 4. For most ROIs one- and two-tissue models provided similar results. This is reflected in the gradients of the fitted correlation lines, which were between 0.9 and 1.1 for all input functions. Correlations between M1T2KVB and M1T2K were high for all input functions ($R^2=0.91$ – 0.96).

Average kinetic parameters for M1T2KVB using C_p^{total} were $K_1=0.03\pm0.01$ ml min⁻¹ ml⁻¹, $k_2=0.03\pm0.01$ min⁻¹ and $V_b=0.04\pm0.04$. A high correlation was observed between V_d M1T2KVB using C_p^{total} as input function and SUV^{20-40} (Fig. 5, $R^2=0.73$). Correlations between SUV^{20-40} , SUV^{20-60} and SUV^{40-60} were very high ($R^2=0.99$ – 1.00).

Discussion

In the present study, three different input functions were used: parent (R)-[¹¹C]PK11195 concentration in plasma (C_p^{pk}), sum of parent (R)-[¹¹C]PK11195 and its nonpolar metabolites concentrations in plasma (C_p^{pk+np}) and total radioactivity concentration in plasma (C_p^{total}). It is likely that all three fractions (i.e. parent, polar and nonpolar metabolite fractions) enter tissue, but with different kinetics. It is not possible to select an optimal model based on theoretical grounds. From a practical point of view, a model should provide good fits to the data and reproducible

Table 2 Number of times a model resulted in the best/second/third/worst fit for the models M1T1KVB, M1T2KVB, M2T3KVB and M2T4KVB according to AIC

	M1T1KVB	M1T2KVB	M2T3KVB	M2T4KVB
C_p^{pk}	3/4/1/25	20/6/7/0	4/16/11/2	6/7/14/6
C_p^{pk+np}	0/6/1/26	20/4/9/0	5/15/10/3	8/8/13/4
C_p^{total}	0/6/1/26	20/5/8/0	4/16/9/4	9/6/15/3

The preference of the models was analysed separately for each input function used.

Table 3 Number of times a model resulted in the best or worst fit for the models M1T2KVB and M2T4K according to AIC

	M1T2KVB	M2T4K
C_p^{pk}	19/14	14/19
C_p^{pk+np}	20/13	13/20
C_p^{total}	21/12	12/21

The preference of the models was analysed separately for each input function used.

results. Since the summed fractions of (R)-[¹¹C]PK11195 and its nonpolar metabolites decreased from $94\pm3\%$ at 3 min to $91\pm3\%$ at 60 min, compared to a constant 100% fraction for C_p^{total} , the shape of C_p^{pk+np} differed little from that of C_p^{total} . This is reflected in the high correlation ($R^2=1.00$) between V_d M1T2KVB using C_p^{pk+np} and C_p^{total} . Correlations between C_p^{pk} and C_p^{total} were lower but still high ($R^2=0.88$, Fig. 3). Due to the high correlations between C_p^{pk} and C_p^{total} , clinical performance (i.e. sensitivity to change) would be similar. Compared with C_p^{pk+np} and C_p^{pk} , C_p^{total} has the advantage that no metabolite fractions and plasma concentrations need to be measured. Therefore C_p^{total} was taken as the input function of choice.

Analysis using AIC indicated that V_d M1T2KVB was the preferred parameter (Tables 1, 2, and 3). Preference for the one-tissue reversible compartment models over the two-tissue reversible compartment models is also indicated by the high correlations between these models ($R^2=0.82$ – 0.92 , Fig. 4a, b), suggesting that little additional information was contained within the second compartment. High correlations were also observed between results with and without inclusion of V_b ($R^2=0.91$ – 0.96 , Fig. 4c, d). Because AIC indicated that the model including V_b was optimal, M1T2KVB was selected as the optimal model.

Correlation coefficients between different SUV measures were very high ($R^2=0.99$ – 1.00) and therefore no difference

Fig. 3 Scatter plot of V_d M1T2KVB values obtained using C_p^{pk} and C_p^{total} as input functions. Different symbols represent different subjects

Fig. 4 Scatter plots between V_d M1T2KVB and V_d M2T4KVB (a, b) and between V_d M1T2K and V_d M1T2KVB (c, d) using either C_p^{pk} (a, c) or C_p^{total} (b, d) as input function. Different symbols represent different subjects

in performance existed between these measures. SUV^{20-60} had lower noise levels than SUV^{20-40} and SUV^{40-60} but apparently this did not lead to increased performance, probably because the noise level in a 20-min scan is already low (e.g. very good correlation between SUV^{20-40} and SUV^{40-60}). SUV^{20-60} has the disadvantage of increased risk of patient movement, increased patient discomfort and longer occupation of the scanner. SUV^{20-40} performed similarly to SUV^{40-60} but is slightly more patient friendly. The high correlation between V_d M1T2KVB and SUV^{20-40}

Fig. 5 Scatter plot between M1T2KVB using C_p^{total} as input function and SUV^{20-40} . Different symbols represent different subjects

($R^2=0.73$, Fig. 5) indicates that SUV^{20-40} is a practical alternative when arterial sampling cannot be performed.

Application of the results to clinical trials is limited by the unknown test-retest variance. Knowledge of this variance is relevant in estimating required sample sizes in clinical studies and needs to be assessed in future studies. Three methodological factors may limit the use of (*R*)-[^{11}C] PK11195 as an inflammation marker in the knee. First, results may be influenced by physiological (normal) uptake of (*R*)-[^{11}C]PK11195. In most of the patients, however, there was a large difference in the clinical condition of the two knees, and no uptake was observed in the knee with no or limited inflammation. Furthermore, no uptake in healthy controls was observed (data not shown), indicating that high uptake is likely to be related to inflammation. Second, uptake of a metabolite that does not bind to the PBR may also influence quantification. Although this cannot be ruled out, nonpolar metabolites are chemically similar to (*R*)-[^{11}C]PK11195 and will likely also bind to the receptor. In addition, the majority of the radioactivity concentration originates from the parent compound, which certainly binds to the receptor. The high correlation between the results obtained with the various input functions indicates, however, that the effect of labelled metabolites is small, at least in the present patient population. Third, treatment such as disease-modifying antirheumatic drugs may influence (*R*)-

[^{11}C]PK11195 metabolism, and introduce bias when results are compared to those from a normal control group. Future studies are needed to investigate the effect of various treatments on (*R*)-[^{11}C]PK11195 metabolism and thus on SUV results.

The kinetics of (*R*)-[^{11}C]PK11195 and its metabolites in the human knee joint affected by RA can best be described by a reversible one-tissue compartment model including a blood volume component. There was a good correlation between distribution volume values independent of whether plasma (*R*)-[^{11}C]PK11195 concentration, plasma (*R*)-[^{11}C]PK11195 and nonpolar metabolite concentration or total plasma radioactivity concentration was used as the input function. Based on practical considerations (no metabolite measurements), use of total plasma radioactivity concentration is preferred. Because of the good correlation between V_d and SUV calculated using the average radioactivity concentration between 20 and 40 min after injection, the latter is a practical alternative for routine clinical studies.

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Conflicts of interest None.

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