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A combined accelerator mass spectrometry-positron emission tomography human microdose study with ^{14}C - and ^{11}C -labelled verapamil

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Abstract

Background and Objective—In microdose studies, the pharmacokinetic (PK) profile of a drug in blood after administration of a dose up to 100 μg is measured with sensitive analytical techniques, such as accelerator mass spectrometry (AMS). As most drugs exert their effect in tissue rather than blood, methodology is needed for extending PK analysis to different tissue compartments. In the present study, we combined, for the first time, AMS analysis with positron emission tomography (PET) in order to determine the PK profile of the model drug verapamil in plasma and brain of humans. In order to assess PK dose-linearity of verapamil, data were acquired and compared after administration of an intravenous (iv) microdose and an iv microdose dosed concomitantly with an oral therapeutic dose.

Methods—Six healthy male volunteers received an iv microdose (0.05 mg) (period 1) and an iv microdose dosed concomitantly with an oral therapeutic dose (80 mg) of verapamil (period 2) in a randomized, cross-over, two-period study design. The iv dose was a mixture of (*R/S*)-[^{14}C]verapamil and (*R*)-[^{11}C]verapamil and the oral dose was unlabelled racemic verapamil. Brain distribution of radioactivity was measured with PET whereas plasma PK of (*R*)- and (*S*)-verapamil was determined with AMS. PET data were analyzed by kinetic modeling to estimate the rate constants for transfer of radioactivity across the blood-brain barrier.

Results—Most PK parameters of (*R*)- and (*S*)-verapamil as well as parameters describing exchange of radioactivity between plasma and brain ($K_1=0.030\pm0.003$ and 0.031 ± 0.005 $\text{mL}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$ and $k_2=0.099\pm0.006$ and 0.095 ± 0.008 min^{-1} for period 1 and 2, respectively) were not statistically different between the two periods although there was a trend for non-linear kinetics for the (*R*)-enantiomer. On the other hand, all PK parameters (except for $t_{1/2}$) differed significantly between the (*R*)- and (*S*)-enantiomers for both periods. C_{max} , $AUC_{(0-24)}$ and $AUC_{(0-\text{inf})}$ were higher and CL , V and V_{SS} were lower for the (*R*)- than for the (*S*)-enantiomer.

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Claudia C Wagner and Marie Simpson contributed equally to this study.

Conclusion—Combining AMS and PET microdosing allows long term PK data along with information on drug tissue distribution to be acquired in the same subjects thus making it a promising approach to maximize data output from a single clinical study.

Background

In microdose studies, the pharmacokinetics (PK) of a drug are assessed after administration of a dose up to 100 µg in humans.^[1, 2] Safety and toxicology testing is reduced for conducting microdose studies, compared to conventional first in human (Phase-1) studies, so that PK properties of drug candidates or drug-drug interactions can be studied at lower cost and earlier along the drug development pathway.^[3]

The issue of dose-linearity is often discussed as a limitation of the microdosing concept, as there is concern that PK data determined after the administration of a microdose might fail to predict PK data of the drug observed at therapeutic doses.^[4] However, previous human microdose studies have shown that for many drugs, microdosing can predict human PK at clinically relevant doses.^[5-8] In a comprehensive microdosing review reporting on a total of 18 drugs with different PK properties,^[9] 15 drugs demonstrated linear or near-to linear PK at a microdose and a therapeutic dose in either animals or humans.

The feasibility of performing clinical microdose studies critically depends on the availability of sensitive analytical methods that are capable of detecting minute drug amounts in plasma and tissue samples.^[10] A commonly employed method is accelerator mass spectrometry (AMS), an ultrasensitive isotope ratio technique, which relies on the use of carbon-14 (¹⁴C)-labelled drugs. In a typical AMS microdosing study, ¹⁴C-labelled drug is administered to human subjects and the drug's plasma concentration is determined by separation of unchanged drug by liquid chromatography followed by AMS analysis, which has a potential sensitivity in the low femtomolar range.^[11, 12]

A limitation of AMS microdosing is that it mainly provides information on drug distribution to the central blood compartment. This is of limited value as most drugs exert their pharmacologic effect in tissue and drug PK in blood may be a poor predictor of drug tissue distribution.^[13] The non-invasive nuclear imaging technique positron emission tomography (PET) has been used to study tissue distribution and PK of drugs labelled with positron-emitting radionuclides, such as carbon-11 (¹¹C) or fluorine-18 (¹⁸F).^[14, 15] For PET imaging, drugs labelled at high specific activity are commonly used, so that the mass of unlabelled drug associated with a PET tracer is usually low enough to satisfy the definition of a microdose. On the other hand, it is difficult to derive standard plasma PK parameters of a drug in PET microdosing studies, due to the short radioactive half-lives of positron-emitting radionuclides (¹¹C: 20.4 minutes, ¹⁸F: 109.8 minutes) resulting in short sampling periods.

In the present pilot study we combined AMS and PET analysis, for the first time, in order to obtain plasma and brain PK in the same subjects, after administration of a mixture of (*R/S*)-[¹⁴C]verapamil and (*R*)-[¹¹C]verapamil in a human microdose study. The calcium channel inhibitor verapamil was used as a model drug, because it is a commonly prescribed drug with a well established safety profile, which can be straightforwardly labelled with ¹¹C and ¹⁴C. In order to assess PK dose-linearity of verapamil in plasma and brain, data were acquired and compared after administration of an intravenous (iv) microdose (period 1) and an iv microdose dosed concomitantly with an oral therapeutic dose (period 2). The iv dose was a mixture of (*R/S*)-[¹⁴C]verapamil and (*R*)-[¹¹C]verapamil and the oral dose was unlabelled racemic verapamil.

Methods

The study was conducted as an open-label, randomized, two-period, cross-over single-iv infusion microdose and single-oral therapeutic dose pilot clinical study. The study was conducted in seven healthy male subjects with a mean age (mean±standard deviation, SD) of 27±4 years and a mean body weight of 81±8 kg. The study was conducted as a pilot study, so no *a priori* sample size calculation was done. Intake of any medication with known interference with cytochrome P450 enzymes or P-glycoprotein (P-gp) within two weeks before the start of the study lead to study exclusion.

The clinical trial was performed as a collaborative study at the Department of Clinical Pharmacology at the Medical University of Vienna and at Xceleron Ltd, York, UK. The clinical phase of the study, the collection of blood samples as well as interpretation of all PET data was performed at the Medical University of Vienna, whereas AMS analysis was conducted at Xceleron Ltd.

The study protocol (including issues related to radiation exposure of study subjects) was approved by the Ethics-Committee of the Medical University of Vienna and the Vienna General Hospital - AKH and was performed in accordance with the Declaration of Helsinki (1964) in the revised version of 2000 (Edinburgh), the Guidelines of the International Conference of Harmonization, the Good Clinical Practice Guidelines and the Austrian drug law (Arzneimittelgesetz). All subjects were given a detailed description of the study and their written consent was obtained prior to the enrolment in the study.

PET imaging and experimental procedures

Each study subject underwent two PET scans of 60 minutes duration on two separate study days, separated by a wash-out period of 14-18 days. On one study day, subjects received a microdose (0.05 mg), containing tracer amounts of (*R/S*)-[¹⁴C]verapamil and (*R*)-[¹¹C]verapamil at the beginning of the PET scan (period 1). On the other study day (period 2), subjects were first administered one oral therapeutic dose (80 mg) of racemic unlabelled verapamil (Isoptin®, 80 mg, Abbott, Vienna). Two hours later (i.e. at the estimated time of maximum concentration (t_{max}) of verapamil in plasma after oral administration^[16]) the subjects received a microdose (0.05 mg) of (*R/S*)-[¹⁴C]verapamil and (*R*)-[¹¹C]verapamil, at the beginning of the PET scan. Subjects underwent period 1 and 2 in a randomized order.

Prior to PET imaging, one venous catheter was placed in a cubital vein of one arm for radiotracer infusion. An arterial catheter was placed in the radial artery of the same arm for arterial blood sampling. A second venous catheter was placed on the other arm for venous blood sampling from 0-24 hours after radiotracer injection. For PET imaging, each subject was positioned supine on the imaging bed of the PET camera with the head in a fixing device in order to avoid movement artifacts. PET images were acquired with an Advance PET scanner (General Electrics Medical Systems, Wukesha, WI, USA) run in 3D mode with a transversal field of view (FOV) of 55 cm and an axial FOV of 15 cm and an axial slice thickness of 4.25 mm. In order to correct for tissue attenuation of photons a transmission scan of 5-minutes duration using two 400 MBq ⁶⁸Ge pin sources was recorded prior to radiotracer injection.

At the start of each PET scan, study medication was iv injected over 20 seconds. Dynamic PET imaging and arterial blood sampling were started at the time of injection. The following frame sequence was used for PET imaging: 1×15 seconds, 3×5 seconds, 3×10 seconds, 2×30 seconds, 3×60 seconds, 2×150 seconds, 2×300 seconds, 4×600 seconds. During both scans arterial blood samples were manually drawn at intervals of 7 seconds during the first 3

minutes after radiotracer injection and subsequently at 3.5, 5, 10, 20, 30, 40 and 60 minutes after radiotracer injection.

Study medication

(*R*)-[^{11}C]verapamil ((*R*)-2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]-([^{11}C]methyl)amino]-2-isopropylpentanenitrile) was synthesized at the Medical University of Vienna from (*R*)-norverapamil (ABX advanced biochemical compounds, Radeberg, Germany) and [^{11}C]methyl triflate with a radiochemical purity >98% and a specific activity at the end of synthesis >20 GBq· μmol^{-1} .^[17] Racemic [^{14}C]verapamil hydrochloride ((*R*/*S*)-2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]-([^{14}C]methyl)amino]-2-isopropylpentanenitrile) was custom-synthesized by BioDynamics Research (Rushden, UK) with a radiochemical and chemical purity >98% and a specific activity >0.9 MBq· μmol^{-1} . For formulation, 0.05 mg of racemic verapamil (Isoptin®, 5-mg vials, Abbott, Vienna, 20 μL) was mixed with (*R*/*S*)-[^{14}C]verapamil dissolved in ethanol (200 μL) and (*R*)-[^{11}C]verapamil dissolved in physiological saline solution/ethanol (9/1, v/v, 3-6 mL) and the mixture filtered through a sterile Millex-GV filter (0.22 μm) (Millipore, Bedford, Mass, USA) into a sterile vial. Prior to iv injection, one aliquot (0.4 mL) was removed from each dose preparation for determining the concentrations of (*R*)- and (*S*)-verapamil by chiral high-performance liquid chromatography (HPLC) as described previously^[18] and for determining the administered ^{14}C -amount by liquid scintillation counting. For iv injection, the final volume of each dose preparation was adjusted to 10 mL with physiological saline solution. The injected radioactivity of (*R*/*S*)-[^{14}C]verapamil and (*R*)-[^{11}C]verapamil was 4.1 ± 0.5 kBq and 407 ± 48 MBq, respectively. The dose preparations contained 37.6 ± 9.1 μg and 26.1 ± 0.1 μg of (*R*)- and (*S*)-verapamil, respectively.

PET data analysis

Reconstruction of the PET data was performed by means of iterative reconstruction using the ordered subsets-expectation maximization method with 28 subsets and 2 iterations. The loop filter (Gaussian) was set to a full-width at half-maximum (FWHM) of 4.3 mm, and a post-filtering algorithm of 6.00 mm FWHM was applied. Attenuation correction was performed using the manufacturer's segmentation algorithm for transmission data. T1-weighted magnetic resonance imaging (MRI) scans had been recorded within one month before the PET scan. MRI and PET data were processed with PMOD 2.6 (PMOD Technologies, Zurich, Switzerland), Analyze 8.0 (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN, USA)^[19] and SPM5 (SPM5, Wellcome Department of Imaging Neuroscience, UCL, London, UK) software^[20] as published in detail before.^[21] A whole brain grey matter region of interest was defined by using the Hammersmith n30r83 three-dimensional maximum probability atlas of the human brain.^[22] Radioactivity concentrations (kiloBecquerels per gram tissue, kBq·g $^{-1}$) were converted into mass concentration values (pg equivalents·mL $^{-1}$) via the specific activity of the radiotracer (i.e. administered ^{11}C -radioactivity amount divided by the mass of (*R*)-verapamil contained in the dose preparation). The concentration data were combined to provide concentration-time curves for the whole observation period.

Blood and metabolite analysis

Radioactivity counts in aliquots of arterial blood and plasma were measured with a Packard Cobra II auto-gamma counter (Packard Instrument Company, Meriden, USA), which had been cross-calibrated with the PET camera. Plasma samples were analyzed for radiometabolites of (*R*)-[^{11}C]verapamil by employing a previously described combined solid-phase extraction (SPE)/HPLC assay.^[23] The polar radiometabolites of (*R*)-[^{11}C]verapamil ([^{11}C]formaldehyde and related species) were determined by SPE, whereas the lipophilic radiometabolites of (*R*)-[^{11}C]verapamil (i.e. the ^{11}C -labelled *N*-dealkylation

products D-617 and D-717) were measured with HPLC. The 10, 20, 30, 40 and 60 minutes plasma samples were analyzed for radiometabolites of (*R*)-[¹¹C]verapamil using the SPE/HPLC assay. Because of time constraints due to the short radioactive half-life of ¹¹C, the 3.5 and 5-minutes samples were only analyzed by the SPE assay. An arterial input function of (*R*)-[¹¹C]verapamil was constructed by correcting the total decay-corrected radioactivity concentrations in arterial plasma (kBq·mL⁻¹) for the fraction of polar radiometabolites of (*R*)-[¹¹C]verapamil, as determined by SPE, and by subsequent interpolation of the radioactivity data.

Kinetic modeling of (*R*)-[¹¹C]verapamil

Standard 1-tissue-2-rate constant (1T2K) or 2-tissue-4-rate constant (2T4K) compartment models were fitted to the concentration-time curves of radioactivity in whole brain grey matter to estimate the rate constants of radioactivity exchange between plasma and brain tissue (K_1 , k_2 , k_3 and k_4) as well as the distribution volume (DV) as described in detail elsewhere.^[21, 24] The first-order rate constants K_1 (mL·mL⁻¹·min⁻¹) and k_2 (min⁻¹) describe transport of radioactivity from plasma to the first tissue compartment and back, whereas k_3 (min⁻¹) and k_4 (min⁻¹) characterize exchange of radioactivity between the first and the second tissue compartment. DV is not identical to the volume of distribution (V) in standard PK analysis. For the 2T4K compartment model, DV is given as $(1+k_3/k_4) \cdot (K_1/k_2)$ and can be considered as an estimate of the brain tissue-plasma partition coefficient of radioactivity at equilibrium. Fits were performed by the method of weighted nonlinear least squares as implemented in the *Optimization Toolbox* of MATLAB (Mathworks, Natick, MA, USA). Goodness-of-fit was assessed by visual inspection of observed and predicted concentrations *versus* time, by the correlation between observed and predicted concentrations, by the randomness of the residuals (runs test), and by estimating parameter uncertainties (variances) from the inverse of the appropriate Fisher information matrix. In order to obtain a model-independent estimate of DV , Logan graphical analysis was applied to the PET and arterial plasma data using MATLAB.^[25] The slope DV of the linear part of the Logan plot was estimated by linear regression of the Logan variables. The linear regression was assessed by the magnitude of the squared linear correlation coefficient (r^2).

Venous blood sampling and AMS analysis

Venous blood samples (4 mL) were drawn at pre-dose as well as at time points of 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after radiotracer injection. AMS analysis of (*R*)- and (*S*)-[¹⁴C]verapamil was performed as described previously.^[18] In brief, to each plasma sample an excess and known amount of unlabelled verapamil was added and the samples extracted into solvent. Aliquots of the extracts were injected onto a C18 HPLC system and verapamil collected as a discrete fraction. This verapamil fraction was injected onto a chiral HPLC system and the fractions corresponding to (*R*)- and (*S*)-verapamil were collected. The ultraviolet (UV) peak areas corresponding to (*R*)- and (*S*)-verapamil were recorded. Fractions containing verapamil enantiomers were converted to graphite and analyzed for ¹⁴C-content by AMS. The mass concentration of verapamil enantiomers in plasma were determined from the ¹⁴C-content measured by AMS relative to the UV peak area (used as an internal standard). The accuracy of this assay, as previously reported^[18] was assessed using quality control samples and the results were within 3% of the true value (13 and 2% coefficient of variation for (*R*)- and (*S*)-verapamil respectively). The limit of quantification of the assay was 1.95-4.81 pg/mL for (*R*)-verapamil and 1.76-3.34 pg/mL for (*S*)-verapamil. Where measured, the mean concentration data was within 2% of the measured concentration (14% coefficient of variation) over a period of at least 48 hours.

Pharmacokinetic analysis

PK analysis of verapamil in plasma was performed using WinNonLin software version 4.1 (Pharsight, Mountain View, CA, USA) using a noncompartmental model. Input consisted of plasma drug concentrations (in $\text{pg}\cdot\text{mL}^{-1}$), recorded times of sample collection (in hours), and doses administered. The following PK parameters were calculated: maximum concentration (C_{max}), terminal half-life ($t_{1/2}$), area under the curve (AUC) for the period studied, and AUC extrapolated to infinity ($AUC_{0-\text{inf}}$), clearance (CL), volume of distribution (V) and volume of distribution under steady-state conditions (V_{ss}).

Statistical analysis

All statistical analysis was performed using the STATISTICA software package (release 6.1, StatSoft, Inc., Tulsa, USA). Model parameters of (*R*)-[^{11}C]verapamil in brain (K_1 , k_2 , k_3 , k_4 , DV) and plasma PK parameters of verapamil for the two periods were compared using the Wilcoxon matched pairs test. A p level of <0.05 was regarded significant.

Results

6 subjects completed both study periods and one subject completed only period 2. Isoptin® at a therapeutic dose (80 mg) was well tolerated without occurrence of severe or serious adverse events. Mild adverse events, possibly related to administration of the study medication were headache in two subjects and dizziness in one subject.

Figure 1a shows mean concentration-time curves of total ^{11}C -radioactivity and (*R*)-[^{11}C]verapamil in arterial plasma, respectively, over 60 minutes following administration of an iv microdose (period 1) and an iv microdose dosed concomitantly with an oral therapeutic dose of verapamil (period 2). There was no significant difference between the AUC of the fractions of polar and lipophilic radiometabolites of (*R*)-[^{11}C]verapamil for the two periods (12.2 ± 1.3 min and 9.6 ± 1.3 min ($p=0.14$) and 12.3 ± 1.7 min and 16.8 ± 4.5 min ($p=0.14$) for period 1 and period 2, respectively) (data not shown). Figure 1b shows concentration-time curves of radioactivity in brain measured with PET imaging for period 1 and period 2.

The 2T4K compartment model provided better fits of the PET data for both periods than the 1T2K compartment model (mean Akaike Information Criterion, AIC –23.86 versus –14.19 and –35.5 versus –28.61 for period 1 and period 2, respectively). Parameter estimates for the exchange of radioactivity between plasma and brain obtained from the 2T4K model are displayed in table I. There were no significant differences in model outcome parameters for the two periods except for k_4 , which was higher for period 2 ($p=0.046$). Compartment-model derived DV values were in good agreement with DV s estimated by Logan graphical analysis (table I).

Figure 2 shows total ^{14}C -radioactivity (figure 2a) and (*R*)-[^{14}C]verapamil (figure 2b) and (*S*)-[^{14}C]verapamil (figure 2c) concentrations in venous plasma for the two periods. Mean PK parameters of (*R*)- and (*S*)-verapamil are given in table II. There was no statistically significant difference in any of the PK parameters for (*R*)-verapamil or (*S*)-verapamil for period 1 and period 2, respectively, except for V_{ss} of (*R*)-verapamil, which was smaller for period 2. On the other hand, all PK parameters (except for $t_{1/2}$) differed significantly between the (*R*)- and (*S*)-enantiomers for the two periods. C_{max} , $AUC_{(0-24)}$ and $AUC_{(0-\text{inf})}$ were higher and CL , V and V_{SS} were lower for the (*R*)- than for the (*S*)-enantiomer (table II).

Figure 3 shows concentration levels of (*R*)-verapamil in arterial and venous plasma measured by gamma counting with SPE/HPLC correction and by HPLC-AMS, respectively, showing that both methods to quantify parent in plasma were in reasonably good agreement.

Discussion

The ultrasensitive analytical techniques AMS and PET have both been used in human microdose studies.^[9, 15] While AMS is useful for obtaining PK over prolonged periods of time after administration of a ¹⁴C-labelled drug, the main asset of PET is that PK data can be obtained in humans from otherwise inaccessible tissues such as brain, by using drugs labelled with short-lived positron emitters such as ¹¹C. In the present study, we demonstrated, for the first time, the feasibility of combining AMS and PET analysis in a single clinical microdose study by administering ¹⁴C- and ¹¹C-labelled verapamil, as a model drug, to human volunteers. Because a major question with microdosing is the predictivity of a drug's PK at higher, therapeutic doses, we administered a ¹⁴C- and ¹¹C-labelled iv microdose (period 1) and a ¹⁴C- and ¹¹C-labelled iv microdose dosed concomitantly with an unlabelled oral therapeutic dose of verapamil (period 2) in a two-period design, in order to assess the dose-linearity of verapamil PK in plasma and brain. It has to be emphasized, however, that such a comparison of a drug's PK at a microdose and a therapeutic dose will not be possible for microdose studies of new drug candidates, because the required safety and toxicology data will usually not be available to allow for administration of therapeutic doses. In the current study however, where an approved drug was investigated, the two-period design allowed for a comparison of the PK to be made at systemic concentrations relevant to both the microdose and therapeutic dose in the same subjects which was considered a more robust approach than using just literature data.

The chosen study design has been used previously to examine the PK linearity of iv administered drugs labelled with an isotopic tracer.^[5, 12, 26, 27] Whilst the plasma drug concentrations from the iv dose alone (period 1) arose entirely from the amount administered iv, the plasma drug concentrations from period 2 arose from the sum of the iv dose and that absorbed following oral dosing. Plasma concentrations of unlabelled verapamil following oral dosing were not measured in the present study. However, the reported oral bioavailability of verapamil is approximately 20% and the expected $C_{\max}$ after a 80 mg oral dose is 130 ng·mL⁻¹ and $AUC_{0-\infty}$ is 450 h·ng·mL⁻¹.^[28] The $C_{\max}$ following iv microdosing in the current study was 0.31 ng·mL⁻¹ and $AUC_{0-\infty}$ was 0.93 h·ng·mL⁻¹ (total for (*R*)- and (*S*)-verapamil) (table II). The observed PK were therefore shown to be essentially linear over an approximate 420-fold plasma concentration range. By administering the iv microdose at the estimated $t_{\max}$ of verapamil after oral administration^[16] we attempted to approach the elimination phases of the iv and the oral drug doses in order to keep the values for the clearance between the two dose routes as equivalent as possible.^[27]

For AMS analysis [¹⁴C]verapamil was administered as a racemic mixture. Plasma samples were subjected to 2-dimensional reversed-phase followed by chiral HPLC analysis, before AMS analysis, to enable plasma concentrations of (*R*)- and (*S*)-verapamil to be measured separately. For PET imaging, however, the ¹¹C-labelled (*R*)-enantiomer of verapamil was used, as PET measures total radioactivity in tissue and does not distinguish between different radiolabelled enantiomers or between unchanged drug and metabolites. (*R*)-[¹¹C]verapamil has been shown to undergo extensive cytochrome P450-mediated metabolism, mainly giving rise to polar radiometabolites ([¹¹C]formaldehyde and related species) and the lipophilic *N*-dealkylation products [¹¹C]D-617 and [¹¹C]D-717.^[23, 29] In the present study, it was determined that 42±8%, 31±8% and 28±9% of total plasma radioactivity were in the form of (*R*)-[¹¹C]verapamil, lipophilic *N*-dealkylation products,

and polar radiometabolites, respectively, at 60 minutes after radiotracer injection. It seems therefore likely that the radiometabolites of (*R*)-[^{11}C]verapamil contributed to the measured brain PET signal. Hence, the brain tissue PK (figure 1b) and rate constants for plasma-tissue exchange of radioactivity (table I) reported in this study are not wholly representative of parent drug but rather of a mixture of parent drug and its radiometabolites. In the case of (*R*)-[^{11}C]verapamil, however, the major fraction of its radiometabolites ([^{11}C]D-617 and [^{11}C]D-717) can be expected to behave similarly to parent^[24] because D-617 and D-717 have been shown, like verapamil itself, to be also transported by P-gp.^[30] Although metabolites of [^{14}C]verapamil were not directly measured, comparing the concentration-time curves of parent drug (figure 2b,c) in plasma with the concentration time-curve of total ^{14}C -radioactivity (figure 2a), also suggested extensive metabolism of verapamil.^[31] At 24 hours after administration of a ^{14}C - and ^{11}C -labelled iv microdose and a ^{14}C - and ^{11}C -labelled iv microdose dosed concomitantly with an unlabelled oral therapeutic dose of verapamil, the sum of mean (*R*)- and (*S*)-[^{14}C]verapamil concentrations in plasma represented only about 3% of total ^{14}C -radioactivity in plasma.

It has been previously reported that after iv administration of racemic verapamil, metabolism of (*S*)-verapamil was higher compared to (*R*)-verapamil which resulted in higher systemic clearance of the pharmacologically more potent (*S*)-enantiomer.^[32] These results were confirmed in the present study where CL , V and V_{ss} were higher for (*S*)-verapamil than for (*R*)-verapamil, for both periods (table II). Accordingly, C_{max} , $AUC_{(0-24)}$ and $AUC_{(0-inf)}$ were lower for (*S*)-verapamil than for (*R*)-verapamil (table II). The value for V_{ss} was approximately 69-76% of V (table II), indicating only moderate drug elimination occurs before the terminal phase is reached. In general, the plasma PK parameters of (*R*)- and (*S*)-verapamil determined in the present study were in good agreement with those previously reported by Eichelbaum et al.^[32]

Clearly, for a drug such as a verapamil, which has a $t_{1/2}$ in the range of 6-8 hours, the plasma concentration-time profile of ^{11}C -labelled parent tracer measured during PET imaging (figure 1a, figure 3) would fail to predict the drug's PK parameters as the drug's elimination phase cannot be captured during the short time course of the PET experiment.^[33] For such drugs a combination of PET imaging for assessment of drug tissue distribution with AMS for plasma PK analysis is particularly powerful. Another possible advantage of the AMS-PET combination could be that the rate constants describing exchange of radiolabelled drug between plasma and brain (table I), which are obtained by compartment modeling of the PET data, can be used to predict long term brain tissue PK data from the venous PK data as measured by AMS analysis as recently described by Bergström and co-workers.^[34] However, this particular approach was not used in the present study due to the possible confounding effect of radiometabolites of (*R*)-[^{11}C]verapamil, which were also taken up into brain tissue.

An important finding of this study was that verapamil displays to a large degree linear PK in plasma and brain at a microdose and therapeutic dose with nearly superimposable plasma and brain concentration-time curves over the dose range from 0.05 to 80 mg (figure 1 and 2), although it is noted that dose-dependent clearance would be expected at higher doses.^[35] This is of particular interest as verapamil is a high-affinity substrate of the efflux transport protein P-gp (ABCB1), which is highly abundant in tissues involved in drug absorption and excretion (kidney, liver, intestines), where it limits absorption and increases excretion of its substrates by active adenosine triphosphate (ATP)-dependent transport.^[36] A moderate degree of non-linearity was observed for (*R*)-verapamil which had higher C_{max} , AUC_{0-24} , AUC_{0-inf} values and lower CL , V and V_{ss} values for period 2 as compared to period 1 (table II). This is also in line with the observation that the fraction of polar radiometabolites of (*R*)-[^{11}C]verapamil was somewhat lower for period 2 than for period 1. Slower metabolism of

PET tracers administered at a therapeutic dose as compared to a microdose has also been reported by other investigators.^[33] As opposed to standard PK parameters, no statistically significant differences were found for (*R*)-[¹¹C]verapamil between the two periods for parameters describing exchange of radioactivity between plasma and brain (table I).

(*R*)-[¹¹C]verapamil has been used before as a PET tracer to measure P-gp mediated transport at the blood-brain barrier (BBB).^[21, 24, 37] Overactivity of P-gp at the BBB has been suggested to be a contributing mechanism to pharmacoresistance in neurologic disorders, particularly in medically refractory epilepsy.^[38] A promising strategy to overcome drug resistance is treatment with P-gp modulating drugs in combination with neurologic drugs.^[39-41] One of the first P-gp modulating drugs employed for overcoming multidrug resistance in cancer was racemic verapamil, which was then followed by (*R*)-verapamil.^[42] Interestingly, analysis of brain tissue PK of ¹¹C-radioactivity following (*R*)-[¹¹C]verapamil injection revealed no major differences in brain distribution parameters (*DV*, *K*₁, *k*₂) for the two periods (table I), which provides strong evidence that therapeutic doses of verapamil are not able to enhance brain uptake of P-gp substrates. Two clinical case reports have recently provided evidence that co-administration of verapamil with antiepileptic therapy might be able to restore treatment response in epilepsy patients. This was at least partly attributed to inhibition of P-gp at the BBB by verapamil, thereby facilitating brain entry of antiepileptic drugs.^[43, 44] The verapamil doses used to treat medically refractory epilepsy (1-1.5 mg·kg⁻¹·day⁻¹)^[43] were comparable to the single oral verapamil dose given in our study (1 mg·kg⁻¹), which questions whether the observed response in patients was indeed mediated by verapamil-induced cerebral P-gp inhibition.

Conclusions

The presented PK data for verapamil in plasma and brain demonstrate the principle of combining AMS and PET microdosing in a single clinical study. Co-administration of ¹⁴C-labelled drug can be incorporated into standard clinical PET study protocols and thereby allows long term PK data along with information on drug tissue distribution to be acquired in the same subjects. In contrast to PET microdosing alone, the combination of AMS and PET microdosing allows for an accurate description of a drug's plasma PK parameters. This approach has the potential to maximize the outcomes of a single clinical study in comparison to obtaining the plasma and tissue distribution data of a drug in individual groups of subjects. In addition, by administering an iv drug microdose as well as an iv microdose over an extravascular therapeutic dose in a two-period design, dose-linearity of a drug's PK at a microdose and plasma concentrations relevant to the therapeutic dose can be assessed. The results of this human pilot study suggest that studying drug disposition and PK with AMS and PET offers numerous benefits and may thus be of interest for early clinical drug development.

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Fig 1. Concentration-time curves (mean $\pm$ SD) of (a) total ^{11}C -radioactivity in arterial plasma (diamonds) and (R) -[^{11}C]verapamil in arterial plasma (circles) and (b) total ^{11}C -radioactivity in whole brain grey matter (diamonds) after administration of an iv microdose (period 1, open symbols, $n=6$) and an iv microdose dosed concomitantly with an oral therapeutic dose of verapamil (period 2, filled symbols, $n=7$).

Fig. 2. Concentration-time curves (mean $\pm$ SD) of (a) total ^{14}C -radioactivity in venous plasma (diamonds), (b) (R)- ^{14}C verapamil in venous plasma (circles) and (c) (S)- ^{14}C verapamil in venous plasma (triangles) after administration of an iv microdose (period 1, open symbols, $n=6$) and an iv microdose dosed concomitantly with an oral therapeutic dose of verapamil (period 2, filled symbols, $n=7$).

Fig. 3. Concentration-time curves (mean $\pm$ SD) of (*R*)-[^{11}C]verapamil in arterial plasma as measured by gamma counting with SPE/HPLC correction (circles) and of (*R*)-[^{14}C]verapamil in venous plasma as measured by HPLC-AMS (triangles) after administration of an iv microdose (period 1, open symbols, $n=6$) and an iv microdose dosed concomitantly with an oral therapeutic dose of verapamil (period 2, filled symbols, $n=7$). Note that the time axis is not set to the full range of venous plasma sampling (24 hours) to facilitate better comparison of data sets.

Table I

Outcome parameters of the 2-tissue-4-rate constant (2T4K) compartment model

Parameter	Microdose (period 1)	Microdose+oral dose (period 2)
K_1 (mL·mL ⁻¹ ·min ⁻¹)	0.030±0.003 (10)	0.031±0.005 (8)
k_2 (min ⁻¹)	0.099±0.006 (49)	0.095±0.008 (40)
k_3 (min ⁻¹)	0.100±0.001 (90)	0.101±0.000 ^a (96)
k_4 (min ⁻¹)	0.092±0.029 (26) ^b	0.159±0.063 (42) ^b
DV (mL·mL ⁻¹)	0.66±0.12 (4)	0.56±0.11 (2)
DV (Logan) (mL·mL ⁻¹)	0.66±0.11 (2)	0.57±0.11 (1)

Data are presented as mean±standard deviation (period 1: $n=6$, period 2: $n=7$). The value in parentheses represents the precision of parameter estimates (expressed as their coefficient of variation in percent), averaged over the 6 study subjects.

K_1 , k_2 , k_3 , k_4 =rate constants for exchange of radioactivity between plasma and first and second tissue compartment; DV =distribution volume; DV (Logan)= DV estimated with Logan analysis

For all statistical comparisons, data from subject 2, who underwent only period 2, were excluded.

^a Rounded to 3 decimal digits.

^b A statistically significant difference was observed between the two periods ($p<0.05$).

Table II

Pharmacokinetic parameters of (*R*)- and (*S*)-[¹⁴C]verapamil given intravenously

Parameter	Enantiomer ^a	Microdose (period 1)	Microdose+oral dose (period 2)
$t_{1/2}$ (h)	<i>R</i>	6.26±1.86	6.92±1.57
	<i>S</i>	7.23±2.50	7.12±2.22
C_{max} (pg·mL ⁻¹)	<i>R</i>	210.06±79.19	243.86±77.65
	<i>S</i>	96.33±28.59	103.46±33.62
$AUC_{(0-24)}$ (h·pg·mL ⁻¹)	<i>R</i>	579.82±107.36	794.04±265.14
	<i>S</i>	272.56±70.68	313.79±59.72
$AUC_{(0-inf)}$ (h·pg·mL ⁻¹)	<i>R</i>	624.48±131.59	843.20±281.07
	<i>S</i>	308.63±79.28	343.29±58.92
CL (L·h ⁻¹)	<i>R</i>	60.99±12.61	46.89±10.88
	<i>S</i>	89.65±24.18	78.18±14.70
$V(L)$	<i>R</i>	528.24±95.14	465.65±133.04
	<i>S</i>	912.88±341.93	789.00±272.77
V_{ss} (L)	<i>R</i>	397.85±89.75 ^b	319.93±68.74 ^b
	<i>S</i>	681.97±166.96	600.56±187.64

$t_{1/2}$ =terminal half-life; C_{max} =maximum concentration; AUC_{0-24} =area under the curve from time 0 to 24 hours; AUC_{0-inf} =AUC extrapolated to infinity; CL =clearance; V =volume of distribution; V_{ss} =volume of distribution under steady-state conditions

For all statistical comparisons, data from subject 2, who underwent only period 2, were excluded.

^aFor all PK parameters except for $t_{1/2}$ a statistically significant difference was observed between the (*R*)- and (*S*)-enantiomers ($p<0.05$).

^bA statistically significant difference was observed between the two periods ($p<0.05$). Data are presented as mean±standard deviation (period 1: $n=6$, period 2: $n=7$).