Combined PET and Microdialysis for In Vivo Assessment of Intracellular Drug Pharmacokinetics in Humans

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Because many drugs possess an intracellular site of action, the knowledge of intracellular concentration–time profiles is desirable. In the present study, PET, which measures total (i.e., intracellular, extracellular, and intravascular) concentrations of radiolabeled drugs in tissue, and microdialysis, which determines unbound drug concentrations in the extracellular space fluid of tissue, were combined to describe the intracellular pharmacokinetics of a model compound—that is, the ^{18}F-labeled antibiotic ^{18}F-ciprofloxacin—in vivo in humans. **Methods:** Ten healthy male volunteers received a mixture of 687 ± 50 MBq of ^{18}F-ciprofloxacin and 200 mg of unlabeled ciprofloxacin as an intravenous bolus infusion over 10 min. The pharmacokinetics of ciprofloxacin in skeletal muscle tissue were assessed by means of combined PET and in vivo microdialysis for 5 h after drug administration. A 3-compartment pharmacokinetic model was fitted to the tissue concentration–time profiles of ciprofloxacin measured by PET to estimate the rate constants of ciprofloxacin uptake and transport. **Results:** In muscle tissue, mean total and extracellular peak concentration (C_{max}) values of ciprofloxacin of 1.8 ± 0.4 μg/mL and 0.7 ± 0.2 μg/mL were attained at 95 ± 34 min and 48 ± 20 min after drug administration, respectively. The extracellular-to-intracellular exchange appeared to be very fast, with an estimated rate constant k_3 of 1.69 ± 0.25 min^{-1}. An intracellular-to-extracellular concentration ratio (C_{intra}/C_{extra}) of 3.2 ± 0.8 was reached at 110 min after injection and followed by sustained intracellular retention of the antibiotic for the remainder of the experiment. The predicted extracellular concentration–time profiles from the compartmental modeling were in good agreement with the measured microdialysis data. **Conclusion:** The results obtained in the present study were in accordance with previous in vitro data describing cellular ciprofloxacin uptake and retention. The presently used PET/microdialysis combination might be useful during research and development of new drugs, for which knowledge of intracellular concentrations is of interest.

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Most drugs exert their effects outside the plasma compartment, in defined target tissues, where drug binding sites are located and into which drugs have to distribute from the central compartment. Target site drug levels may substantially differ from corresponding plasma levels and drug distribution processes may be characterized by a high intertissue variability, leading to suboptimal target site concentrations and the potential risk of therapeutic failures (*1*).

Several methods, such as tissue biopsy, magnetic resonance spectroscopy, PET, and microdialysis, have been used previously to assess drug disposition in humans (*2–4*). Biopsies, which have frequently been applied to measure drug concentrations in tissue, are an invasive procedure that, for ethical reasons, is difficult to perform repeatedly in the same subject, which thus precludes detailed information on tissue concentration–time courses. PET, a noninvasive nuclear imaging method, can be used for a dynamic measurement of radiolabeled drug concentrations in human body tissues over several hours (*5*). The PET signal comprises the intracellular, the extracellular, and the intravascular fraction of a radiolabeled drug and its metabolites in a given volume of tissue. In vivo microdialysis is a minimally invasive sampling technique that has been used to assess unbound drug concentrations in humans in the extracellular space fluid of peripheral tissues (*6*). However, for several drugs, such as certain antiinfective and anticancer agents, the site of drug action is not the biophase surrounding the cells but rather an intracellular compartment. Therefore, the knowledge of intracellular rather than extracellular drug concentrations may be more relevant in many cases. The in vivo assessment of intracellular drug pharmacokinetics in human

body tissues, however, has so far been hampered by a lack of appropriate technology.

We have recently labeled the fluoroquinolone antibiotic ciprofloxacin with the positron-emitter ^{18}F (half-life, 109.8 min) (*7*) and have used this radiotracer—that is, ^{18}F-ciprofloxacin—to assess the pharmacokinetics of ciprofloxacin in various body tissues of healthy volunteers (*8*) and patients with bacterial infections (*9*) with PET. In the present study we used ^{18}F-ciprofloxacin as a model compound to simultaneously perform PET and microdialysis in healthy volunteers in an attempt to describe intracellular drug pharmacokinetics in vivo in human muscle tissue by the combined use of both techniques.

MATERIALS AND METHODS

The study took place at the Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria. The study protocol was approved by the local Ethics Committee 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 before enrollment in the study.

Healthy Volunteers

Ten healthy male volunteers (mean age ± SD, 31 ± 7 y; mean weight, 78 ± 6 kg; mean height, 179 ± 5 cm) were included in the present study. Before inclusion in the study, each volunteer was subjected to a screening examination. Subjects were excluded if they had taken any prescription medication or over-the-counter drugs within the 2 wk before the study or if they had undergone any diagnostic test with radioactive tracers or x-rays during the 12 mo preceding the study.

Study Design and Study Medication

This study was performed as a descriptive, exploratory, single-center, nonrandomized study. Subjects received a mixture of ^{18}F-labeled ciprofloxacin (^{18}F-ciprofloxacin; mean injected activity, 687 ± 50 MBq) and 200 mg of unlabeled ciprofloxacin (Ciproxin 200 mg, Infusionsflaschen; Bayer Leverkusen) as an intravenous infusion over 10 min. The pharmacokinetics of ciprofloxacin in skeletal muscle tissue were assessed by means of combined PET and microdialysis for 5 h after drug administration.

PET

The total concentration−time profile of ciprofloxacin in skeletal muscle [$C_{PET}(t)$] was measured by PET of ^{18}F-ciprofloxacin. The PET signal comprises the intracellular, the extracellular, and the intravascular fraction of ^{18}F-ciprofloxacin−derived radioactivity in a given volume of tissue.

In Vivo Microdialysis

The concentration of ciprofloxacin in the extracellular compartment of skeletal muscle (C_{extra}, in μg/mL) is given by: $C_{extra} = C_{extra\text{-}unbound} + C_{extra\text{-}bound}$, where $C_{extra\text{-}unbound}$ and $C_{extra\text{-}bound}$ refer to the concentration of free extracellular ciprofloxacin and the concentration of ciprofloxacin bound to proteins present in the extracellular space fluid, respectively. $C_{extra\text{-}unbound}(t)$ was measured by high-performance liquid chromatography (HPLC) analysis of samples obtained by in vivo microdialysis. This technique is based on the sampling of analytes from the extracellular space by means of a semipermeable membrane at the tip of a microdialysis probe. The probe is implanted into the tissue and perfused with a physiologic solution at a flow rate of 1.5 μL/min. Substances that are present in the extracellular fluid at concentration $C_{extra\text{-}unbound}$ diffuse out of the extracellular fluid into the probe, resulting in a concentration in the perfusion medium ($C_{dialysate}$). For most analytes, equilibrium between $C_{extra\text{-}unbound}$ and $C_{dialysate}$ is incomplete; therefore, $C_{extra\text{-}unbound} > C_{dialysate}$. The factor by which the concentrations are interrelated is termed "relative recovery." For calibration of the microdialysis probe, the in vivo recovery (in percent) was assessed before each experiment by the retrodialysis method following procedures that have been described in detail previously (*10,11*). $C_{extra\text{-}unbound}$ was calculated as $100 \cdot (C_{dialysate}/\text{in vivo recovery})$. The fraction of ciprofloxacin bound to extracellular proteins was assumed to be 0.1. This value was obtained as described by Araki et al. (*12*) by multiplying the fraction of ciprofloxacin protein binding in human plasma (0.33) (*13*) by the tissue-to-plasma albumin ratio in human skeletal muscle tissue (0.3) (*14*) assuming that extracellular ciprofloxacin binds mainly to albumin. The total extracellular concentration of ciprofloxacin (C_{extra}) was therefore estimated as follows: $C_{extra} = C_{extra\text{-}unbound}/0.9$.

Measurement of Ciprofloxacin Concentrations in Microdialysates and Plasma Samples

Ciprofloxacin was quantified by a previously described HPLC assay using fluorescence detection with levofloxacin as an internal standard (*15*). For analysis of plasma, proteins were removed by methanol precipitation before HPLC analysis. The limit of quantification of ciprofloxacin was 0.1 μg/mL.

Synthesis and Quality Control of ^{18}F-Ciprofloxacin

^{18}F-Ciprofloxacin was synthesized and purified as previously described (*7*). Before injection into volunteers, quality control was performed for each batch of synthesized ^{18}F-ciprofloxacin (*7*). For intravenous administration, an aliquot of the formulated radiotracer solution (2–4 mL) was mixed with 100 mL of an infusion solution containing unlabeled ciprofloxacin at a concentration of 2 mg/mL.

Study Protocol

On the study day, approximately 3 h before radiotracer injection, volunteers were positioned supine on a bed and a venous catheter was placed in each arm (one for infusion of the study medication and one for blood sampling). Then, a commercially available microdialysis probe (CMA 10, Microdialysis AB; outer diameter: 0.5 mm, overall length: 86 mm, membrane length: 16 mm) with a molecular cutoff of 20,000 was inserted as previously described into the left thigh muscle (*11*). The microdialysis system was first perfused for 30 min with Ringer's solution (Mayrhofer Pharmazeutika) at a flow rate of 1.5 μL/min using a Precidor microinfusion pump (Infors-AG). Microdialysis probes were calibrated in all individuals according to the retrodialysis method (*10*) by using a ciprofloxacin concentration of 3.0 μg/mL. The in vivo calibration period was followed by a 30-min washout period. Volunteers were subsequently positioned supine on the PET scanner bed with the thigh muscles within the field of view (FOV) of the PET camera. For PET, an Advance PET scanner (GE Healthcare) with a transversal FOV of 55 cm and an axial FOV of 15 cm was used. To correct for tissue attenuation of photons, a transmis-

sion scan of 5-min duration using two 400-MBq ^{68}Ge pin sources was recorded before radiotracer injection. Then, the study medication was administered as an intravenous infusion over 10 min. Serial PET, microdialysis sampling, and venous blood sampling were initiated at the start of bolus infusion and were continued for 5 h. The PET protocol consisted of the following frame sequence: during the first 60 min after injection, dynamic images were acquired (frame lengths, 12 × 5 min). Approximately 70, 130, 190, 250, and 295 min after tracer injection, a transmission scan (5-min duration) followed by a 10-min emission scan over one axial FOV was performed. At the end of the last emission scan, a disposable injection needle (0.8 × 60 mm), which had been flushed with diluted radiotracer solution, was aligned with the microdialysis probe and a dynamic emission scan (frame lengths, 4 × 30 s) was started to localize the microdialysis probe in the PET images. During the study period, the microdialysis system was perfused with Ringer's solution at a flow rate of 1.5 μL/min and microdialysates were collected at 20-min intervals. Microdialysates were stored at −80°C until HPLC analysis. During the study period, venous blood samples (9 mL) were collected into heparinized tubes at designated time points after radiotracer injection. Blood samples were centrifuged and radioactivity in 1-mL aliquots of plasma was measured in a Packard Cobra II auto-γ-counter (Packard Instrument Co.). Plasma samples were stored at −80°C until HPLC analysis. At the end of the PET study, subjects were asked to empty their bladders and a 50-mL aliquot of urine was counted for radioactivity in a well counter. Then, a 0.2-mL aliquot of postdose urine was analyzed by a previously described reversed-phase radio-HPLC system (*8*).

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 postfiltering algorithm of 6.00-mm FWHM was applied. Attenuation correction was performed using the manufacturer's segmentation algorithm for transmission data. The final dynamic image sequence was used to outline the course of the microdialysis probe in the investigated muscle tissue. A region of interest with an area of approximately 2–3 cm^2 was drawn around the tip (length, 16 mm) of the microdialysis probe and transferred to all other images of the time sequence. Radioactivity concentrations (in kBq/mL) were combined and decay-corrected to the time of tracer injection to obtain time−radioactivity curves for the whole observation period. Radioactivity concentrations were converted into absolute concentration units of ciprofloxacin (μg/mL) via the specific activity of the administered ^{18}F-ciprofloxacin (i.e., 3.4 ± 0.3 kBq/μg). Individual plasma, extracellular, and total concentration−time profiles of ciprofloxacin were used to calculate the following pharmacokinetic parameters using the Kinetica 2000 version 3.0 (InnaPhase Corp.) software package: peak concentration (C_{max}, μg/mL), time to C_{max} (t_{max}, min), elimination half-life ($t_{1/2}$, min, calculated from the last data points), and area under the concentration−time curve (from time zero to the last measurement, $AUC_{0\text{-}n}$, μg·min·mL^{-1}).

Modeling of Ciprofloxacin Data

A 3-compartment, 4-rate-constant (4K) model for ciprofloxacin tissue concentrations in skeletal muscle was used to estimate rate constants of ciprofloxacin uptake and transport (*16,17*). The model assumes that the transport of ciprofloxacin between compartments can be described by first-order kinetics, that compartments are of homogeneous composition, and that venous plasma concentration of ciprofloxacin can be used to approximate capillary concentration. The 4K model (Fig. 1) is described by:

$$\frac{dC_1(t)}{dt} = K_1C_p(t) - (k_2 + k_3)C_1(t) + k_4C_2(t). \quad C_1(0) = 0$$

$$\frac{dC_2(t)}{dt} = k_3C_1(t) - k_4C_2(t). \quad C_2(0) = 0$$

$C_p(t)$ is the input function to the 4K model; $C_p(t)$ was obtained by fitting a linear 3-compartment pharmacokinetic model to the plasma concentration data of ciprofloxacin (*18*). $C_1(t)$ is the extracellular concentration of ciprofloxacin normalized to intracellular volume V_{intra}: $C_1(t) = (V_{extra}/V_{intra})C_{extra}$. $C_2(t)$ denotes the intracellular concentration of ciprofloxacin. The first-order rate constants K_1 (mL·mL^{-1}·min^{-1}) and k_2 (min^{-1}) describe transport of ciprofloxacin from plasma to extracellular space and back, whereas k_3 (min^{-1}) and k_4 (min^{-1}) characterize exchange of ciprofloxacin between extracellular and intracellular space. For a detailed discussion of the model equations—in particular, their derivation from the respective mass balance equations—see Bertoldo et al. (*17*). The total tissue concentration $C(t)$ of ciprofloxacin was described by the equation:

$$C(t) = (1 - V_b)[C_1(t) + C_2(t)] + V_bC_b(t),$$

where V_b is the vascular volume fraction of the total tissue volume and $C_b(t)$ is the concentration−time course of ciprofloxacin in the whole blood. For ciprofloxacin, which binds to about 50% to cellular components of blood, $C_b(t) = C_p(t)$ (*8*). The model equations were solved numerically using the ode15s solver of MATLAB (Mathworks). The kinetic parameters K_1, k_2, k_3, k_4 as well as the vascular volume fraction V_b were estimated by fitting the 4K model to the measured PET data $C_{PET}(t_j)$ using the operational equation:

$$\hat{C}_{PET}(t_j) = \frac{1}{te_j - ts_j}\int_{ts_j}^{te_j} C(t)dt,$$

where ts_j and te_j denote start and end time of the jth PET time frame and t_j is its midpoint. Fits were performed by weighted nonlinear least squares as implemented in the lsqcurvefit function of the Optimization Toolbox of MATLAB and data were weighted by $(te_j - ts_j)/C_{PET}(t_j)$ (*17*). For the ratio of intracellular to extra-

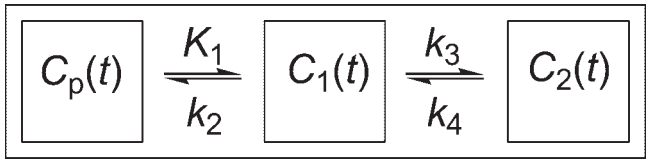

FIGURE 1. Diagram of 3-compartment, 4-rate-constant (4K) model to describe ciprofloxacin pharmacokinetics in human skeletal muscle. $C_p(t)$ is the input function, $C_1(t)$ denotes the extracellular concentration, and $C_2(t)$ denotes the intracellular concentration of ciprofloxacin. K_1, k_2, k_3, and k_4 are first-order rate constants describing exchange of ciprofloxacin between plasma and extracellular space as well as between extracellular and intracellular space.

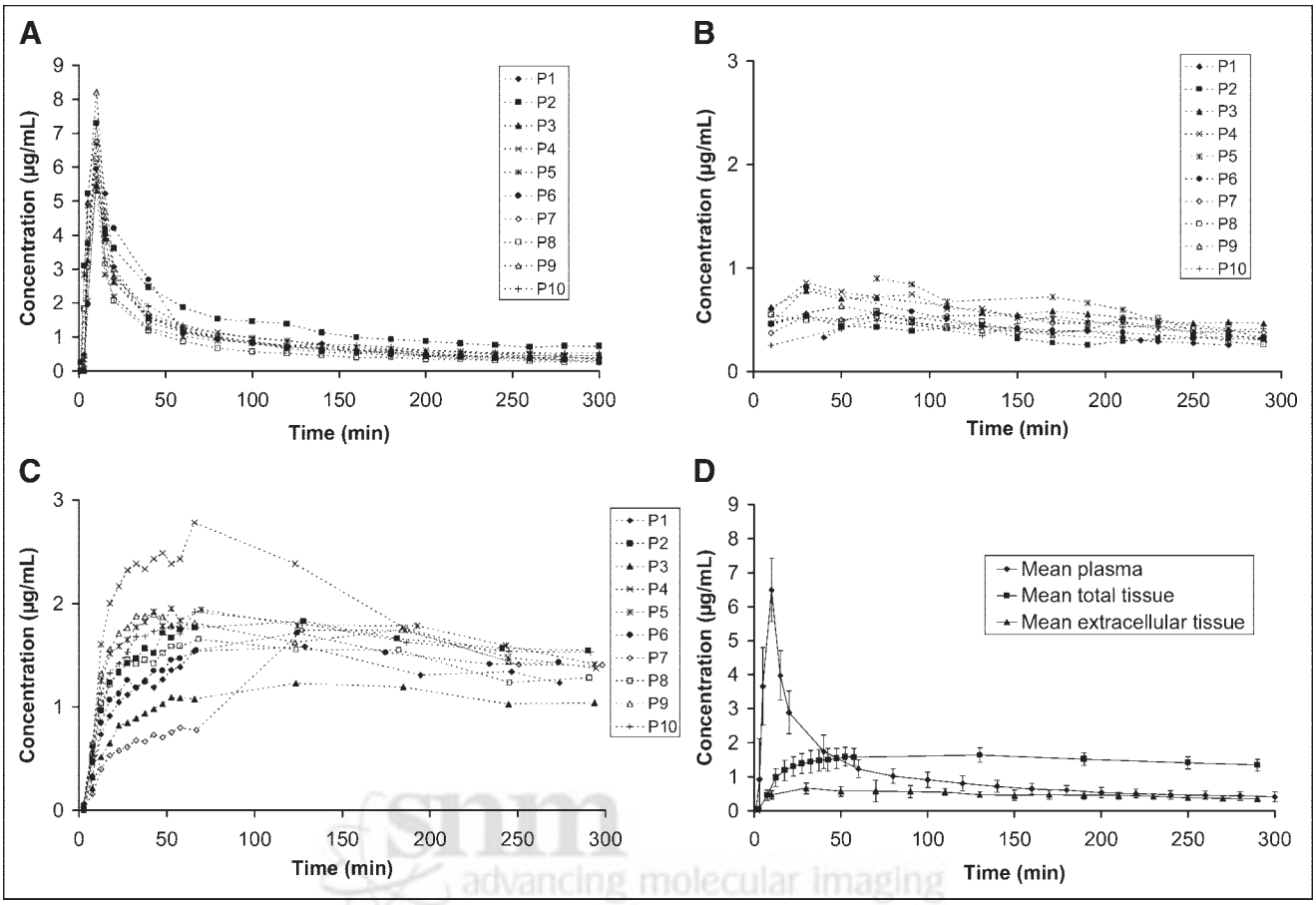

FIGURE 2. Individual (A–C) and mean ± SD (D) concentration–time profiles of ciprofloxacin in plasma (A), extracellular space of skeletal muscle tissue (B), and total skeletal muscle (C) measured by combined microdialysis and PET after intravenous administration of a mixture of 687 ± 50 MBq of ^{18}F-ciprofloxacin and 200 mg of unlabeled ciprofloxacin to 10 healthy male volunteers (P1–P10 indicate individual subjects).

cellular volume, a constant value of $V_{intra}/V_{extra} = 0.88/0.12 \approx 7.33$ was applied (*14*).

RESULTS

All study procedures were well tolerated by all volunteers. No serious adverse event and no side effects occurred from the administration of the study medication. Figure 2 shows individual and mean concentration–time curves of ciprofloxacin in different tissue compartments measured with combined PET and microdialysis after bolus infusion of a mixture of unlabeled ciprofloxacin and ^{18}F-ciprofloxacin. In plasma, a mean C_{max} of ciprofloxacin of 6.5 ± 0.9 μg/mL was reached at 10 min after start of infusion (Table 1). The mean elimination half-life ($t_{1/2}$) of ciprofloxacin from plasma was 208 ± 49 min and the mean $AUC_{0\text{-}n}$ was 290 ± 30 μg·min·mL^{-1}. A mean total (i.e., intracellular + extracellular + intravascular) C_{max} of ciprofloxacin of 1.8 ± 0.4 μg/mL was attained in muscle tissue at 95 ± 34 min after infusion start with a mean $t_{1/2}$ of 528 ± 142 min and a mean $AUC_{0\text{-}n}$ of 434 ± 76 μg·min·mL^{-1} (Table 1). The mean extracellular C_{max} ($C_{extra\text{-}unbound}$) of ciprofloxacin in muscle tissue was 0.7 ± 0.2 μg/mL with a t_{max} of 48 ± 20 min and a mean $AUC_{0\text{-}n}$ of 132 ± 33 μg·min·mL^{-1} (Table 1). A 3-compartment, 4-rate-constant (4K) pharmacokinetic model (Fig. 1) was fitted to the tissue data of ciprofloxacin (C_{PET}). Estimates for the rate constants K_1, k_2, k_3, and k_4 that describe the exchange of ciprofloxacin between plasma and extracellular space as well as between extracellular and intracellular space are given in

TABLE 1
Pharmacokinetic Parameters (Mean ± SD) of Ciprofloxacin in Blood and Skeletal Muscle Tissue After Intravenous Administration of a Mixture of 687 ± 50 MBq of ^{18}F-Ciprofloxacin and 200 mg of Unlabeled Ciprofloxacin to 10 healthy Male Volunteers

Compartment	C_{max} (μg/mL)	t_{max} (min)	$t_{1/2}$ (min)	$AUC_{0\text{-}n}$ (μg · min · mL^{-1})
Plasma	6.5 ± 0.9	10 ± 0	208 ± 49	290 ± 30
Total tissue [$C_{PET}(t)$]	1.8 ± 0.4	95 ± 34	528 ± 142	434 ± 76
Extracellular tissue [$C_{extra\text{-}unbound}(t)$]	0.7 ± 0.2	48 ± 20	233 ± 88	132 ± 33

TABLE 2
Parameter Estimates (Mean ± SD) of 3-Compartment, 4-Rate-Constant Pharmacokinetic Model Used for Description of Ciprofloxacin Pharmacokinetics in Human Skeletal Muscle Tissue

Parameter	Value
K_1 [mL · mL^{-1} · min^{-1}]	0.02 ± 0.01
k_2 [min^{-1}]	0.21 ± 0.07
k_3 [min^{-1}]	1.69 ± 0.25
k_4 [min^{-1}]	0.07 ± 0.01
V_b	0.04 ± 0.01

Table 2. Figure 3 shows the concentration−time profiles of ciprofloxacin in different muscle tissue compartments of 2 representative subjects together with the fits obtained from the 4K model. For the predicted intracellular curves, a mean C_{max} of 1.9 ± 0.2 μg/mL was reached at 103 ± 14 min after injection. At 110 min and 290 min after injection, the mean intracellular-to-extracellular concentration ratios (C_{intra}/C_{extra}) were 3.2 ± 0.8 and 3.5 ± 0.8, respectively. At the end of the PET experiment, urine was collected and counted for radioactivity. A mean of 42% ± 13% (n = 6) of totally administered activity was excreted in postdose urine. As shown in a representative radio-HPLC chromatogram (Fig. 4), the majority (>85%) of the excreted activity represented unmetabolized ^{18}F-ciprofloxacin. Two unidentified radiolabeled metabolites (indicated by an arrow) eluting after the parent compound were observed on HPLC.

DISCUSSION

In the present study, the PET and the microdialysis technique were combined in an attempt to characterize in vivo intracellular drug concentrations in humans. Although some PET/microdialysis combinations have already been reported in the literature (*4*), none of these measured the pharmacokinetics of a drug molecule. We used the radiolabeled fluoroquinolone antibiotic ^{18}F-ciprofloxacin as a model compound. Ciprofloxacin is well suited for such a combination for several reasons. First, ciprofloxacin contains fluorine in its native structure and can be radiolabeled with a comparatively long-lived PET radionuclide (i.e., ^{18}F, half-life: 109.8 min) without changing its structure (*7*). Second, as shown in a previous study in healthy volunteers (*8*), ^{18}F-ciprofloxacin possesses a high degree of metabolic stability and radiolabeled metabolites are therefore unlikely to contribute to the PET signal measured in peripheral tissue. In the present study, we analyzed postdose urine collected from the study participants with radio-HPLC (Fig. 4) and found that the majority (>85%) of renally excreted activity represented unchanged ^{18}F-ciprofloxacin, which gave additional support for the metabolic stability of the radiotracer. Third, ciprofloxacin displays moderate plasma protein binding (about 30%) (*13*) and attains measurable concentrations in the extracellular space fluid of peripheral tissue (*19–21*). Finally, unlike other antibiotics whose distribution is restricted to the extracellular space fluid of tissue (e.g., β-lactam antibiotics), fluoroquinolones are known to penetrate from plasma into blood and body tissue cells, attaining concentrations that exceed those in serum and extracellular fluid by severalfold (*22,23*). Inside the cell, fluoroquinolones remain mostly free in the cytosol as the microbiologically active compound rather than being sequestered and bound to subcellular organelles (*24,25*).

The tissue chosen for the present investigation was skeletal muscle. Whereas the accumulation of ciprofloxacin in phagocytic blood cells (neutrophilic granulocytes and monocytes) has been investigated in vitro and ex vivo

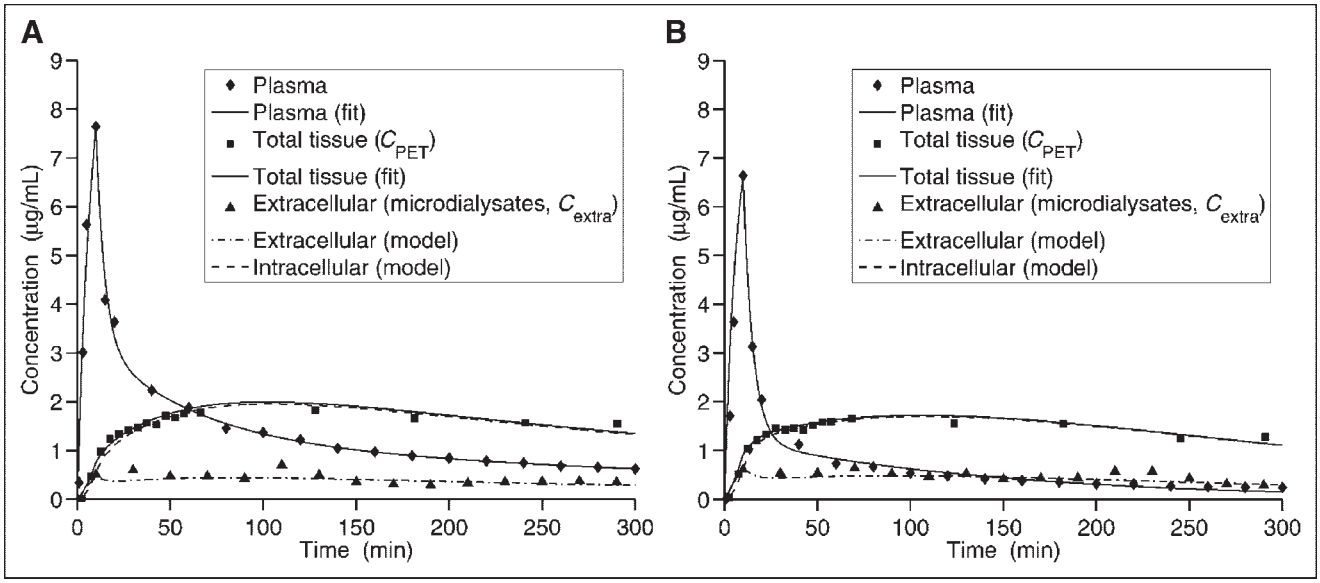

FIGURE 3. Blood and tissue concentration−time profiles of ciprofloxacin and fits obtained from 3-compartment, 4-rate-constant pharmacokinetic model from 2 representative subjects (A, subject 2; B, subject 8). Microdialysis data points (C_{extra}) were corrected for extracellular protein binding.

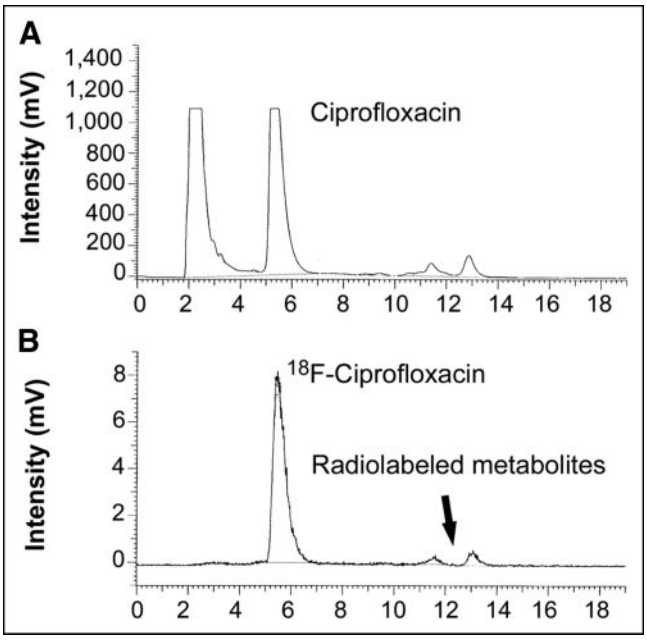

FIGURE 4. Representative reversed-phase HPLC chromatogram of postdose urine obtained from 1 healthy volunteer at the end of PET experiment. HPLC eluate was monitored in series for ultraviolet absorption (wavelength, 280 nm; A) and for radioactivity (B). Arrow indicates 2 unidentified radiolabeled metabolites.

before (*26–28*), little is known about myocytal uptake of this agent. From a therapeutic point of view, muscle cell uptake of antibiotics is of minor importance, since intracellular pathogens are generally not encountered in these cells. However, skeletal muscle tissue can be regarded as a useful model tissue, where a PET and microdialysis combination is easily feasible.

In the present experiments, PET provided a measure of the total tissue concentrations of ciprofloxacin comprising the intracellular, the extracellular, and the intravascular fraction of the drug. However, because the volume contribution of blood vessels in skeletal muscle tissue is very small (<2%) (*17*) and because the extracellular space fraction in human muscle tissue is only about 0.1 (*14*), the PET signal can be expected to be dominated by the intracellular concentration−time profile of the radiolabeled antibiotic. The volume of interest (VOI) for the analysis of the PET data was positioned in such a way that total tissue concentrations of ^{18}F-ciprofloxacin were measured in the muscle segment that surrounded the microdialysis probe. It should be noted, however, that placing the VOI in a muscle segment that was distant from the microdialysis probe provided a similar kinetic profile of ^{18}F-ciprofloxacin, thus indicating that insertion of the microdialysis probe did not cause any tissue damage. The recovery-corrected concentration−time profiles measured by HPLC analysis of microdialysates were used to describe the free extracellular ciprofloxacin concentrations [$C_{extra\text{-}unbound}(t)$]. We corrected these free concentrations for extracellular protein binding to provide an estimate of the total extracellular ciprofloxacin concentrations $C_{extra}(t)$. The correction was done as described in the literature (*12*) by assuming that ciprofloxacin bound mainly to albumin present in the extracellular space fluid and that protein binding of ciprofloxacin was linearly related to the protein concentration. It should be noted that the temporal resolution of microdialysis sampling was lower than that of the dynamic PET measurements. Microdialysis sampling intervals shorter than 20 min could not be used because of difficulties in handling small liquid volumes (<30 μL) in the HPLC assay (*15*) used for quantification of extracellular ciprofloxacin. The plasma and extracellular concentration−time curves obtained in the present study (Fig. 2) were similar to the profiles measured previously after administration of a comparable amount of ciprofloxacin to healthy subjects (*19,20*). The approximately 3-fold higher total-tissue *AUC* as compared with extracellular ciprofloxacin and the slow elimination of radioactivity from muscle tissue (Table 1) pointed to cellular uptake and retention of the antibiotic.

A 3-compartment, 4-rate-constant pharmacokinetic model (Fig. 1) was fitted to the concentration−time curves of ciprofloxacin in tissue (C_{PET}). As depicted in Figure 3, the fitted curves were in good agreement with the measured data. The measured microdialysis data points were used to validate the modeling results. As shown in Figure 3 for 2 representative volunteers, the predicted extracellular concentration−time profiles of ciprofloxacin were in good agreement with the microdialysis data. These results indicate that, in the case of ^{18}F-ciprofloxacin, extracellular profiles can be predicted by modeling of the PET data only. The mean intracellular-to-extracellular concentration ratio (C_{intra}/C_{extra}) (3.2 ± 0.8 at 110 min after injection) was in accordance with a previously published study that estimated total tissue concentrations of ciprofloxacin in rats by tissue biopsy and reported a C_{intra}/C_{extra} of 3.4 at 120 min after injection (*12*). Ciprofloxacin uptake from the extracellular fluid into cells appeared to occur very fast (k_3 = 1.69 ± 0.25 min^{-1}; Table 2). The efflux rate constant k_4 from the intracellular space (Fig. 1) was more than one order of magnitude smaller than the influx constant k_3 (Table 2). These observations most likely accounted for the almost irreversible behavior of ^{18}F-ciprofloxacin in muscle tissue and were in good agreement with previous in vitro data that demonstrated rapid cellular uptake and sustained intracellular retention of ciprofloxacin when blood cells (*27,28*) or bacterial cells (*29*) were incubated with the antibiotic.

In the present study, the vascular volume fraction (V_b) was estimated to be about 4% of the total tissue volume (Table 2), which indicated that the contribution of blood radioactivity to the PET signal was rather small. Similar V_b values (i.e., 0.3%–2%) were reported in a previous study, where the behavior of ^{18}F-FDG in human skeletal muscle was modeled (*17*). A possible limitation of the kinetic modeling performed in the present work is the fact that a venous rather than an arterial blood curve was used as an input function. Arterial blood sampling was avoided because of its invasiveness. However, in a previous PET study

in healthy subjects (*8*) we found that arterial and venous blood equilibrated within 10–20 min after administration of ^{18}F-ciprofloxacin as a single intravenous bolus over 30 s. Because the length of the bolus infusion in our study (10 min) was approximately of the same order as the arterial/venous blood equilibration time, we assumed that a venous blood curve would serve as a good approximation of the input function.

CONCLUSION

A combination of PET and microdialysis was used to characterize the intracellular pharmacokinetics of the model compound ^{18}F-ciprofloxacin in vivo in human skeletal muscle. The obtained results pointed to cellular uptake and retention of ciprofloxacin in muscle tissue, which was in accordance with previous in vitro data. The approach used in the present study might evolve into a useful application in the development of new drug candidates, for which the knowledge of intracellular concentrations is important.

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