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Characterization of Peripheral-Compartment Kinetics of Antibiotics by In Vivo Microdialysis in Humans

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The calculation of pharmacokinetic/pharmacodynamic surrogates from concentrations in serum has been shown to yield important information for the evaluation of antibiotic regimens. Calculations based on concentrations in serum, however, may not necessarily be appropriate for peripheral-compartment infections. The aim of the present study was to apply the microdialysis technique for the study of the peripheral-compartment pharmacokinetics of select antibiotics in humans. Microdialysis probes were inserted into the skeletal muscle and adipose tissue of healthy volunteers and into inflamed and noninflamed dermis of patients with cellulitis. Thereafter, volunteers received either cefodizime (2,000 mg as an intravenous bolus; $n = 6$), cefpirome (2,000 mg as an intravenous bolus; $n = 6$), fleroxacin (400 mg orally $n = 6$), or dirithromycin (250 mg orally; $n = 4$); the patients received phenoxymethylpenicillin (4.5×10^6 U orally; $n = 3$). Complete concentration-versus-time profiles for serum and tissues could be obtained for all compounds. Major pharmacokinetic parameters (elimination half-life, peak concentration in serum, time to peak concentration, area under the concentration-time curve [AUC], and AUC/MIC ratio) were calculated for tissues. For cefodizime and cefpirome, the AUC_{tissue}/AUC_{serum} ratios were 0.12 to 0.35 and 1.20 to 1.79, respectively. The AUC_{tissue}/AUC_{serum} ratios were 0.34 to 0.38 for fleroxacin and 0.42 to 0.49 for dirithromycin. There was no visible difference in the time course of phenoxymethylpenicillin in inflamed and noninflamed dermis. We demonstrated, by means of microdialysis, that the concept of pharmacokinetic/pharmacodynamic surrogate markers for evaluation of antibiotic regimens originally developed for serum pharmacokinetics can be extended to peripheral-tissue pharmacokinetics. This novel information may be useful for the rational development of dosage schedules and may improve predictions regarding therapeutic outcome.

The selection of an antimicrobial agent for the treatment of an infection is usually based on information given by the MICs and the time-versus-concentration profile of the drugs in serum (i.e., pharmacokinetic data). Thus, when possible, individual drug doses and dosing intervals are tailored to achieve concentrations in serum that are above the MIC throughout the dosing interval. The MIC, however, is only one of the factors that need to be considered for the selection of an appropriate antimicrobial agent and dosing regimen. Several pharmacokinetic surrogate markers, e.g., peak concentration of drug in serum (C_{max})/MIC ratio, area under the inhibitory curve (area under the concentration-time curve [AUC]/MIC ratio), and time above the MIC ($T >$ MIC), have been examined for various classes of antibiotics, and it has become clear that clinical outcome is determined by a complex combination of pharmacokinetic and microbiological parameters (11).

Prediction of clinical outcome by pharmacokinetic surrogate markers has almost exclusively been performed by recalculation from concentrations in serum (11, 27). However, this approach is appropriate only for conditions in which the central compartment is the main site of infection, e.g., septicemia or endocarditis, but it may not necessarily be appropriate for infections of extravascular compartments, e.g., soft-tissue infections. In these cases the pharmacokinetic profile in tissue rather than in serum determines the clinical outcome of antimicrobial therapy. Hence, dosing regimens based on target-tissue kinetics would be desirable, but mostly because of methodological limitations, characterization of complete time-versus-tissue concentration profiles of drugs at the site of infection is not readily feasible.

The concentrations of antibiotic agents in tissues have typically been estimated by extrapolation from algorithms based on the level in plasma (27). This approach has clear limitations since it can serve only as a surrogate for direct measurement of the concentrations in tissues. Several experimental approaches are available for the quantification of concentrations of antibiotics in tissues, e.g., the skin blister fluid method (22), magnetic resonance imaging (13), positron emission tomography (8), or biopsy and then tissue homogenization (24), each of which shares inherent limitations for tissue pharmacokinetic studies.

Recently, the microdialysis technique has been adapted for clinical studies (4, 16, 18–21). Microdialysis (33) is an in vivo sampling technique for the continuous monitoring of analytes in the fluid of the interstitial space, the compartment where the majority of infections are localized (25). This technique provides the unique opportunity to obtain near-complete concentration profiles of drugs in anatomically clearly defined tissues and organs. Thus, a description of pharmacokinetic parameters for peripheral compartments and measurement of ratios of the concentration in tissue/concentration in serum as a measure of drug penetration becomes possible. This can provide a novel way of assessing peripheral compartment kinetics, thereby supporting the selection of appropriate antimicrobial therapy.

In the present study, the serum and interstitial tissue fluid kinetics of select antibiotics were measured by microdialysis in

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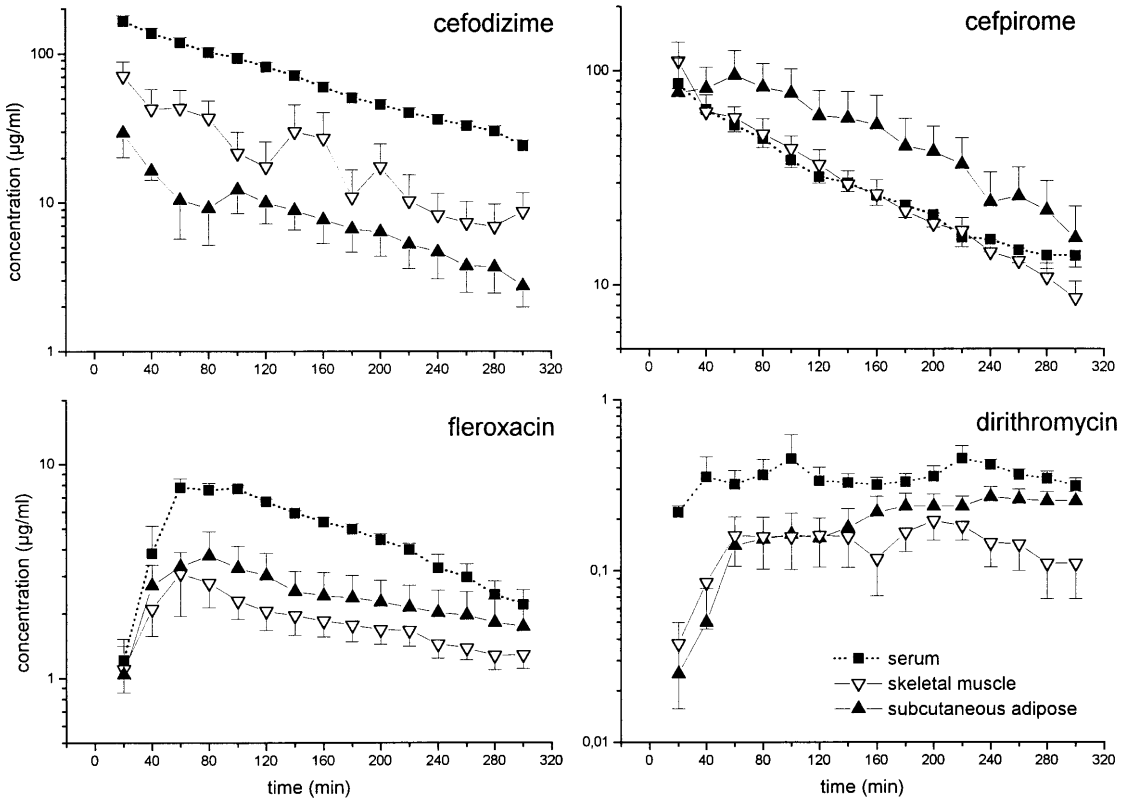

FIG. 1. Time versus concentration in serum and interstitial fluid for muscle and subcutaneous adipose tissue following the administration of cefodizime (single i.v. dose of 2 g over 5 min; $n = 6$), cefpirome (single i.v. dose of 2 g over 5 min; $n = 6$), fleroxacin (single oral dose of 400 mg; $n = 6$), or dirithromycin (single oral dose of 250 mg; $n = 4$) for healthy volunteers. Results are presented as means ± standard errors. The drugs were administered at time zero.

healthy volunteers and a limited number of patients with cellulitis. The study drugs were selected on the basis of previous studies reporting good deep-compartment penetration for the beta-lactams cefodizime (1, 15), cefpirome (14), and phenoxymethylpenicillin (31), the quinolone fleroxacin (6, 23), and the macrolide dirithromycin (3), which renders them suitable for the treatment of localized tissue infections. However, data on the pharmacokinetics of these drugs in tissues are scarce for humans and rely on one-point measurements of concentrations in tissue biopsy specimens (6), animal studies (1), or measurements of concentrations in skin blister fluid (15). Data derived from our experiments may be useful for the identification of drugs with optimal tissue penetration rates and, more generally, for critically reassessing dosage regimens for antibiotic therapy.

MATERIALS AND METHODS

The study was approved by the local ethics committee. All volunteers and patients were given a detailed description of the study, and their written consent was obtained. The study was performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines of the European Commission (EC-GCP guideline).

Healthy volunteers. The study population included six healthy male volunteers (ages 21 to 32 years; weight, 82.8 ± 7.2 [standard deviation] kg). Each subject passed a screening examination including history and physical examination; a 12-lead electrocardiographic examination complete blood count with differential; urinalysis; a urine drug screen; tests for levels of serum electrolytes, bilirubin (total and conjugated), blood urea nitrogen, creatinine, cholesterol, γ-glutamine transferase, glucose, inorganic phosphate, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, total protein, triglycerides, and uric acid; activated prothrombin time, normotest and thrombin time; and fibrinogen, hepatitis B virus surface antigen, and human immunodeficiency virus antibody tests. Subjects were excluded from the study if they had taken any prescription medication or nonprescription drugs within a period of 2 weeks prior to the study. For each study day, volunteers fasted for 12 h prior to the start of the experiments.

Patients. Three patients (one female and two males) who were admitted to the dermatology ward of the University Hospital Vienna because of a clinically confirmed diagnosis of cellulitis of a lower extremity and who were scheduled to receive systemic antibiotic therapy were studied.

Microdialysis. The principles of microdialysis have previously been described in detail (16, 33). Briefly, microdialysis is based on the sampling of analytes from the interstitial space with a semipermeable membrane at the tip of a microdialysis probe. The probe is constantly perfused with a physiological solution (perfusate) at a flow rate of 0.5 to 10 μl/min. Once the probe is implanted into the tissue, substances present in the interstitial fluid at a detectable concentration (C_{tissue}) are filtered by diffusion out of the interstitial fluid into the probe, resulting in a concentration ($C_{dialysate}$) in the perfusion medium. The samples are collected and analyzed. For most analytes, equilibrium between interstitial tissue fluid and the perfusion medium is incomplete; therefore, C_{tissue} is greater than $C_{dialysate}$. The factor by which the concentrations are interrelated is termed recovery.

Assessment of microdialysis probe recovery. (i) In vitro experiments. Prior to designing the in vivo experiments, the correlation between drug concentrations in the dialysate and drug concentrations in a surrounding medium was studied for each drug. Dialysis probes were placed in glass beakers containing different concentrations of antibiotics (0.5 to 300 μg/ml). The probes were perfused at a flow rate of 1.5 μl/min at room temperature (20°C). The drug concentrations in the dialysate were measured and were expressed as the percentage of the concentration in the surrounding medium. In vitro recoveries were concentration independent over a wide concentration range for all study drugs, as indicated by linear regression ($r > 0.95$). In vitro recovery was 28% for cefodizime, 47% for cefpirome, 46% for fleroxacin, 71% for dirithromycin, and 64% for phenoxymethylpenicillin.

(ii) In vivo experiments. To obtain absolute concentrations in the interstitial fluid from the concentrations in the dialysate, the microdialysis probes were calibrated for in vivo recovery rates by the retrodialysis method (18). The principle of this method relies on the assumption that the diffusion process is quantitatively equal in both directions through the semipermeable membrane. Therefore, the study drug was added to the perfusate at a concentration of 5 to 150 μg/ml (150 μg/ml for cefodizime, 50 μg/ml for cefpirome, 30 μg/ml for

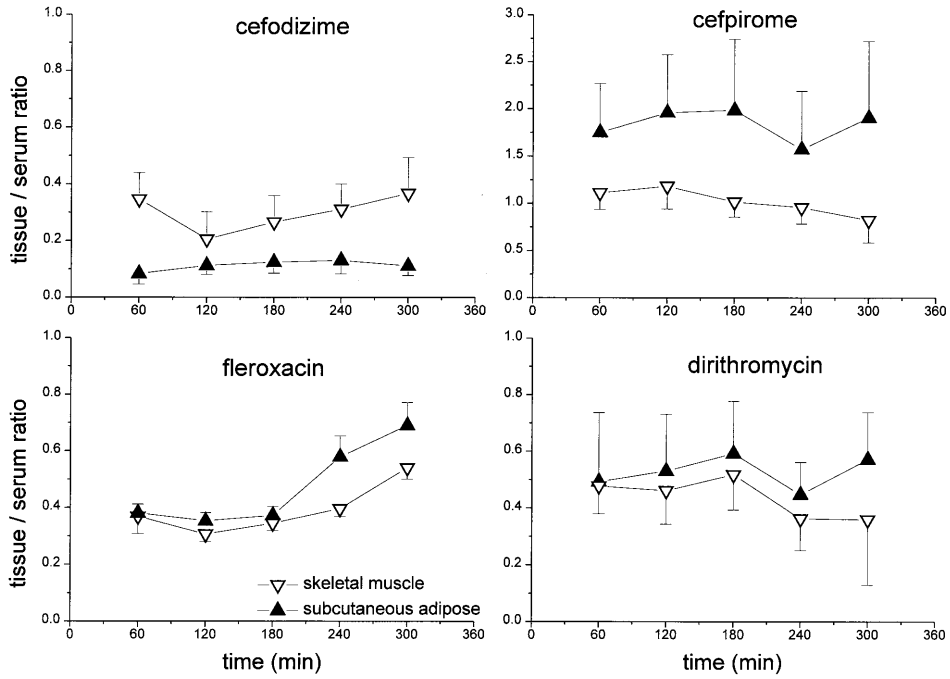

FIG. 2. Time course of the ratios of the concentration in interstitial fluid of muscle or subcutaneous to the concentration in serum for cefodizime, cefpirome, fleroxacin, and dirithromycin for the experiments whose results are presented in Fig. 1. The results are presented as means ± standard errors. The drugs were administered at time zero.

fleroxacin, and 5 μg/ml for dirithromycin), and the disappearance rate through the membrane was taken as the in vivo recovery rate. The in vivo recovery value was calculated as recovery (percent) = 100 − (100 · analyte concentration$_{\text{dialysate}}$ · analyte concentration$_{\text{perfusate}}^{-1}$)

Study drugs. Healthy volunteers received cefodizime (Timecef; Albert Rousell Pharma, Vienna, Austria) and cefpirome (Cefrom; Albert Rousell Pharma) as a single intravenous (i.v.) dose of 2 g over 5 min (mean dose, 25.0 ± 4.4 mg/kg), fleroxacin (Quinodis; Hoffmann-La Roche, Vienna, Austria) as a single oral dose of 400 mg (mean dose, 5.0 ± 0.9 mg/kg), and dirithromycin (Dimac; Lilly, Vienna, Austria) as a single oral dose of 250 mg (mean dose, 3.1 ± 0.6 mg/kg). Each of the six volunteers received cefodizime, cefpirome, fleroxacin, and dirithromycin according to a randomized crossover design once on separate study days with a minimum washout period of 14 days. For technical reasons, data from only four experiments with dirithromycin were available for analysis.

Patients received a single oral dose of 4.5×10^6 U of phenoxymethylpenicillin (Ospen; Biochemie, Vienna, Austria). Each patient was studied only once, immediately after administration of the first dose.

Study protocol. The volunteers were in a supine position throughout the study period. A plastic cannula (Venflon) was inserted into an antecubital vein to monitor the concentrations of the antibiotics in serum at 20-min intervals. The skin at the site of microdialysis probe insertion was cleaned and disinfected. One probe was inserted into a medial vastus muscle and one was inserted into the periumbilical subcutaneous layer without anesthesia by a previously described procedure (18). Subsequently, the microdialysis system was connected and perfused with Ringer's solution at a flow rate of 1.5 μl/min with a microinfusion pump (Precidor; Infors-AG, Basel, Switzerland). After a 30-min baseline sampling period, in vivo probe calibration was performed in the study with the healthy volunteers as described above for a period of 30 min, and two 15-min samples were taken. In vivo recovery was calculated on the basis of the mean value for the two 15-min samples. Prior to the administration of exogenous drugs at the doses mentioned above, the perfusate was changed to Ringer's solution and the system was flushed for 30 min. Sampling was continued at 20-min intervals for up to 5 h.

For the study with patients, procedures were carried out as described for the study with healthy volunteers. However, one dialysis probe was inserted at the site of the dermatological lesion into the inflamed dermis and one was inserted into the unaffected dermis at the corresponding site of the contralateral leg.

Analyses. For the analysis of cefodizime and cefpirome in serum and dialysates, a microagar diffusion bioassay (9) with a detection limit of 0.075 μg/ml was used. Inter- and intraassay coefficients of variation were <5% in the concentration range of 1 to 100 μg/ml. Serum and dialysate fleroxacin and dirithromycin concentrations were measured by reversed-phase high-pressure liquid chromatography on unmodified silica with fluorescence detection (for fleroxacin, emission at 445 nm and extinction at 277 nm; detection limit, 50 ng/ml) or UV detection (for dirithromycin, at 277 nm; detection limit, 2.5 ng/ml), respectively. Inter- and intraassay coefficients of variation were <5% in the concentration range of 0.1 to 10 μg/ml. The dialysates were analyzed directly without prior extraction.

Calculations and data analysis. (i) Calculations for microdialysis experiments. Absolute concentrations in interstitial fluid were calculated from the concentrations in dialysates by the following equation: concentration in interstitial fluid = 100 · concentration in sample · in vivo recovery value^{-1}. For the study with healthy volunteers, the concentrations obtained with the probes were corrected for in vivo recovery; therefore, the results are given as concentrations in interstitial fluid. For the study with patients, the concentrations obtained with the probes were not corrected for recovery; therefore, the results are given as concentrations in dialysates.

Pharmacokinetic calculations. Data were fitted by a two-compartment model to the following equation for concentrations in serum: $C\ (t) = B\ e^{-K_\alpha t} + A\ e^{-K_1 t}$, where K_α is the elimination rate constant for serum, K_1 is the elimination rate constant for the second compartment, i.e., muscle or subcutaneous tissue, B is the back extrapolated intercept with the ordinate for the alpha phase, A is the back extrapolated intercept with the ordinate for the second compartment, C is concentration, and t is time. Data for concentrations in tissues were fitted by a one-compartment model to the following equation: $C\ (t) = A\ e^{-K_1 t} - C\ e^{-K_a t}$, where K_a is the absorption rate constant and the other abbreviations are as defined above. Subsequently, the transfer rate constant from the central compartment to the peripheral compartment (k_{12}) was calculated by the equation $K_\alpha + K_1 = k_{12} + k_{21} + k_{13}$ from $K_\alpha \cdot K_1 = k_{21} \cdot k_{13}$, where k_{21} is the rate constant from the peripheral compartment to the central compartment and k_{13} is the rate constant of excretion from the central compartment. AUCs from time zero to 5 h (AUC_{0-5} [in micrograms · minute · milliliter^{-1}]) for individual drugs were determined for serum and tissues by the trapezoidal rule. Single-dose data were extrapolated to a multiple-dose situation given a dose interval (τ) of 6 h according to the equation $C_{ss} = AUC/\tau$, where C_{ss} is the concentration at steady state. Penetration ratios for tissues were determined as AUC_{tissue}/AUC_{serum} and $C_{max\text{-}tissue}/C_{max\text{-}serum}$. For cefodizime and cefpirome, the terminal half-life of elimination ($t_{1/2\beta}$) was calculated by a direct fit (nonlinear computer-assisted iteration) by the least-squares curve-fitting equation to the entire curve describing a monoexponential decay (using a Gauss-Newton algorithm). Since the elimination phase was not reached during the observation period for fleroxacin ($t_{1/2\beta}$ = 14 h [10]) and dirithromycin ($t_{1/2\beta}$ = 44 h [29]), $t_{1/2\beta}$, C_{ss}, and k_{12} were not calculated for these drugs. The area under the inhibitory curve values (AUC/MIC ratio [in micrograms · minute · milliliter^{-1}]) were calculated for tissues by the following equation (32):

$$\text{AUC/MIC ratio} = \int_{t_{mic1}}^{t_{mic2}} \text{concentration in tissue} \cdot dt - \int_{t_{mic1}}^{t_{mic2}} \text{MIC}\ dt$$

TABLE 1. Pharmacokinetic parameters for serum, skeletal muscle, and subcutaneous adipose tissue following administration of cefodizime, cefpirome, fleroxacin, or dirithromycin to healthy volunteers[a]

Drug	C_{max} (μg/ml)			T_{max} (min)[b]			AUC (μg · min · ml^{-1})		
	Muscle	Adipose tissue	Serum	Muscle	Adipose tissue	Serum	Muscle	Adipose tissue	Serum
Cefodizime	79.1 ± 35.5	31.2 ± 21.3	164.8 ± 38.9	26.6 ± 16.4	26.6 ± 16.4	20.0 ± 0.0	7,656 ± 4,424	2,740 ± 1,362	21,507 ± 3,963
Cefpirome	121.3 ± 47.5	111.5 ± 74.5	86.8 ± 11.8	30.0 ± 16.7	33.3 ± 20.6	20.0 ± 0.0	10,563 ± 2,795	15,923 ± 12,074	8,983 ± 1,617
Fleroxacin	3.02 ± 1.67	3.39 ± 1.30	8.98 ± 0.64	68.0 ± 25.0	63.3 ± 14.9	70.0 ± 16.7	529 ± 228	593 ± 260	1,384 ± 140
Dirithromycin	0.20 ± 0.08	0.20 ± 0.12	0.55 ± 0.28	200.0 ± 16.4	160.0 ± 56.6	200.0 ± 67.4	41.7 ± 21.4	47.4 ± 27.4	99.2 ± 26.8

[a] Data are means ± standard deviations. See text for dosages and administration schedules.
[b] T_{max}, time to C_{max}.
[c] Significant difference for drug penetration ratio between tissues ($p < 0.05$).

where t_{mic1} (in minutes) is the time point at which the antibiotic concentration equals MIC for the first time following drug administration, and t_{mic2} (in minutes) is the time point at which the antibiotic concentration equals the MIC for the last time following administration. AUC/MIC ratios were calculated on the basis of the following MICs at which 90% of strains are inhibited (MIC_{90}s) for methicillin-susceptible *Staphylococcus aureus*, an important isolate from soft-tissue infections: cefodizime, 8 μg/ml (30); cefpirome, 0.5 μg/ml (12); fleroxacin, 0.5 μg/ml (5); and dirithromycin, 4 μg/ml (2).

Statistical calculations. For comparison of the different penetration rates into skin and muscle, *t* tests for paired observations were performed. A *P* value of <0.05 was considered the level of significance. All data are presented as means ± standard deviations.

RESULTS

In vivo recovery measurements. In vivo recovery values measured by the retrodialysis method for human skeletal muscle were 89% for dirithromycin, 55% for fleroxacin, 34% for cefodizime, and 30% for cefpirome. In vivo recovery values for subcutaneous adipose tissue were 91% for dirithromycin, 47% for fleroxacin, 39% for cefodizime, and 33% for cefpirome.

Kinetics in serum, muscle, and subcutaneous adipose tissue (volunteer study). The results from experiments in which probes were inserted simultaneously into the medial vastus muscle and into the periumbilical subcutaneous adipose tissue of healthy volunteers are presented in Fig. 1. The time courses of the ratios of the concentration in tissue interstitial fluid/concentration in serum ratios are presented in Fig. 2. Pharmacokinetic data are given in Table 1. k_{12} for muscle was 0.4705 ± 0.0243 for the experiments with cefpirome and 0.3935 ± 0.1117 for the experiments with cefodizime. k_{12} for subcutaneous adipose tissue was 0.4770 ± 0.0153 for the experiments with cefpirome and 0.3718 ± 0.0924 for the experiments with cefodizime. C_{ss} for muscle was 29.3 ± 7.8 μg/ml for the experiments with cefpirome and 21.3 ± 12.3 μg/ml for the experiments with cefodizime. C_{ss} for subcutaneous adipose tissue was 44.2 ± 32.5 μg/ml for the experiments with cefpirome and 7.6 ± 3.5 μg/ml for the experiments with cefodizime.

Kinetics in serum, cellulitic tissue, and subcutaneous adipose tissue (patient study). The results from experiments in which probes were inserted simultaneously into the inflammatory lesion and into the subcutaneous adipose tissue of patients to compare the pharmacokinetics of phenoxymethylpenicillin in inflamed and noninflamed tissues are presented in Fig. 3.

DISCUSSION

In the present study we estimated by means of microdialysis key pharmacokinetic parameters of select antimicrobial drugs in anatomically clearly defined peripheral compartments, i.e., skeletal muscle, subcutaneous adipose tissue, and inflamed and noninflamed dermis. In addition, by relating data for tissue to serum pharmacokinetics, the kinetics of the distribution of these antimicrobial drugs into these tissues could be characterized much more precisely than by estimation from concentrations in serum alone.

For the beta-lactams cefodizime and cefpirome, which are distributed predominantly in the interstitial space (7, 26), the AUCs for concentrations in interstitial fluid after i.v. administration were 12 to 35% and 120 to 179% of the corresponding concentrations in serum respectively. These results are in agreement with those from previous studies reporting concentrations in skin blister fluid of about 31 to 54% and about 123% of the corresponding concentrations in serum for cefodizime (15) and cefpirome (14), respectively. However, the high AUC/penetration ratio for cefpirome must be highlighted as an unexpected and unexplained finding. Given a protein binding of 80% for cefodizime and of 10% for cefpirome, approximately 60 to 175% (cefodizime) and 130 to 200% (cefpirome) of the corresponding free concentrations in serum, respectively, were reached in interstitial fluid. There were considerable differences in the concentration profiles and the equilibration rates between muscle and subcutaneous adipose tissue for both compounds (Fig. 2). Cefodizime penetration into skeletal muscle, as expressed by penetration ratios, was significantly higher ($P < 0.02$) than that into subcutaneous tissue. The opposite was true for cefpirome. Cefpirome penetration from serum into subcutaneous interstitial fluid was fast and resulted in concentrations in interstitial fluid exceeding the concentrations in serum at 40 min postdosing. Thus, even when antibiotics share similar physicochemical properties, the penetration into the interstitial fluid of various tissues may vary considerably. Differences in the rate and extent of drug penetration into specific tissues, such as the differences between cefodizime and cefpirome, may reflect differences in protein or tissue binding or transcapillary drug transport.

For the beta-lactams the $T > MIC$ was shown to be the most appropriate surrogate marker for predicting clinical outcome (11). In our experiments the free cefodizime concentrations in the interstitial fluid of muscle and subcutaneous adipose tissue were above the MIC_{90} for *Streptococcus pyogenes* (0.04 μg/ml) during the entire study period. For *S. aureus* (MIC_{90}, 8 μg/ml), however, inhibitory concentrations were maintained in subcutaneous tissue only until 140 min ($T > MIC = 140$ min) postadministration. Cefpirome concentrations in muscle and subcutaneous adipose tissue were above the MIC_{90}s for *S. pyogenes* (0.12 μg/ml) and *S. aureus* (0.5 μg/ml), the most important causative agents of soft-tissue infections, during the entire study period ($T > MIC > 300$ min). Thus, on the basis of our findings, cefpirome may be superior to cefodizime for the treatment of soft-tissue infections, at least from a pharmacokinetic perspective.

For fleroxacin, which is not only distributed in the extracellular space but whose concentration also reaches equilibrium

TABLE 1—*Continued*

AUC/MIC ($\mu g \cdot min \cdot ml^{-1}$)			$t_{1/2\beta}$ (min)			AUC_{muscle}/AUC_{serum}	$AUC_{adipose\ tissue}/AUC_{serum}$	C_{max} in muscle/C_{max} in serum	C_{max} in adipose tissue/C_{max} in serum
Muscle	Adipose tissue	Serum	Muscle	Adipose tissue	Serum				
5,637 ± 4,159	1,248 ± 813	17,440 ± 3,338	102 ± 7	132 ± 105	106 ± 15	0.35 ± 0.17[c]	0.12 ± 0.05[c]	0.48 ± 0.17[c]	0.48 ± 0.10[c]
10,413 ± 2,795	15,790 ± 12,086	8,833 ± 1,617	92 ± 10	92 ± 22	103 ± 17	1.20 ± 0.34	1.79 ± 1.32	1.38 ± 0.49	1.32 ± 0.91
379 ± 509	443 ± 260	1,233 ± 142				0.34 ± 0.20	0.38 ± 0.20	0.38 ± 0.15	0.42 ± 0.15
0 ± 0	0 ± 0	0 ± 0				0.42 ± 0.20	0.49 ± 0.32	0.39 ± 0.18	0.41 ± 0.26

with that in the intracellular space (34), the concentrations in the interstitial fluid of muscle and subcutaneous tissue were about 40% of the corresponding concentrations in serum at 1 h and about 60% at 5 h following oral administration. Thus, an increase in the concentration in tissue/concentration in serum ratios over time could be observed. Given a level of protein binding of 25% for fleroxacin (10), approximately 50 to 80% of the corresponding free concentrations in serum were reached in the interstitial fluid. In contrast to the beta-lactams, the time course of the concentration in subcutaneous adipose tissue closely resembled the time course in muscle, although the concentrations in muscle were somewhat lower (Fig. 1). However, we were not able to confirm previous results reporting concentration in whole tissue/concentration in plasma ratios of >1 for fleroxacin (6). This discrepancy may be explained by the short observation period of our experiments (5 h postdosing) in comparison with those of other studies and may also reflect the slow equilibration of fleroxacin within the intracellular compartment, leading to concentration in the intracellular compartment/concentration in the extracellular compartment ratios of >5 (34). Furthermore, previous studies were grinding tissue studies and concentrations in the intracellular compartment were measured. The concentrations in the intracellular compartment, however, are not detectable by microdialysis.

For quinolone agents the ratio of C_{max} to MIC is considered to be the most relevant surrogate marker and proved to be predictive of bacterial eradication (11). Although a 99% killing can be obtained by quinolones at a low ratio, e.g., 3 for ciprofloxacin, bacterial regrowth and development of bacterial resistance may occur unless higher ratios, e.g., 8 for ciprofloxacin, are reached (11). In our experiments a C_{max}/MIC ratio for methicillin-susceptible *S. aureus* of 18 was obtained for serum and a ratio of 6 was obtained for muscle and subcutaneous tissue. C_{max}/MIC ratios may thus differ significantly between the central and peripheral compartments, reaching effective ratios in one and ineffective ratios in another.

For dirithromycin, concentrations in interstitial fluid were about 40 to 60% of the corresponding concentrations in serum following oral administration. Given a level of protein binding of 30% for dirithromycin (29), concentrations approximately 60 to 85% of the corresponding free concentrations in serum were reached in the interstitial fluid. Again, the time course of the concentration in subcutaneous adipose tissue resembled the time course of the concentration in muscle, at least during the first 2 h (Fig. 1). Dirithromycin concentrations in serum and interstitial fluid of muscle and subcutaneous adipose tissue were below the MIC_{90} for methicillin-susceptible *S. aureus* (MIC_{90}, 4 μg/ml) during the entire observation period. Previous studies reported concentrations in tissue up to 40 times higher than corresponding concentrations in serum at 12 to 24 h after the administration of the last oral dose (3). This discrepancy of our findings in comparison with the results obtained for whole-tissue homogenates was even more striking than the discrepancy described above for fleroxacin. Like fleroxacin, dirithromycin slowly accumulates by a calcium-dependent uptake in the intracellular compartment reaching concentration in the intracellular compartment/concentration in the extracellular compartment ratios of up to 47 (17) and thereby exerting high levels of activity against intracellular pathogens. Microdialysis measurements which selectively mirror the concentrations in the extracellular space may thus underestimate the total concentrations of antibiotics that accumulate in the intracellular space of tissues. Although microdialysis seems ideal for drugs that distribute exclusively in the interstitial space, like beta-lactams, it may be inferior to other techniques like biopsy tissue sampling for antibiotics like dirithromycin or fleroxacin. In conjunction with biopsy specimens, however, microdialysis may provide information on concentration in the intracellular compartment/concentration in the extracellular compartment ratios of antibiotics. However, biopsy data need to be interpreted with extreme caution. For drugs like beta-lactams, they underestimate concentrations in the interstitial fluid. For drugs like fluoroquinolones and macrolides, they overestimate the concentrations in the interstitial fluid because of the concentrated admixture of intracellular drug and underestimates the concentration in the intracellular compartment because of the admixture of lower concentration in interstitial fluid.

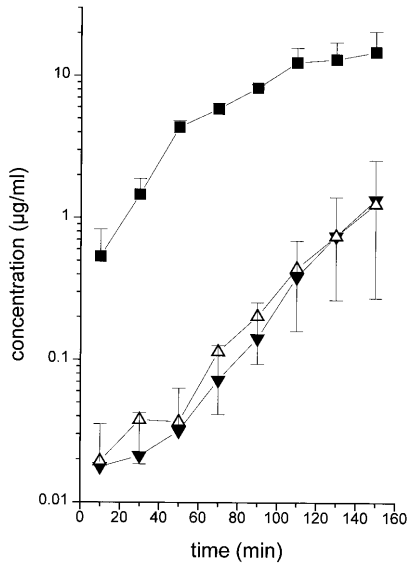

FIG. 3. Time versus concentration in serum (closed squares) and interstitial fluid dialysate of inflamed (open triangles) and noninflamed (closed triangles) dermis for phenoxymethylpenicillin following the administration of a single oral dose of 4.5×10^6 U of phenoxymethylpenicillin ($n = 3$). Results are presented as means ± standard errors. The drugs were administered at time zero.

The penetration of antibiotics into inflamed and noninflamed tissues may differ considerably. To study the influence of inflammation on drug penetration into tissue, we conducted experiments with a small number of patients with cellulitis following the administration of a single oral dose of phenoxymethylpenicillin. In these experiments the time to C_{max} for phenoxymethylpenicillin was not reached in serum, probably because of the nonfasting state of the patients. Phenoxymethypenicillin concentrations in inflamed and noninflamed dermis dialysates were approximately 10% of the corresponding concentrations in serum during the entire observation period. There was no visible difference in the concentration profile and equilibration rates between inflamed and noninflamed dermis; however, because of the small sample size, our study had an inadequate statistical power to detect small differences in concentration profiles. Nevertheless, these preliminary findings do not support the concept that antibiotic penetration into cellulitic tissue differs considerably from penetration into normal dermis.

Pharmacokinetic and pharmacodynamic surrogates have been shown to be important markers for the evaluation of antibiotic regimens and the prediction of the clinical outcome of antimicrobial therapy (11). In the present study we were able to demonstrate that, by microdialysis, this concept, originally developed for serum pharmacokinetics, can also be applied to peripheral target tissues. This may be of particular importance for those cases in which therapeutic efficacy cannot be predicted on the basis of in vitro data. Differences in clinical outcome may be due to different susceptibilities of the microorganisms or the impaired permeation of antibiotics to the target site. By comparison of penetration ratios (AUC_{tissue}/AUC_{serum}, k_{12}) for different antibiotics and by calculation of pharmacokinetic surrogates for target tissues (e.g., C_{ss}), microdialysis measurements may provide useful information.

In comparison with traditional attempts to measure antibiotic concentrations in tissues, like mathematical modeling from time courses in plasma or skin blister fluid or biopsy tissue sampling, microdialysis offers several advantages. Microdialysis is only minimally invasive and provides information about a clearly defined anatomical compartment, the interstitial space, an important compartment for the growth of bacteria (27). Technically, microdialysis may be applied to kinetic studies of all tissues and organs that are accessible to ultrasound-controlled puncture. For many organs and tissues of interest, including the brain (28), such studies are also ethically acceptable under special circumstances. Furthermore, microdialysis provides a relatively high temporal resolution, which is of particular importance for antibiotics with rapid distribution and elimination kinetics. Particularly for antibiotics which distribute predominantly in the interstitial fluid, like beta-lactams, microdialysis is superior to whole-tissue measurements, which substantially underestimate the actual concentrations present in the interstitial fluid (7, 26). Differences in tissue-specific kinetics, as was demonstrated for cefodizime and cefpirome, cannot be detected by a calculation of peripheral compartment kinetics merely based on the concentration in serum or skin blister sampling, but they are detectable by microdialysis.

A major limitation of the microdialysis technique for antibiotic studies is the requirement for sensitive analytical techniques because of the small sample volumes and the low concentrations obtained by microdialysis. The duration of microdialysis experiments is limited by the inconvenience caused to the experimental subject, particularly the requirement of resting in a supine position. This may be a limiting factor for studies of drugs with long $t_{1/2\beta}$s.

In conclusion, we were able to demonstrate by microdialysis that the concept of pharmacokinetic/pharmacodynamic surrogate markers for the evaluation of antibiotic regimens can be extended to peripheral-tissue pharmacokinetics. Such information may be useful for the rational development of dosage schedules, and it may improve predictions regarding therapeutic outcomes.

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