Pharmacokinetics of Single- and Multiple-Dose Oral Clarithromycin in Soft Tissues Determined by Microdialysis[∇]

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The antimicrobial spectrum of clarithromycin renders this antibiotic a frequently used option in the treatment of skin and soft-tissue infections. In most cases, these infections are caused by extracellularly proliferating microorganisms. Thus, clarithromycin concentrations achieved in the interstitial space are considered particularly important for clinical efficacy. In the present study, clarithromycin concentrations in plasma and interstitial-space fluid of subcutaneous adipose tissue and skeletal muscle of six healthy male volunteers were assessed by means of the microdialysis technique after oral single-dose administration of 250 mg and multiple doses of 500 mg of clarithromycin twice a day (b.i.d.). The ratios of the area under the concentration-time curve of free clarithromycin from 0 to 24 h calculated for a single dose of 250 mg ($fAUC_{0-24}$) in interstitial-space fluid to the $fAUC_{0-24}$ in plasma were 0.29 ± 0.17 and 0.42 ± 0.18 for subcutis and skeletal muscle, respectively. For 500 mg of clarithromycin at the steady state (3 to 5 days of intake twice daily), the $fAUC_{0-24(b.i.d.)}$ ratios at the steady state were 0.39 ± 0.04 and 0.41 ± 0.19 for subcutis and skeletal muscle, respectively. The half-life was around 2 h after a single dose but increased to approximately 4 h in plasma and tissues after repetitive clarithromycin administration. Based on subsequently performed pharmacokinetic-pharmacodynamic calculations, a dosing regimen of 500 mg b.i.d. may be ineffective in the treatment of soft-tissue infections caused by pathogens with a drug MIC higher than 0.125 mg/liter.

Clarithromycin, a 14-membered ring macrolide, is antimicrobially active against a broad range of gram-positive and certain gram-negative pathogens frequently isolated from soft-tissue infections and bite wounds (11). Clarithromycin is considered a therapeutic alternative in special cases of minor soft-tissue infections and penicillin allergy or in cases of nontuberculous mycobacterial skin infections (19, 20). High tissue concentrations of the class of the macrolides have been reported previously in the literature (10, 13, 21). Indeed, intracellular accumulation of macrolides in isolated peripheral blood phagocytes, alveolar macrophages, and tissue culture cells of human origin has been demonstrated previously (9, 16). To date, investigations of in vivo tissue pharmacokinetics (PK) of clarithromycin have been confined to concentrations derived from homogenized biopsy samples of the upper and lower respiratory tract and epithelial lining fluid collection obtained by bronchoalveolar lavage (10, 13, 21). The results derived from homogenized biopsy samples, as frequently used in previous studies, represent an average concentration of all tissue components extracted, including blood cells, intracellular fluid, interstitial fluid, and structural tissue components, and may therefore cause confusion with regard to the actual concentration of an antimicrobial agent in a defined compartment. These data, thus, provide only limited insight into the time course of concentration at the relevant site of most bacterial infections, namely, the extracellular-space fluid. Hence,

we used the microdialysis technique, which is capable of the continuous assessment of unbound, i.e., microbiologically active concentrations of clarithromycin in the interstitial-space fluid of soft tissues (15).

Knowledge about the concentration-time profiles of free clarithromycin in the interstitial-space fluid of soft tissues can be considered a prerequisite for dosage recommendations in the treatment of extracellularly proliferating bacteria causing soft-tissue infections. Hence, the aim of the present study was to determine free interstitial concentrations of clarithromycin in subcutaneous adipose tissue and skeletal muscle after oral single- and multiple-dose administration to healthy male volunteers.

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 the study was performed in accordance with the Declaration of Helsinki 1964 (including current revisions), the Austrian Drug Law, and the Good Clinical Practice Guidelines.

Healthy volunteers. Seven healthy male volunteers between the ages of 25 and 37 years were enrolled into the study. Written informed consent was obtained from each volunteer prior to any study-related investigation or intervention. Each volunteer underwent a screening examination consisting of the following: medical history, physical examination, routine laboratory tests, heart rate, blood pressure, and a 12-lead electrocardiography. These assessments were performed prior to inclusion and after completion of the study. All volunteers were initially drug free and received standardized meals on study days and were instructed to avoid caffeine and grapefruit juice during the entire study period.

Study protocol. (i) Study day 1 (250 mg clarithromycin single dose). The volunteers were admitted to the clinical research ward in the morning of study day 1. A plastic cannula was inserted into an antecubital vein to monitor blood concentrations of clarithromycin at defined time points. Concentrations in interstitial-space fluid of skeletal muscle and subcutaneous adipose tissue were determined by microdialysis. The principle of microdialysis has been described

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TABLE 1. Main pharmacokinetic indices of clarithromycin following administration of a single oral dose of 250 mg ($n = 6$)^a

Compartment	AUC ₀₋₈ (mg · h/liter)	AUC ₀₋₂₄ ^b (mg · h/liter)	AUC ₀₋₂₄ tissue/ fAUC ₀₋₂₄ plasma	C _{max} (mg/liter)	t _{max} (h)	t _{1/2} β (h)	V _z (liters)	CL (liters/h)
Plasma (total)	3.63 ± 1.32	4.45 ± 1.94		1.09 ± 0.35	2.6 ± 0.5	1.9 ± 0.6	94.0 ± 25.0	37.2 ± 14.9
Plasma (free)	0.99 ± 0.28	1.21 ± 0.35		0.31 ± 0.14	2.6 ± 0.5	1.9 ± 0.6		
Subcutis	0.24 ± 0.16 ^c	0.35 ± 0.25 ^c	0.29 ± 0.17	0.07 ± 0.03	3.0 ± 0.5	2.4 ± 1.2		
Muscle	0.29 ± 0.14 ^c	0.48 ± 0.23 ^c	0.42 ± 0.18	0.08 ± 0.03	3.0 ± 0.3	3.1 ± 0.9		

^a Values represent means ± standard deviations.

^b Calculated for single dose of 250 mg. Abbreviations: C_{max}, maximum concentration of drug; t_{max}, time to maximum concentration; t_{1/2β}, half-life at β phase; AUC, area under the concentration-time curve; fAUC, AUC of free drug; V_z, volume of drug distribution at the terminal phase after single dose; CL, total clearance.

^c $P < 0.03$ compared to free plasma results.

previously in detail (15). In brief, a microdialysis probe with a molecular weight cutoff of 20,000 (CMA12; CMA/Microdialysis AB, Solna, Sweden) was inserted into one thigh muscle and into the subcutaneous adipose tissue at the ventrolateral side of the thigh under aseptical conditions by use of a guidance cannula. The probe was constantly perfused with Ringer's solution at a flow rate of 1.5 μl/min by means of a precision pump (Precidor; Infors-AG, Basel, Switzerland). After a 60-min equilibration period, 250 mg of clarithromycin (Klacid 250 mg tablet; Abbott, Abbott Park, IL) was administered orally to the fasting volunteer. Sampling of microdialysates and venous blood was performed at 20-min intervals from h 0 to 4 and at 30-min intervals from h 4 to 8. After completion of the 8-h sampling period, the individual recovery values of clarithromycin were determined by use of the "retrodialysis" method (3). For that reason, clarithromycin was added at a concentration of 5 mg/liter to the perfusion fluid and its rate of disappearance through the microdialysis membrane was determined. The individual recovery was calculated by using the mean of two measurements by the following equation: recovery (%) = $100 - (100 \times C_{\text{dialysate}}/C_{\text{perfusate}})$, where C represents the concentration. Blood was collected in tubes containing the lithium salt of heparin, kept on ice for a maximum of 30 min, and centrifuged at $1,600 \times g$ for 5 min at 4°C. Plasma and microdialysates were stored at minus 80°C until analysis.

At 12 h after the initial single dose of 250 mg, each volunteer continued oral intake of clarithromycin at a dosage of 500 mg twice a day (b.i.d.) for 3 to 5 days until the morning of study day 2.

(ii) **Study day 2 (steady state; 500 mg clarithromycin b.i.d.).** After clarithromycin intake b.i.d. over a period of 3 to 5 days, the last dose of 500 mg of clarithromycin was administered in the morning of study day 2 under the supervision of the study staff. Before the last dosage of clarithromycin was taken, a baseline blood sample was drawn and a microdialysate for the determination of the through concentrations in tissue was collected over 2 h. The setting of study day 2 was identical to that of study day 1.

Chemical analysis. Clarithromycin concentrations in plasma and microdialysates were analyzed by use of a validated high-performance liquid chromatography method (23), applying moderate modifications. Pure clarithromycin was a gift from Abbott (Abbott Laboratories, Abbott Park, IL). Pure roxithromycin and all other chemicals were purchased from Sigma-Aldrich (Steinheim, Germany). In brief, 150 μl plasma containing the internal standard roxithromycin (1 mg/liter) and 10 μl of 1 M sodium hydroxide were extracted with 2 ml of *tert*-butyl methyl ether. The organic layer was evaporated to dryness, and the residue was dissolved with 50 μl of the mobile phase. The mobile phase consisted of 0.05 M citrate buffer (pH 6.5) and acetonitrile (71:29 [vol/vol]). The flow rate was 0.450 ml/min. Separation was performed isocratically on a reverse-phase column (Synergi max RP; Phenomenex, Torrance, CA) (150 by 2 mm; particle size, 4 μm) at ambient temperature. Microdialysates were spiked with the internal standard at a final concentration of 0.05 mg/liter and analyzed without further preparation. Clarithromycin and roxithromycin in the eluent were detected with an amperometric detector (BAS; West Lafayette, IN) at +950 mV oxidation potential. The lower limits of quantification were 0.04 mg/liter and 0.012 mg/liter in plasma and microdialysates, respectively. Intraday and interday inaccuracy were <9%. Intraday and interday imprecision were <12%.

Protein binding studies. Protein binding of clarithromycin was determined individually for each volunteer. Aliquots of 300 μl plasma from samples drawn 80 and 180 min after administration of the drug (for both single doses and steady state) were ultrafiltrated by use of centrifugal filter units with a low-binding regenerated-cellulose membrane (Ultrafree-MC; Millipore Corp., Bedford, MA) (nominal relative molecular weight cutoff, 5,000) at $5,000 \times g$ for 30 min at ambient temperature. Ultrafiltrates were analyzed as described above for plasma. For determination of the binding of clarithromycin to the ultrafiltration

membrane during the filtration process, standards in Ringer's solution were ultrafiltrated and analyzed in the same way. The ultrafiltrate concentrations were subsequently corrected (corr) by the mean membrane binding of 5% ($C_{\text{ultrafiltrate corr}}$). The protein binding was calculated using the following equation: protein binding (%) = $100 - (100 \times C_{\text{ultrafiltrate corr}}/C_{\text{plasma total}})$.

Pharmacokinetic calculations and statistical analysis. The individual protein binding values were used for the determination of free clarithromycin concentrations in plasma. The absolute interstitial concentrations were calculated by use of the following formula: interstitial concentration = $100 \times (C_{\text{dialysate}}/\text{recovery})$.

Pharmacokinetic calculations were carried out by use of commercially available computer software (Kinetic, version 3.0; Innaphase, Philadelphia, PA). Concentrations at 12 h and 24 h were calculated by the following equation: $C = C_8 \times e - k_{el} \times t$, where C is the concentration at 12 h or 24 h, C_8 is the last concentration measured in vivo (at 8 h), k_{el} is the elimination rate constant, and t is the time difference between C_8 and C . The areas under the concentration-time curves from 0 to 8 h (AUC₀₋₈), 0 to 12 h (AUC₀₋₁₂), and 0 to 24 h (AUC₀₋₂₄) in plasma and interstitial fluid were calculated by use of the linear trapezoidal rule. For calculation of the total drug clearance (CL) and the apparent volume of drug distribution during the terminal phase after a single dose (V_z) and at the steady state (V_{ss}), the oral dose of clarithromycin was corrected for a bioavailability value (F) of 55% (6). CL, V_{ss} , and V_z of clarithromycin were calculated for plasma as follows: $CL = \text{dose} \times (F)/AUC_{0-\infty}$, where AUC_{0-∞} represents the AUC from 0 to infinity, $V_{ss} = CL \times MRT$ (mean residence time), and $V_z = \text{dose} \times (F)/(AUC_{0-\infty} \times k_{el})$. The AUC_{0-24(b.i.d.)} for 500 mg at the steady state was corrected for dosing twice daily by the equation $AUC_{0-24(500 \text{ mg b.i.d.})} = AUC_{0-12} \times 2$. Wilcoxon's paired test was used for comparison of AUC values in plasma and interstitial fluids within individuals. A two-sided P value of <0.05 was considered significant.

RESULTS

The present study set out to test the ability of clarithromycin to penetrate the interstitial-space fluid of subcutaneous adipose tissue and skeletal muscle in healthy volunteers. The results for one volunteer had to be excluded because plasma concentrations were almost 0, indicating noncompliance of the subject with respect to the study protocol. Thus, results of six volunteers were eligible for pharmacokinetic analysis.

The mean plasma protein binding of clarithromycin was $71.3 \pm 7.4\%$ for a 250 mg single dose and $76.9 \pm 8.1\%$ for 500 mg b.i.d. at the steady state (drug intake for 3 to 5 days). The mean individual in vivo recovery values for clarithromycin in microdialysis were $57.7 \pm 17.2\%$ and $54.3 \pm 19.0\%$ for adipose and muscle tissue, respectively. In separate in vitro experiments (data not shown), we demonstrated that recovery was not dependent on concentration and time. Variability between probes was minimal.

Pharmacokinetic data for a single 250 mg dose of clarithromycin. Main pharmacokinetic data are summarized in Table 1.

The concentration-time profiles for free clarithromycin in the interstitial-space fluid of adipose tissue resembled closely the concentration-time profiles for skeletal muscle. Detectable interstitial concentrations were observed about 1 h after drug

FIG. 1. Clarithromycin concentrations in plasma and interstitial-space fluid of soft tissues from six male healthy volunteers after a single dose of 250 mg (mean $\pm$ standard deviation).

FIG. 2. Clarithromycin concentrations in plasma and interstitial-space fluid of soft tissues from six male healthy volunteers after multiple doses of 500 mg b.i.d. (mean $\pm$ standard deviation).

administration (Fig. 1). The ratios of the $fAUC_{0-24}$ values in tissues to the $fAUC_{0-24}$ values in plasma were 0.29 ± 0.17 (range, 0.14 to 0.61) and 0.42 ± 0.18 (range, 0.17 to 0.60) for subcutaneous adipose and skeletal muscle tissue, respectively, after intake of a single oral dose of 250 mg clarithromycin. The differences between $fAUC_{0-24}$ values for plasma and tissues were significant ($P < 0.03$).

Pharmacokinetic data of 500 mg clarithromycin at the steady state. Main pharmacokinetic data are summarized in Table 2.

Interstitial-space fluid concentrations of free clarithromycin in subcutaneous adipose tissue and skeletal muscle were descriptively identical (Fig. 2). The ratios of the $fAUC_{0-24(b.i.d.)}$ values in tissues to the $fAUC_{0-24(b.i.d.)}$ values in plasma were 0.39 ± 0.04 (range, 0.32 to 0.44) and 0.41 ± 0.19 (range, 0.19 to 0.75) for subcutaneous adipose tissue and skeletal muscle, respectively. The differences between $fAUC_{0-24(b.i.d.)}$ values for plasma and tissues were significant ($P < 0.03$).

The clearance values at steady state differed from the single dose data because dose and $AUC_{0-\infty}$ did not increase proportionally (nonlinear pharmacokinetics).

Safety and tolerability. The study drug was well tolerated by all subjects. Metal-like taste sensation and mild gastrointestinal disturbance were observed in one volunteer. Both adverse events subsided within the study period without therapeutic

measures. No adverse events related to the microdialysis procedure were observed.

DISCUSSION

Excellent tissue penetration characteristics are an attribute commonly ascribed to the entire class of the macrolides (1), and clarithromycin is considered a very typical representative of this class. In its label information, one can read that clarithromycin distributes readily into body tissues and that tissue concentrations are higher than serum concentrations (Abbott Laboratories, Biaxin prescription information, January 2005). These statements are based on the presence of high concentrations of clarithromycin measured in biopsy homogenates and isolated tissue culture cells (10, 13, 16). We set out to measure its concentrations in the interstitium of soft tissues, the site of infection.

We found, interestingly, that the $fAUC$ values for clarithromycin in the interstitial-space fluid of soft tissues did not confirm the hypothesis of a significant accumulation of clarithromycin in the interstitial space at doses of up to 500 mg administered twice daily (Table 1 and Table 2). These findings can be explained by (i) incomplete penetration of the drug from the central compartment into the interstitial-space fluid,

TABLE 2. Main pharmacokinetic indices of clarithromycin at the steady state after administration of 500 mg b.i.d. ($n = 6$)^a

Compartment	AUC_{0-8} (mg · h/liter)	AUC_{0-24} ^b (mg · h/liter)	AUC_{0-24} tissue/ $fAUC_{0-24}$ plasma	C_{max} (mg/liter)	t_{max} (h)	$t_{1/2\beta}$ (h)	V_{ss} (liters)	CL (liters/h)
Plasma (total)	10.59 ± 1.78	26.81 ± 5.49		2.21 ± 0.33	3.2 ± 0.3	3.7 ± 0.6	126.5 ± 17.4	18.7 ± 5.2
Plasma (free)	2.26 ± 0.71	5.85 ± 1.79		0.58 ± 0.19	3.2 ± 0.3	3.7 ± 0.6		
Subcutis	0.84 ± 0.19^c	2.22 ± 0.59^c	0.39 ± 0.04	0.23 ± 0.10	4.0 ± 0.9	3.4 ± 1.2		
Muscle	0.92 ± 0.43^c	2.42 ± 1.23^c	0.41 ± 0.19	0.23 ± 0.09	3.8 ± 0.9	3.8 ± 1.1		

^a Values represent means $\pm$ standard deviations.

^b Values calculated on the basis of dosing twice daily. Abbreviations: C_{max} , maximum concentration of drug; t_{max} , time to maximum concentration; $t_{1/2\beta}$, half-life at β phase; AUC, area under the concentration-time curve; $fAUC$, AUC of free drug; V_{ss} , volume of distribution at the steady state; CL, total clearance.

^c $P < 0.03$ compared to free plasma results.

(ii) forced intracellular uptake of clarithromycin, or (iii) spontaneous degradation of clarithromycin in tissues. Spontaneous degradation and impaired transport of clarithromycin across the capillary barrier is unlikely to account for the observation reported above because (i) clarithromycin is highly stable and (ii) the high density of negative charges in the basement membrane of the capillary endothelium should facilitate the diffusion of lipophilic basic drugs to the extracellular-space fluid (12). Probably, fast and high-level intracellular uptake of clarithromycin into lysosomes, likely assisted by a phenomenon called "ion-trapping," is one explanation for the unexpectedly low concentrations of clarithromycin in the interstitium (5, 9).

The elimination half-life of clarithromycin in tissues and plasma was about 2 h in our study collective after a single dose of 250 mg. As observed previously in other studies (7, 22), a nonlinear plasma and tissue pharmacokinetic profile of clarithromycin was detected following administration of the higher dose of 500 mg twice a day in a 12-h interval (Table 2). The nonlinear increase in AUC values and prolongation of half-life is most likely attributable to the inhibition of the activity of cytochrome P₄₅₀ 3A4 caused by clarithromycin itself after repetitive dosing (25).

For the class of macrolides, the ratio of the AUC_{0-24 plasma} value to the MIC has been shown to be the most predictive PK-pharmacodynamics (PK-PD) index for survival of animals (2, 24). In literature, there is circumstantial evidence that optimal bacterial eradication of *Streptococcus pneumoniae* and survival of animals can be expected when the $fAUC_{0-24 \text{ plasma}}/MIC$ ratio of macrolides is not lower than around 35 (2, 24). Studies respecting PK-PD breakpoints for bacteria other than *S. pneumoniae* are currently almost completely lacking or show conflicting results. Another source of confusion at this point is that some authors reported ratios of total AUC to MIC values and did not correct for plasma protein binding (24). However, provided that a $fAUC_{0-24 \text{ plasma}}/MIC$ ratio target of at least 35 is also valid in humans for pathogens other than *S. pneumoniae* and taking a calculated AUC_{tissue} to AUC_{plasma} ratio of 0.40 for tissues into account, then the corresponding $fAUC_{0-24 \text{ tissue}}/MIC$ ratio should be around 14. Thus, six out of the six volunteers would have had a high probability of a cure with 500 mg of clarithromycin b.i.d. in the case of soft-tissue infection caused by pathogens with drug MICs of less than 0.125 mg/liter. However, if the MIC for a given pathogen is ≥ 0.25 mg/liter (11), it is tempting to conclude that the 500 mg b.i.d. regimen would not work sufficiently well for subcutaneous adipose tissue for any of the subjects and would be active for skeletal muscle for only three out of six subjects.

In general, one may argue that clarithromycin undergoes extensive hepatic metabolism and is converted to 14-(R)-OH-clarithromycin, which is a major active metabolite exerting in vitro activity similar to that of the parent compound (14, 17). Data of four previous publications about the plasma concentrations of the 14-hydroxy metabolite in humans are largely in agreement, with an overall ratio of the AUC_{metabolite} to the AUC_{parent compound} of $34.9 \pm 2.1\%$ (4, 7, 8, 18). Interstitial concentrations of the more hydrophilic 14-hydroxy metabolite are unknown at present. However, based on previous investigations as well as on the chemical and physical properties of the metabolite, it may be concluded that the ratio of the AUC_{metabolite} to the AUC_{parent compound} in the interstitium may be similar to

that seen with plasma (13, 21). Since it is unlikely that interstitial concentrations of 14-(R)-OH-clarithromycin of one-third of the concentration of the parent compound would significantly affect antimicrobial action at the target site, we did not include an estimation of concentrations of the active metabolite in the present PK-PD calculations. Thus, the concentrations of 14-hydroxy clarithromycin were not measured in the present study.

Another potential limitation of the present study is that steady-state conditions may not have been reached for all volunteers, even though subjects were asked to take the study drug twice daily over a period of at least 3 days. From a pharmacokinetic point of view, steady-state concentrations should have been reached within this period provided that the study drug was taken as foreseen in the protocol and that the elimination half-life did not increase with further duration of dosing. An advanced increase in the half-life of clarithromycin in tissues and plasma could theoretically lead to higher concentrations of clarithromycin and its active metabolite in the interstitium.

However, intake of the study drug was monitored by pill count; thus, inadequate compliance of subjects would have required more sophisticated and subtle methods. Nevertheless, in one subject the plasma concentrations of clarithromycin were below the limit of quantification, as mentioned in Materials and Methods. Thus, the level of compliance of some volunteers was most probably not optimal.

In summary, our results indicate that plasma pharmacokinetics of clarithromycin may lead to overestimation of its interstitial concentrations in unaffected soft tissues. The results of PK-PD calculations support the idea that even a 500 mg dose b.i.d. may be ineffective in the therapy of skin infections caused by extracellular pathogens with drug MICs higher than 0.125 mg/liter. However, subsequent studies looking at the interstitial concentrations of clarithromycin and its active metabolite after 1 week of therapy are necessary to confirm these data.

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