Penetration of Cefaclor into the Interstitial Space Fluid of Skeletal Muscle and Lung Tissue in Rats

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Purpose. To measure and compare the penetration of cefaclor from the plasma compartment into the interstitial space of lung and skeletal muscle in rats and to integrate the data in a pharmacokinetic model.

Methods. Unbound interstitial concentrations in muscle and lung were measured by *in vivo* microdialysis following i.v. bolus doses of 50 and 75 mg/kg cefaclor. Unbound muscle concentrations were also measured after a primed, continuous i.v. infusion at an infusion rate of 0.3 mg/kg/min.

Results. The cefaclor half-life in plasma, muscle and lung was approximately 1 h. Unbound cefaclor concentrations in muscle and lung were found to be virtually identical. A 2-compartment body model was fitted to the data with a tissue penetration factor ($AUC_{tissue(unbound)}/AUC_{plasma(unbound)}$) of approximately 0.26 independent of dose, tissue and mode of administration.

Conclusions. Unbound concentrations of cefaclor in the interstitial space fluid of lung and skeletal muscle are of similar magnitude and lower than those in plasma. Using total plasma concentrations would overestimate the antibacterial activity of the drug and therefore its clinical efficacy. Instead, therapeutically active levels of cefaclor at the site of action should be taken into account. Microdialysis allows direct measurement of these unbound concentrations.

KEY WORDS: pharmacokinetics; free tissue concentrations; microdialysis; muscle; lung; rat; cefaclor; β-lactam antibiotics.

INTRODUCTION

Most infections with few exceptions like septicemia do not occur in the central compartment but in defined target tissues. Therefore the ability of antibiotics to distribute to particular target sites is of great importance and is considered a key determinant of clinical outcome. However, in most cases a complete and lasting equilibration between the plasma and the target sites cannot be taken for granted. To support the design of optimal dosing guidelines, several studies have therefore tried to measure antibiotic concentration-time profiles directly in target tissues. One particularly suitable technique in this respect is microdialysis because it is the sole technique that enables the measurement of the active, unbound drug fraction in the interstitial fluid, i.e., the space which is considered the anatomical effect site for most infections (1–4).

A class of antibiotics that has gained considerable attention in recent years are oral cephalosporins because they are considered a safe, effective, and also cost-effective means to treat various infections in outpatient settings. Despite the frequent prescription of oral cephalosporins, only sparse data is available in the literature to support the concept that oral administration of cephalosporins actually leads to therapeutically effective drug concentrations at the target site. Data on target site penetration of oral cephalosporins, however, could provide an explanation for therapeutic failures (5,6), also in cases where the causative organisms were susceptible to the drug *in vitro*.

Cefaclor was selected as a study drug because it is an orally available broad-spectrum cephalosporin antibiotic that can be used for the treatment of soft tissue and respiratory infections. Previous studies with beta-lactam antibiotics have indicated that rats are a suitable test system to investigate tissue distribution using microdialysis (1–3). The present study aimed at quantifying cefaclor penetration to two important target sites, i.e., muscle and lung in an established rat model.

METHODS

Experimental Design

The study was performed under GLP conditions and with the approval of the Institutional Animal Care and Use Committee (IACUC) of the University of Florida. The animals were housed in the Animal Resources Department of the University of Florida and kept according to the Animal Resources standard husbandry procedures. The research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

Altogether 17 male Wistar rats (Breeder: Harlan Sprague Dawley, Inc.) were used. Their body weights were approximately 300–400 g (animals with similar body weights were used), corresponding to approximately 2 months of age. Rats received I.V. doses of either 50 mg/kg (n = 7) or 75 mg/kg (n = 7) of cefaclor. A separate set of rats (n = 3) were administered a loading dose of cefaclor (15 mg/kg) followed by a continuous IV infusion (0.3 mg/Kg/min). Plasma concentrations as well as unbound tissue concentrations in thigh muscle and lung were measured. Animals were sacrificed at the end of the study. The data was analyzed both by noncompartmental and compartmental pharmacokinetic approaches.

Anesthetic Procedure

The animals were initially anesthetized by inhalation of methoxyflurane gas (Metofane) in a glass jar. A dose of 1.25 g/kg of ethylcarbamate was administered intraperitoneally.

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Surgical anesthesia resulted after about 4–5 min, and was confirmed by the absence of reflexes after pinching the footpads. Subsequent doses of ethylcarbamate (equal to one half the first dose) were administered to the animal whenever consciousness signs were observed. Animals were immobilized in a supine position; body temperature was kept at 37°C with a lamp.

Dosing Schedule for I.V. Bolus Experiments

Fourteen male Wistar rats were used for this set of experiments. Seven of the 14 male animals received 50 mg/kg of cefaclor IV and the other seven received 75 mg/kg of cefaclor IV. Cefaclor was injected as an IV bolus injection over a 1-min period in the femoral vein of the right hind leg.

Dosing Schedule for Continuous Infusion Experiments

From the i.v. bolus experiments, it was observed that there is a difference between the free plasma concentrations and the free tissue concentrations of cefaclor. A continuous infusion experiment was performed to investigate if what was seen in the single-dose experiments would also happen in a steady-state situation. A separate set of rats (n = 3) were administered a loading dose of cefaclor (15 mg/kg) followed by a continuous IV infusion (0.3 mg/kg/min). Plasma samples were taken every 30 min and free muscle concentrations were obtained by microdialysis every 20 min for a total of 5 h.

Blood Samples

For all blood sampling a small incision was made in the neck area and a catheter (Venflon, 20–22 gauge) was inserted in the carotid artery at the beginning of the experiment. The artery was irrigated after each sampling with an equal volume of heparinized saline (25 UI of sodium heparin/mL of normal saline). Blood samples (400–500 μL) were collected in heparinized tubes (sodium heparin), before cefaclor administration (time 0) and at times 5, 10, 15, 30, 45, 60, 90, and 120 min after administration. The blood samples were centrifuged 3 min at room temperature at approximately 9170 rpm and the plasma kept at -70°C until analyzed. Approximately 400–500 μL of blood/animal/sampling time were obtained, a sufficient volume to obtain approximately 250 μL of plasma/animal/sampling time for subsequent determination of the cefaclor levels by HPLC.

Microdialysis Samples

Microdialysis probes were implanted both in muscle and lung tissue. Microdialysis samples were collected simultaneously for lung and muscle, over 20-min intervals at times 20, 40, 60, 80, 100 and 120 min after administration of the IV cefaclor dose.

Probe Calibration

The microdialysis probes were CMA/10 custom made microdialysis probes, shaft length 70 mm, membrane length 16mm, 20 kD cutoff. Both probes were initially perfused with Ringer's (NaCl 0.9%, KCl 0.03%, $CaCl_2$ 0.03%) at a flow rate of 3 μL/min using a Harvard Apparatus 22 injection pump, model 55-4150. The probes were allowed to equilibrate for 20 min after insertion. After equilibration, the probes were calibrated by retrodialysis. The syringes were changed and a solution of 10 mg/mL of cefaclor was perfused through the probes for 40 min at a flow rate of 3 μL/min. Samples were collected during the following periods: 10–30 min (after the beginning of retrodialysis), and 30–50 min. The first 10 min of the retrodialysis infusion were not collected, to account for the dead space in the tubing. After the retrodialysis samples were obtained, the syringes were changed back and the probes were again perfused with Ringer's solution at a flow rate of 3 μL/min for 20 min to allow for washout of the retrodialysis solution. The solution used for the retrodialysis was kept frozen at −70°C until analyzed. After washout was complete, the IV cefaclor dose was administered. Calibration for the muscle and lung probes was done in each animal before the experiment was performed. Due to the variability in the results obtained by retrodialysis, the recovery was reassessed in muscle by the no net flux method in a separate set of four rats. Four different concentrations of cefaclor were perfused: 2, 4, 10 and 15 μg/mL, while keeping a constant tissue concentration of approximately 2.5 μg/mL.

Muscle Microdialysis

The left hind leg muscle was used for insertion of a microdialysis probe after skin removal. The probe was introduced into the muscle through the tip of a 20-gauge needle that was removed after placing the probe.

Lung Microdialysis

The rats were intubated through a tracheotomy and artificially ventilated with room air throughout the experiment using a rodent respirator (Harvard Apparatus; model 683), with a frequency of 62–66 min^{-1} and a volume of 2 mL. The right lung was exposed through partial resection of the ribs. A microdialysis probe was inserted into the intermediate lobe of the right lung through a small incision made in the pleura. The probe was held in place with ties around the probe shaft and the lung and then the lobe was carefully put back in place.

Sample Assay

Samples were analyzed by a validated reverse-phase HPLC assay with UV detection at λ = 254nm. The procedure, which was modified from a previously published assay, used a 97%:3% 0.05M NaAc/Hac:Isopropanol pH: 5.9 mobile phase (7). Plasma samples were precipitated with 12% perchloric acid in a 5:2 ratio and 50 μL of the supernatant were injected into the HPLC system (8). Microdialysis samples (20 μL) were directly injected into the system without pre-treatment. Runs were performed at room temperature, with flow rates of 1.5–1.0 mL/min, and LLOQ of 0.2/0.5 μg/mL for microdialysis and plasma samples respectively.

Non-Compartmental Pharmacokinetic Analysis

The following parameters were calculated for each animal.

Plasma

The terminal elimination rate constant (k_e) was calculated by linear regression of the natural logarithms of the last n (n > 4) plasma concentrations. Terminal half-life was cal-

culated as $\ln(2)/k_e$. The initial concentration C_0 was determined by logarithmic back-extrapolation to t = 0 using the first two data points. The area under the curve (AUC) was calculated using the trapezoidal rule up to the last data point (C_x) and adding the extrapolated terminal area, calculated as C_x/k_e. The area under the first moment curve (AUMC) was calculated from a plot of Ct vs. t using the trapezoidal rule up to the last data point (C_x) at time tx and adding the extrapolated terminal area, calculated as $C_x t_x/k_e + C_x/k_e^2$. The mean residence time (MRT) was calculated as AUMC/AUC. The volume of distribution of the central compartment (Vc) was calculated as D/C_0, where D is the dose. The volume of distribution at steady state (Vdss) was calculated as $D \cdot AUMC/AUC^2$. The clearance (CL) was calculated as D/AUC.

Muscle and Lung

Unbound concentrations in muscle and lung were calculated from the measured microdialysate concentrations and the measured recovery from retrodialysis samples. Parameters (k_e, $t_{½}$, AUC, AUMC, MRT) were calculated as described for plasma. The tissue distribution (or tissue penetration) factor f_T was calculated as the ratio of the unbound AUC in tissue and the unbound AUC in plasma (AUC_T/f_uAUC), where fu is the fraction unbound in plasma.

Compartmental Pharmacokinetic Analysis

Plasma

For the compartmental pharmacokinetic data analysis of the plasma concentrations, a two-compartment body model with bolus input was used. The respective equation for the plasma concentration C is:

$$C = a \cdot e^{-a \cdot t} + b \cdot e^{-\beta \cdot t}$$

where α and β are the hybrid constants representing distribution and elimination, a and b are the respective intercepts, and t is time.

Muscle and Lung

Unbound muscle and lung concentrations (C_T) were fitted simultaneously with the respective plasma concentrations. The respective equation for the unbound concentration in the tissue is:

$$C_T = \frac{f_T \cdot f_u \cdot (a \cdot \beta + b \cdot \alpha)}{(\beta - \alpha)} \cdot (e^{-\alpha \cdot t} - e^{-\beta \cdot t}) \qquad (2)$$

where α, β, a and b have been defined above, f_u is the fraction unbound in plasma and f_T is a proportionality factor characterizing the degree of tissue distribution.

The data was analyzed by nonlinear regression using the program Scientist (MicroMath, Salt Lake City, Utah). For each animal, the plasma concentrations were fitted to both models. The coefficient of determination (CD) as well as the Model Selection Criterion (MSC) were used as a criterion for the goodness of the resulting curve fits. The closer CD is to 1, the better the agreement between measured and calculated values. The higher MSC, the more appropriate the selected model.

Statistical Analysis

Statistical analysis for recovery values and tissue penetration factors was done by ANOVA. In case of a statistically significant difference ($P < 0.05$), ANOVA was followed by a Tukey multiple comparison test.

RESULTS

Recovery

The mean recovery results for all animals (n = 14) calculated by retrodialysis were 39 ± 12% and 33 ± 7% for muscle and lung, respectively. The recovery values determined by the no-net-flux method was 33 ± 20% and it was found not to be statistically significantly different from those found in both muscle and lung by retrodialysis.

Comparison of Plasma, Muscle and Lung Pharmacokinetics after I.V. Administration

Non-compartmental Pharmacokinetic Analysis

The results of the non-compartmental pharmacokinetic data analysis for cefaclor after I.V. administration of 50 and 75 mg/kg for plasma and the interstitiual space of muscle and lung concentrations are listed in Table I. Parameters were

Table I. Results of the Non-Compartmental Analysis for the 50 and 70 mg/kg Dose

Matrix	Plasma				C_T muscle				C_T lung			
	50mg/kg		75 mg/kg		50 mg/kg		75mg/kg		50mg/kg		75mg/kg	
Parameters	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
$t_{1/2}$ [min]	81.9	41.0	61.1	17.9	61.7	20.2	46.5	11.6	64.0	23.6	58.5	22.2
C_{max} [μg/mL]	270.2	62.1	221.2	69.7	28	5.4	22.5	6.2	29.2	10.4	25.4	7.5
AUC[μg/mL·min]	10184	5871	6504	2404	1547	576	983	324	1499	776	1107	312
MRT[min]	102.1	57.4	72.2	23.8	77.9	27.3	56.3	13.4	78.6	31.1	68.8	24.2
Vc [mL/g]	0.19	0.04	0.37	0.11	–	–	–	–	–	–	–	–
Vdss [mL/g]	0.50	0.08	0.86	0.23	–	–	–	–	–	–	–	–
Vdarea [mL/g]	0.60	0.11	1.07	0.30	–	–	–	–	–	–	–	–
CL [mL/min/kg]	6.3	3.1	12.8	4.5	–	–	–	–	–	–	–	–
AUC_f[μg/mL·min]	6518	3757	4163	1539	1547	576	983	324	1499	776	1107	312
f_T	–	–	–	–	0.27	0.08	0.25	0.07	0.25	0.09	0.28	0.06

calculated individually for each animal. Results are expressed as means, coefficients of variation (C.V.), and median values.

Compartmental Pharmacokinetic Analysis

A two-compartment model with bolus input was able to produce a good curve fit of the average plasma concentrations (Fig. 1). The coefficient of determination (r^2) was 0.978 and 0.977, for 50 and 75 mg/kg, respectively, and the model selection criterion (MSC) was 3.2, indicating reasonable curve fits. Half-lives were found to be 43 and 36 min for the 50 and 75 mg/kg dose, respectively. The tissue penetration factors were 0.23 and 0.24 for the two doses, respectively. As has been observed in many other studies before, the first data point of both muscle and lung concentration is not well described by the model as the distribution equilibrium is not established yet. However, in the terminal phase of the curve, the model describes both plasma and unbound tissue concentrations very well.

Continuous Infusion Experiment

Figure 2 shows the average cefaclor concentration-time profile for total and free plasma concentrations, and for free tissue concentrations, after continuous i.v. infusion at an infusion rate of 0.3 mg/kg/min. Areas under the curve were calculated for mean total and free plasma and free tissue concentrations. The mean $C_{tissue,\ free}/C_{plasma,\ total}$ -ratio was found to be 0.15, the respective tissue penetration factor was 0.25.

DISCUSSION

The present experiments aimed at simultaneously measuring the target site concentrations of cefaclor in two different tissues using a rat model. Cefaclor plasma concentrations were compared to unbound concentrations in muscle and lung as measured by microdialysis.

The main finding of the present study was the observation that unbound concentrations in muscle and lung are comparable which suggests that unbound concentrations measured in muscle maybe reasonable predictors for therapeutically relevant unbound concentrations in lung.

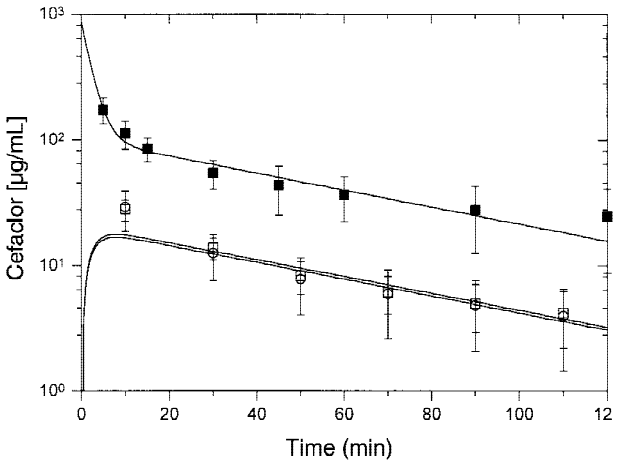

Fig. 1. Mean plasma concentrations (±S.D.) of cefaclor (■) and mean unbound concentrations (±S.D.) in muscle (□) and lung (○) following an i.v. bolus dose of 50 mg/kg. The lines represent the respective curve fits from nonlinear regression using simultaneous compartmental pharmacokinetics (n = 7).

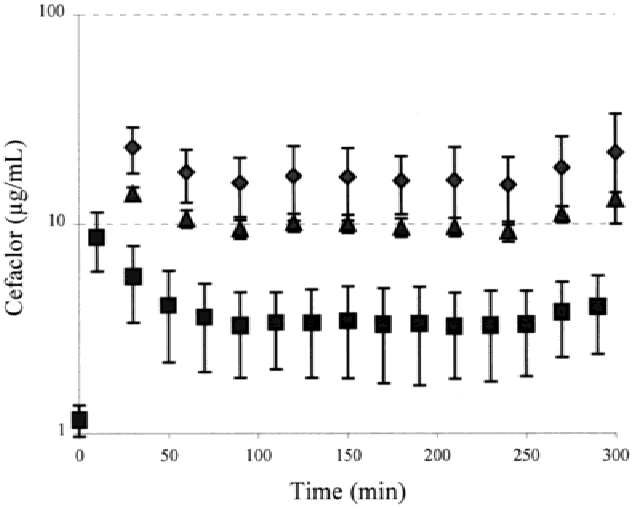

Fig. 2. Mean (±S.D.) total plasma (♦), free plasma (▲) and free tissue (■) concentrations of cefaclor during continuous I.V. administration at an infusion rate of 0.3 mg/kg/min (n = 3).

Another important finding of this study was that both tissues and doses showed statistically not significantly different tissue penetration factors ($P > 0.88$), with tissue cefaclor concentrations of approximately 26% of unbound plasma concentrations. This has potential implications as the concentration of the unbound drug fraction at the target site is an important determinant of antibiotic efficacy. The present data also corroborates previous findings of significantly lower unbound tissue concentrations of other anti-infectives (6).

A potential explanation for the plasma to tissue gradient for cefaclor is the fact that cefaclor is a zwitterion, and as such, differences in pH between the plasma water and the interstitial fluid may cause it to be ionized and hinder its diffusion across membranes. Other possible explanations include the presence of active transport mechanisms or peripheral elimination. Peripheral elimination may be due to the inherent, well-documented instability of cefaclor (9–13). Cefaclor is a chemically unstable molecule that may be undergoing chemical degradation in the tissues faster than in blood.

From a physiological point of view this finding implies that for select analytes unrestricted diffusion across capillaries cannot be taken for granted. The interaction between cefaclor and the cascade of factors that determine blood-to-interstitium transfer such as local blood flow, local capillary density, and capillary permeability to surface area product may also have an influence. Alterations in capillary permeability in the present experimental setup could be caused by the effects of anaesthesia or the hypovolemia due to blood loss. Further studies are needed to address this issue.

Although both doses did not confirm dose-proportionality the measured concentrations in lung and muscle and also the half-lives in plasma, muscle, and lung were almost identical. Given the lack of information in the literature about pharmacokinetic parameters of cefaclor in rats, the 50 and 75 mg/kg doses were chosen based on analytical capabilities. Taking into account the variability in the data, these doses are probably too similar and the sample size too small to allow

evaluation of dose proportionality. However, dose proportionality was not the main objective of the present study. Thus, it is likely that the study was underpowered for that purpose. It was possible to describe the data with a pharmacokinetic two-compartment body model. The model included a tissue penetration factor ($f_T = AUC_{tissue,\ free}/AUC_{plasma,\ free}$) to account for the difference between free plasma and free tissue concentrations observed in both the single-dose and the continuous infusion experiments.

In conclusion the present study indicates that using total plasma concentrations would overestimate the antibacterial activity of cefaclor and therefore probably also its clinical efficacy. Instead, therapeutically active levels of cefaclor at the site of action should be taken into account. Microdialysis is a suitable experimental technique to directly measure these unbound concentrations.

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