

Quantification and Prediction of Skin Pharmacokinetics of Amoxicillin and Cefuroxime

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ABSTRACT: The purpose of this project was to develop and validate a pharmacokinetic model and to quantify the rate and extent of distribution between plasma and skin of two β -lactam antibiotics, amoxicillin (AMX) and cefuroxime (CFX), which are frequently administered systemically to treat skin and skin structure infections. Dosing regimens are usually based on plasma concentration, however, concentrations at the target site are better correlated with the effect. For each antibiotic, three different i.v. bolus doses were administered to three female rabbits according to a randomized cross-over design and plasma samples were collected serially. Skin concentrations were obtained by continuous microdialysis. Skin and unbound plasma concentrations were fitted simultaneously using a semi-physiological model and the transfer constants plasma/skin (K_{in}) and skin/plasma (K_{out}) were estimated. K_{in} and K_{out} were then used to predict skin concentrations from the plasma levels obtained from an oral administration of AMX or from an i.v. bolus of CFX. The predicted skin profiles were similar to those measured by microdialysis during the actual experiments. In conclusion, this study shows that it is possible to generate a reasonable prediction of skin pharmacokinetics from any plasma level once a careful characterization of the transfer process between plasma and skin has been made. Copyright © 2009 John Wiley & Sons, Ltd.

Key words: microdialysis; pharmacokinetics; antibiotics; modeling; prediction

Introduction

The skin is often the target of infections which vary from relatively mild and localized to severe and life threatening, leading to diverse treatment regimens. Simple skin infections can be cured with locally applied formulations; conversely, severe infections such as cutaneous anthrax frequently require multiple oral or intravenous

antimicrobial drug administrations [1]. Bacteria causing skin infections are found extravascularly in the interstitial fluid of the skin and not in the plasma. It is believed that the success of a β -lactam antibacterial drug therapy will depend on the time period the drug concentrations stay above the minimum inhibitory concentration (MIC) of the infecting bacteria [2]. Unfortunately, there is limited information on the pharmacokinetics of antibiotics in the dermis; hence current dosing regimens are based on plasma concentrations under the assumption that it mimics the variations of drug in the skin. Even though this could be a valid assumption, it has never been proved. Indeed, the concentration of drug in the

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skin interstitial fluid may be affected by a number of factors such as blood perfusion, binding to skin-structure components, partitioning into skin cells, and uptake by the lymphatic system. Only the unbound drug in the interstitial fluid will possibly be able to interact with the infecting bacteria. Microdialysis is a technique that allows the measurement of unbound drug concentrations in the tissue interstitial fluid and has been used successfully to determine the kinetics of several drugs in the skin [3–5]. Therefore, it would be wise to use microdialysis concentrations rather than plasma levels to effectively establish dosing regimens for antibiotics to treat skin and skin structure infections. However, microdialysis is still less popular than blood sampling in a clinical setting, consequently it would be desirable to be able to predict skin concentrations from plasma levels.

The goal of this work was to propose a model and an experimental design to allow for the prediction of skin concentration from any plasma level. In order to achieve this goal the distribution between plasma and dermis of two model antibiotics, amoxicillin (AMX) and cefuroxime (CFX), was studied in a rabbit model by concurrent measurement of skin concentrations via microdialysis and plasma levels via serial sampling. AMX is usually administered orally to treat skin infections such as cutaneous anthrax [6–8], while CFX is administered intravenously or intramuscularly to treat skin and skin structure infections such as impetigo, folliculitis and cellulitis caused by *Staphylococcus aureus* and *Streptococcus pyogenes* [9,10]. The linearity of the distribution between plasma and skin was first assessed by administering increasing doses of the antibiotics. Plasma and skin concentrations from i.v. bolus administrations were then fitted simultaneously to determine the parameters that characterize the transport between plasma and skin and vice versa. If the transfer process between plasma and skin is linear and time invariant, the same parameters can be used to predict skin concentrations from any plasma concentration within that range. The model developed in the present study can be used to support or re-examine current dosing regimens and to expedite the selection of appropriate dosing regimens to treat skin infections during drug development.

Materials and Methods

Chemicals and reagents

All chemical were analytical grade or higher in quality. AMX and CFX for standard solutions, monobasic and dibasic sodium phosphates, trichloroacetic acid 99% and triethylamine 99% were purchased from Sigma Chemical, Co. (St Louis, MO, USA). AMX sodium injection was purchased from Ranbaxy Laboratories Ltd, Ahmedabad, India (Batch No. 9071563), AMX oral suspension (25 mg/ml) was obtained from Teva Pharmaceuticals Inc. (Sellersville, PA, USA). Cefuroxime sodium for injection was purchased from Baxter Healthcare Corporation, Deerfield, IL, USA (Lot No. 06035.1). HPLC grade water was from Mallinckrodt, Inc. (Phillipsburg, NJ). Dichloromethane and acetonitrile were from EM Science (Gibbstown, NJ); methanol and phosphoric acid were from J. T. Baker Inc., (Phillipsburg, NJ, USA), lactated Ringer's solution USP was from B. Barun, (Irvine, CA).

HPLC assay

The chromatographic apparatus consisted of a WatersTM 717 plus autosampler, a Hitachi L-4250 UV-Vis detector, a Hitachi L-6200 intelligent pump and a PE Nelson 900 series interface. Data acquisition and chromatographic analysis were carried out using TotalChrom Workstation Version 6.2.1 (PerkinElmer, Inc., Waltham, MA). AMX and CFX concentrations were determined using isocratic, reverse phase HPLC methods [11,12].

The microdialysis samples of AMX were analysed using an Ascentis, Supelco, 10 cm × 2.1 mm, 3 μm column (Bellefonte, PA) while AMX plasma samples were analysed using a Phenomenex, Synergi, 15 cm × 3 mm, 4 μm column (Torrance, CA). The microdialysis samples were eluted using a mobile phase that consisted of 90% 0.05 M phosphate buffer and 10% methanol containing 0.1% triethylamine and the pH was adjusted to 3 using phosphoric acid, while a mobile phase that consisted of 97% 0.05 M phosphate buffer and 3% methanol containing 0.1% triethylamine adjusting the pH to 3 with phosphoric acid was used for the AMX plasma samples. The mobile phase flow rate was 0.5 ml/min and the detection wavelength was 230 nm.

The injection volume for microdialysis samples was 8 μ l, while for plasma samples it was 15 μ l.

The CFX microdialysis and plasma samples were analysed using a C₁₈ column (Xorbax, Eclipse XDB-C₁₈, 4.6 \times 150 mm, 5 μ m, Agilent, CA) and were eluted using a mobile phase that consisted of 25% methanol and 75% 0.1 M phosphate buffer (pH 5). The flow rate was 1 ml/min and the detection wavelength was 280 nm. The injection volume for microdialysis samples was 10 μ l, while for plasma samples it was 50 μ l.

A typical chromatogram of the blank and drug spiked plasma and skin dialysate samples showed a sharp and symmetric peak corresponding to AMX and CFX. No interfering peaks from endogenous substances were observed at the retention time of AMX and CFX. The typical retention time for AMX in skin microdialysis samples was 2.1 min and in plasma samples it was 12.3 min, while for CFX samples it was 5.3 min for both microdialysis and plasma samples. The assays were validated according to the FDA guidelines for small molecules [13]. The LLOQs were 0.1 μ g/ml and 0.5 μ g/ml for AMX in microdialysis and plasma samples, respectively, while for CFX the LLOQs were 0.1 μ g/ml and 1 μ g/ml, respectively. AMX calibration curves in Ringer's solution were linear in the 0.1–100 μ g/ml ($r^2 = 0.99$) range with an intra-day CV% of 2.3 and a percentage error of 2.5 at 0.1 μ g/ml and a CV% of 0.2 and a percentage error of 1.4 at 100 μ g/ml; while AMX concentrations in plasma samples were linear in the range 0.5–3000 μ g/ml ($r^2 = 0.99$) with an intra-day CV% of 17.6 and a percentage error of 19.2 at 0.5 μ g/ml and a CV% of 2.7 and a percentage error of 0.1 at 3000 μ g/ml. CFX calibration curves in Ringer's solution were linear in the 0.1–10 μ g/ml ($r^2 = 0.99$) range with an intra-day CV% of 14.0 and a percentage error of 5.8 at 0.1 μ g/ml and a CV% of 3.5 and a percentage error of 0.9 at 10 μ g/ml; while CFX calibration curves in plasma were linear in the range 1–300 μ g/ml ($r^2 = 0.99$) with an intra-day CV% of 2.9 and a percentage error of 14.9 at 1 μ g/ml and a CV% of 0.1 and a percentage error of 0.2 at 300 μ g/ml.

Microdialysis system

The microdialysis system consisted of a CMA/102 microdialysis pump (CMA/Microdialysis

AB, Stockholm, Sweden) equipped with 1 ml Exmire microsyringe type I (Ito Corporation, Fuji, Japan) and a CMA/142 fraction collector (CMA/Microdialysis AB, Stockholm, Sweden). Disposable microdialysis probes were made in our laboratory according to a procedure described by Stagni *et al.* [14]. A probe consisted of two 7–8 cm segments of polyimide tubing, 200 μ m diameter (MicroLumen, Tampa, FL), connected by a tubular polyacrylonitrile microdialysis membrane with a molecular weight cutoff of 50 kDa, (AN69 HF Hospal-Gambro, Inc., CA). The microdialysis membrane was fixed to the polyimide tubes by cyanoacrylate glue leaving a membrane window 1 cm long. An internal stainless steel wire (0.0015 in. diameter, Molecu-Wire Corp., Wall Township, NJ) reinforced the probes.

In vitro microdialysis

One microdialysis probe was inserted in the inner chamber (2 ml total volume) of a custom-made glass cell, donated by Novartis (Somerville, NJ) in such a way that the membrane window was positioned exactly in the center of the chamber. A circulator (Vanderkamp, Edison, NJ) maintained the temperature of the circulating water in the cell jacket at 35°C to mimic the skin temperature. The solution in the cell was stirred continuously with a star-headed magnetic stirrer. The microdialysis flow rate was 2 μ l/min and sampling was carried out at 8 min intervals for 80 min. The solution of the inner chamber was sampled in triplicates. When studying recovery from the bulk solution (gain), the cell was filled with 2 ml of the following concentrations of AMX or CFX in lactated Ringer's solution: 5, 10 and 20 μ g/ml or 1, 4 and 8 μ g/ml, respectively; and the probe was perfused with blank lactated Ringer's solution. Relative recovery (RR) was calculated as the slope of the linear regression of drug concentration in the dialysate ($C_{\text{dialysate}}$) and drug concentration in the cell (C_{bulk}):

$$C_{\text{dialysate}} = RR * C_{\text{bulk}} \quad (1)$$

For the retrodialysis studies (loss), the cell was filled with a blank lactated Ringer's solution and the probe was perfused with 5, 10 and 20 μ g/ml concentrations of AMX or 1, 4 and 8 μ g/ml of

CFX in lactated Ringer's solutions. Recovery (loss) was calculated as:

$$C_{\text{perfusate}} - C_{\text{dialysate}} = RR * C_{\text{perfusate}} \quad (2)$$

where $C_{\text{perfusate}}$ is the concentration of the perfusing solution.

The *in vitro* gain and loss (mean \pm SD) for AMX were $48 \pm 2.2\%$ ($n = 9$) and $53 \pm 1.4\%$ ($n = 9$), respectively; while gain and loss for CFX were $50 \pm 2.5\%$ ($n = 9$) and $48 \pm 1.6\%$ ($n = 9$), respectively. Both 'dialysate' versus 'bulk' concentrations and 'loss' versus 'perfusing' concentrations exhibited a linear relationship at the concentrations tested demonstrating no dependence of recovery on concentration.

Plasma extraction

AMX was extracted from plasma as described by Du *et al.* [15]. Briefly, 100 μl of rabbit plasma was added with 300 μl of acetonitrile to precipitate proteins. After centrifugation at 10 000 rpm at 10°C for 5 min, the supernatant was added with 1 ml of dichloromethane, gently mixed, and centrifuged again at 10 000 rpm at 10°C for 5 min; 15 μl of supernatant was injected in the HPLC column. The method was applied to the concentrations: 1, 10, 50, 100 and 1000 $\mu\text{g}/\text{ml}$. The AMX plasma extraction efficiency compared with Ringer's solution was $104 \pm 5.78\%$ (mean \pm SD) ($n = 15$). CFX was extracted from plasma according to the method described by Al-Said *et al.* [16], 500 μl of plasma was deproteinized with 100 μl of 12.5% trichloroacetic acid, thoroughly vortex-mixed for 30 s, and centrifuged at 10 000 rpm at 10°C for 10 min. 50 μl of the supernatant was then injected onto the column. The method was applied to the concentrations: 1, 6 and 10 $\mu\text{g}/\text{ml}$. The extraction efficiency compared with Ringer's solution was $78 \pm 3.14\%$ (mean \pm SD) ($n = 9$).

In vivo studies

The institutional Animal Care and Use Committee (IUCAC) at Long Island University, Brooklyn, New York, approved all animal procedures. The experiments were performed in eleven female, pathogen free New Zealand albino rabbits weighing 3.6–5.2 kg. The rabbits were housed under standard laboratory conditions ($22 \pm 1^{\circ}\text{C}$; relative humidity 40–60%) and were fed with

normal rabbit chow and provided with regular drinking water from the tap. At the time when the experiments were performed the animal age ranged from 1–2 years. The rabbits were divided into three groups. One group of three rabbits received AMX as i.v. bolus doses of 25, 50 or 100 mg/kg according to a randomized cross-over design. Another group of three rabbits received CFX i.v. bolus doses of 25, 50 or 100 mg/kg, in a randomized cross-over experimental design. The remaining five rabbits received an oral dose of 25 mg/kg AMX suspension in cherry syrup using a 5 ml needle-less syringe. Rabbits seemed to like the cherry flavor of the AMX suspension. Experiments on the same animal were separated by at least a 1 week washout-period.

The day before the experiment the dorsum of the rabbits was shaved carefully with an electric animal hair clipper. On the day of the experiment, the rabbits were tranquilized with 5 mg/kg i.m. acetopromazine maleate injection (ACE) and allowed a 20 min period for the tranquilization to take place. Two microdialysis probes were implanted in parallel on the rabbit dorsum in the upper and lower half of the back according to the technique described by Stagni *et al.* [17] as superficially as possible using a 25G \times 1.5 inches needle as a guide cannula. The actual depth of the probe was not measured because the appropriate instrument was unavailable. However, the needle was carefully inserted under the skin in such a way that consistently resulted in the needle being clearly visible through the superficial skin layer. In these conditions the depth of the probe was not greater than 2 mm that corresponds to the deep dermis. After about 45 min to allow the skin to recover from the insertion trauma, the probes were connected to the microdialysis pump via Teflon tubing and perfused at a constant flow rate of 2 $\mu\text{l}/\text{min}$. Retrodialysis with a solution of AMX (20 $\mu\text{g}/\text{ml}$) or CFX (8 $\mu\text{g}/\text{ml}$) was performed for 80 min before the administration of the i.v. bolus. Then, the probes were washed with lactated Ringer's solution for 60 min as it was experimentally confirmed that AMX and CFX were below the limit of detection after this time. Finally, the i.v. bolus was administered and dialysis samples were collected every 8 min for AMX and every 10 min for CFX. A 0.8 mL blood sample was

withdrawn from the ear artery immediately before dosing and then at 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min after drug administration. The plasma was separated immediately and stored at -20°C until assayed.

Data analysis

Dialysate skin concentrations were corrected by the retrodialysis recovery value to estimate the actual peri-probe concentrations of drug in the skin. The skin collection times were also corrected to account for the time necessary to travel the dead volume in the outlet tube. The total plasma concentrations were transformed to unbound concentrations by multiplying by the fraction of drug unbound to proteins. Protein binding was estimated *in vitro* by ultrafiltration using a Centrifree[®] Centrifugal filter device (Bedford, MA) at 37°C . The fraction bound was 0.21 ($n = 18$) for AMX and 0.50 ($n = 18$) for CFX. Pharmacokinetic analysis was performed using WinNonlin 5.0.1 (Pharsight Corporation, Mountain View, CA).

Pharmacokinetic analysis

Individual data-set analysis. All individual data set from plasma and skin were analysed by non-compartmental analysis. The following parameters were estimated: *AUC*, clearance, volume of distribution, elimination half-life and C_{max} for plasma; and *AUC*, elimination half-life and C_{max} for skin. The linearity of pharmacokinetics in the skin was assessed by plotting *AUC* from i.v. administrations versus dose. Compartmental analysis was applied to plasma data following i.v. administrations to obtain the initial estimates for the simultaneous fitting presented in the next section. Model selection was based on the Akaike information criteria [18].

Simultaneous fitting of plasma and skin data. Skin and plasma data following iv bolus experiments were fitted simultaneously according to the models shown in Figure 1. The skin compartment was modeled as linked to the plasma by a first order transfer constant but not considered as part of the mass balance for plasma. Plasma concentrations were fitted with a two-compartment

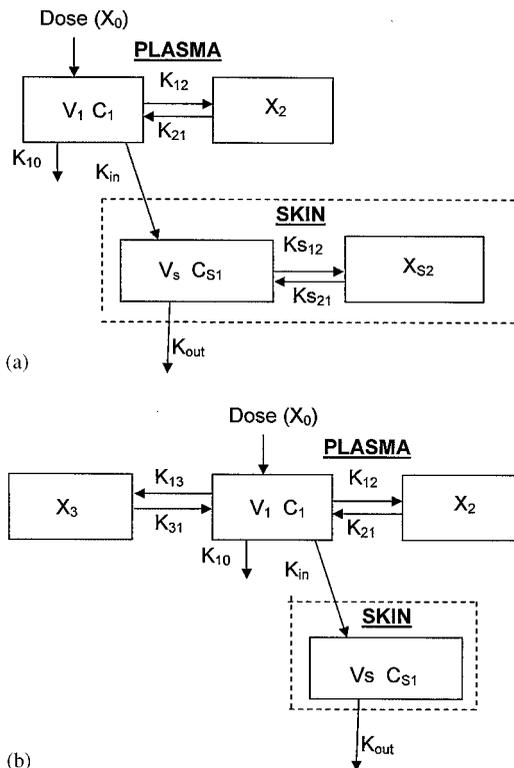


Figure 1. Schematic representation of the models used for simultaneous fitting of plasma and skin pharmacokinetic data: (a) amoxicillin and (b) cefuroxime

model for AMX and three-compartment for CFX. The following differential equations were used for AMX plasma:

$$\frac{dC_1}{dt} = -(K_{12} + K_{10})C_1 + \frac{K_{21}}{V_1} X_2 \quad (3)$$

$$\frac{dX_2}{dt} = -K_{21}X_2 + K_{12}C_1V_1 \quad (4)$$

and for CFX:

$$\frac{dC_1}{dt} = -(K_{13} + K_{12} + K_{10})C_1 + \frac{K_{21}}{V_1} X_2 + \frac{K_{31}}{V_1} X_3 \quad (5)$$

$$\frac{dX_2}{dt} = -K_{21}X_2 + K_{12}C_1V_1 \quad (6)$$

$$\frac{dX_3}{dt} = -K_{31}X_3 + K_{13}C_1V_1 \quad (7)$$

The total amount of AMX in the skin (X_{STot}) can be expressed as:

$$\frac{dX_{STot}}{dt} = Q_s C_{In} - Q_s C_{Out} = \frac{dX_{S1}}{dt} + \frac{dX_{S2}}{dt} \quad (8)$$

where Q_s is the skin perfusion blood flow; C_{in} is the concentration of drug entering the skin and it is considered to be equal to C_1 , the unbound concentration in the plasma compartment; C_{out} is the concentration of drug leaving the skin that is considered to be equal to the concentration measured by microdialysis in the interstitial fluid. X_{S1} is the amount of drug in the skin compartment sampled by microdialysis, and X_{S2} is the amount of drug in the second skin compartment. Defining V_s as the volume of the skin compartment where the microdialysis probe is located, we can write:

$$\frac{dX_{S1}}{V_s dt} = \frac{Q_s C_1}{V_s} - \frac{Q_s C_{S1}}{V_s P} - \frac{K_{S12}}{V_s} X_{S1} + \frac{K_{S21}}{V_s} X_{S2} \quad (9)$$

where K_{S12} and K_{S21} are first order transfer rate constants between the two skin compartments and

$$\frac{dX_{S2}}{dt} = K_{S12} X_{S1} - K_{S21} X_{S2} \quad (10)$$

Skin was modeled as a single compartment for CFX:

$$\frac{dX_{S1}}{V_s dt} = \frac{Q_s C_1}{V_s} - \frac{Q_s C_{S1}}{V_s P} \quad (11)$$

P is the partition ratio and is defined as the ratio of unbound concentration of drug in the skin to the concentration of unbound drug in the plasma. Since Q_s , V_s and P cannot be calculated independently, i.e. they would be highly correlated if set as independent parameters, they were lumped in a single parameter as follows:

$$K_{in} = \frac{Q_s}{V_s} \quad (12)$$

$$K_{out} = \frac{Q_s}{V_s P} \quad (13)$$

Note that from Equations (12) and (13) the partition ratio (P) can also be obtained as a ratio of K_{in} and K_{out} .

Therefore Equation (9) becomes:

$$\frac{dX_{S1}}{V_s dt} = K_{in} C_1 - K_{out} C_{S1} - \frac{K_{S12}}{V_s} X_{S1} + \frac{K_{S21}}{V_s} X_{S2} \quad (14)$$

and Equation (11) becomes:

$$\frac{dX_{S1}}{V_s dt} = K_{in} C_1 - K_{out} C_{S1} \quad (15)$$

The parameter K_{in} can be interpreted as the transfer rate constant between plasma and skin and K_{out} as the transfer rate constant between skin and plasma.

In summary Equations (3), (4), (14) and (10) were implemented into a WinNonlin model-program to fit simultaneously plasma and skin data for AMX; while Equations (5), (6), (7) and (15) were used for CFX.

Model validation. For AMX the parameters K_{in} , K_{out} , K_{S12} and K_{S21} estimated following i.v. bolus doses were used to predict skin concentrations from an oral administration of 25 mg/kg to five different rabbits by using Equations (14) and (10) where C_1 is the plasma concentration measured following the oral administration. The predicted skin concentrations of AMX were then compared with those actually measured by microdialysis following the oral administrations.

Since CFX is clinically administered i.v. or i.m., the K_{in} and K_{out} estimated from the high (100 mg/kg) and low (25 mg/kg) doses were used to predict the skin concentrations following the medium dose (50 mg/kg); that is the plasma concentrations measured from the medium dose were plugged in Equation (15), where the K_{in} and K_{out} were those estimated from the other administrations. Again the predicted skin concentrations of CFX were compared with those actually measured by microdialysis following the i.v. dose of 50 mg/kg.

Prediction of pharmacological effects. The proposed model was then used to predict both plasma and skin concentrations following clinically relevant doses (7.14 mg/kg for AMX and 10.71 mg/kg for CFX) typically used to treat skin and skin structure infections. The time above the

minimum inhibitory concentrations against two different strains of *Bacillus anthracis* (ST-1, MIC: 0.06 µg/ml and Sterne, MIC: 0.125 µg/ml [19]) for AMX and against *Staphylococcus aureus* (MIC: 0.5 to 2 µg/ml [20]) and *Streptococcus pyogenes* (MIC: 0.5 µg/mL [21]) for CFX, were measured as a PK/PD index.

Statistical analysis

ANOVA and *t*-test were performed using SPSS 15 (SPSS Inc., Chicago, IL). The Scheffé *post hoc* procedure for multiple comparisons was applied to detect differences between means. The significance level was 0.05. Linear regression for calibration curves was performed with Microsoft® Office Excel 2003 (Microsoft, Corp, Seattle, WA).

Results

The rabbits did not show any signs of intolerance for the microdialysis probes during the 7–8 h long experiments. Microdialysis probes were inserted easily in the skin without topical anesthesia. Visual inspection of rabbit skin at the end of the experiments and after 24 and 48 h did not show any signs of irritation. The rabbits tolerated well the i.v. bolus and oral doses, however, diarrhea was observed in one rabbit about 48 h after an AMX i.v. bolus dose of 100 mg/kg. *In vivo* average retrodialysis recovery for AMX was $53 \pm 4.0\%$ ($n = 14$) and for CFX was $55 \pm 1.8\%$ ($n = 16$) and there was no significant statistical difference between different experiments (*t*-test, $p > 0.05$), confirming that recovery was consistent and reproducible.

Individual data set analysis

Amoxicillin. Figure 2 shows the average unbound plasma and skin concentrations. The plasma concentration–time profiles had a remarkable distribution phase with a bi-exponential decay. The skin concentrations increased according to a first order input and decreased with a bi-exponential decay. The maximum concentration in skin was reached approximately at the end of the first linear phase of the plasma

concentration profile on semi-log paper. After that, skin concentrations decreased in an almost parallel fashion with respect to plasma. Pharmacokinetic parameters are reported in Table 1. Data collected at each microdialysis probe were analysed independently. Sometimes, a probe stopped functioning during the experiment therefore n is sometimes less than 6. The area under the plasma and skin concentration curve increased linearly with the dose ($r^2 = 0.99$). Also the ratios $AUC_{\text{skin}}/AUC_{\text{plasma-Ub}}$ were constant suggesting that the exposure of AMX in skin and plasma was proportional and dose independent. The terminal half-lives in both plasma and skin were similar and independent of dose ($p > 0.05$).

Cefuroxime. Figure 2 shows that plasma concentration–time profiles had a tri-exponential decay, and skin concentration increased according to a first order input and decreased with a mono-exponential decay. Also in this case, the maximum concentration in skin (Table 1) was reached at the end of the first linear decline of the plasma concentration profile on semi-log paper in plasma. Pharmacokinetic parameters are reported in Table 1. The area under the plasma and skin concentration curve increased linearly with respect to dose ($r^2 = 0.99$). The $AUC_{\text{skin}}/AUC_{\text{plasma-Ub}}$ ratios suggest that the exposure of CFX in skin and plasma are

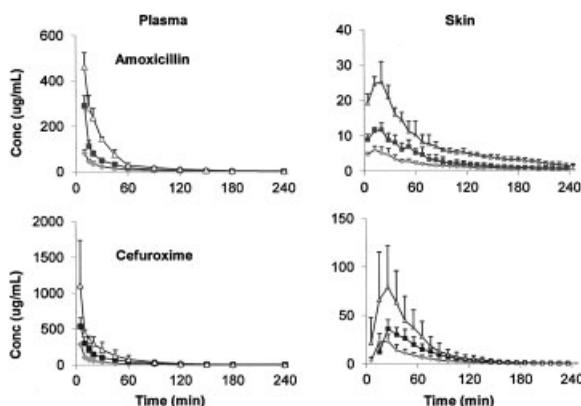


Figure 2. Upper panels: time course of AMX in plasma and skin (mean \pm SD, $n = 3$) following i.v. boluses of 25 (○), 50 (■) and 100 (△) mg/kg; Lower panel: time course of CFX in plasma and skin (mean \pm SD, $n = 3$) following i.v. boluses of 25 (○), 50 (■) and 100 (△) mg/kg

Table 1. Pharmacokinetic parameters (mean \pm SD) in skin and plasma following i.v. bolus dose of 25, 50, 100 mg/kg for AMX and CFX

	Amoxicillin			Cefuroxime		
	25 mg/kg (n = 3)	50 mg/kg (n = 3)	100 mg/kg (n = 3)	25 mg/kg (n = 3)	50 mg/kg (n = 3)	100 mg/kg (n = 3)
Half-life (min)	Plasma 87 \pm 20 (n = 3)	79 \pm 7 (n = 3)	111 \pm 54 (n = 3)	90 \pm 23 (n = 3)	104 \pm 32 (n = 3)	78 \pm 28 (n = 3)
C_{max} (μ g/ml)	Skin ^a 84 \pm 14 (n = 4)	66 \pm 27 (n = 5)	69 \pm 13 (n = 4)	30 \pm 6 (n = 6)	32 \pm 1 (n = 6)	30 \pm 2 (n = 6)
	Plasma 101 \pm 33 (n = 3)	286 \pm 71 (n = 3)	515 \pm 143 (n = 3)	286 \pm 17 (n = 3)	538 \pm 120 (n = 3)	1111 \pm 626 (n = 3)
	Skin ^a 6.2 \pm 1.1 (n = 4)	12 \pm 1.3 (n = 5)	28 \pm 2.9 (n = 4)	29 \pm 3 (n = 6)	36 \pm 9 (n = 6)	94 \pm 37 (n = 6)
AUC (min \cdot μ g/ml)	Plasma 2425 \pm 129 (n = 3)	5750 \pm 974 (n = 3)	11 408 \pm 2068 (n = 3)	4273 \pm 1100 (n = 3)	12 640 \pm 2337 (n = 3)	19 530 \pm 4837 (n = 3)
	Skin ^a 443 \pm 62 (n = 4)	841 \pm 215 (n = 5)	1907 \pm 89 (n = 4)	937 \pm 137 (n = 6)	2307 \pm 397 (n = 6)	4076 \pm 1515 (n = 6)
T_{max} (min)	14 \pm 4 (n = 4)	15 \pm 8 (n = 5)	16 \pm 5 (n = 4)	18 \pm 6 (n = 6)	25 \pm 0 (n = 6)	22 \pm 6 (n = 6)
Volume of distribution (l/kg)	Plasma 0.3 \pm 0.08 (n = 3)	0.2 \pm 0.05 (n = 3)	0.2 \pm 0.06 (n = 3)	0.3 \pm 0.1 (n = 3)	0.2 \pm 0.1 (n = 3)	0.2 \pm 0.1 (n = 3)
Clearance (ml/min/kg)	Plasma 10.3 \pm 0.6 (n = 3)	8.9 \pm 1.4 (n = 3)	9.0 \pm 1.7 (n = 3)	6.1 \pm 1.7 (n = 3)	4.0 \pm 0.7 (n = 3)	5.3 \pm 1.3 (n = 3)

^aIn skin, n represents data analysis for each microdialysis probe.

proportional and dose independent. The elimination half-lives in both plasma and skin were also independent of dose ($p > 0.05$). However, the terminal half-lives in skin and plasma were different ($p < 0.05$), showing that the drug cleared faster from the skin than from the plasma.

Simultaneous analysis of plasma/skin data

Skin and plasma concentrations were fit simultaneously for each experiment. Since the dermis concentrations of AMX and CFX collected at the two probes during the same experiment were similar (t -test, $p > 0.05$) the average skin concentrations were used for the simultaneous fitting of plasma and skin data. The transfer constants between plasma/skin (K_{in}) and skin/plasma (K_{out}) could be estimated with fair precision as shown by a CV% not larger than 79 (Table 2). Figure 3 shows an example of simultaneous fitting of plasma and skin for AMX and CFX, respectively. The values of parameters K_{in} and K_{out} (mean \pm SD) are reported in Table 2. ANOVA showed that the transfer constants estimated from the administration of different doses were not statistically different ($p > 0.05$) suggesting that the transfer constants were independent of dose. Consequently this shows that the estimated transfer constants K_{in} and K_{out} could possibly be used to predict the skin concentrations following any plasma concentration.

Model validation

Figure 4 shows the average skin concentrations, predicted using the previously estimated transfer constants (K_{in}) and (K_{out}), compared with those actually measured by microdialysis for AMX ($n = 5$) and CFX ($n = 3$), respectively. It can be seen that the predicted values were always within the standard deviation of the measured concentrations. Plots of observed skin concentrations versus model-predicted concentrations were linear, further confirming the validity of the predictions. These results demonstrate that the predicted concentrations are similar to those measured, and therefore it can be concluded that the estimated transfer constants (K_{in}) and (K_{out}) allow a reliable prediction of skin concentrations of AMX and CFX from plasma levels.

Table 2. Transfer constants and partition ratios for AMX ($n = 3$) and CFX ($n = 3$) following i.v. bolus doses reported as mean \pm SD

	Dose (mg/kg)	K_{in} (1/min)	CV% ^a	K_{out} (1/min)	CV% ^a	P (K_{in}/K_{out})	P ($AUC_{skin}/AUC_{plasma-ub}$)
AMX	25	0.02 ± 0.01	45	0.08 ± 0.05	42	0.22 ± 0.02	0.18 ± 0.02
	50	0.01 ± 0.01	61	0.06 ± 0.02	56	0.22 ± 0.08	0.15 ± 0.05
	100	0.01 ± 0.00	79	0.05 ± 0.01	75	0.16 ± 0.03	0.16 ± 0.03
CFX	25	0.04 ± 0.03	55	0.1 ± 0.1	49	0.32 ± 0.07	0.23 ± 0.04
	50	0.05 ± 0.04	17	0.2 ± 0.1	14	0.26 ± 0.05	0.18 ± 0.03
	100	0.03 ± 0.01	22	0.1 ± 0.1	18	0.24 ± 0.02	0.20 ± 0.03

^aCV% is the coefficient of variation calculated as the parameter-standard-error divided by the corresponding parameter-estimate*100 [18].

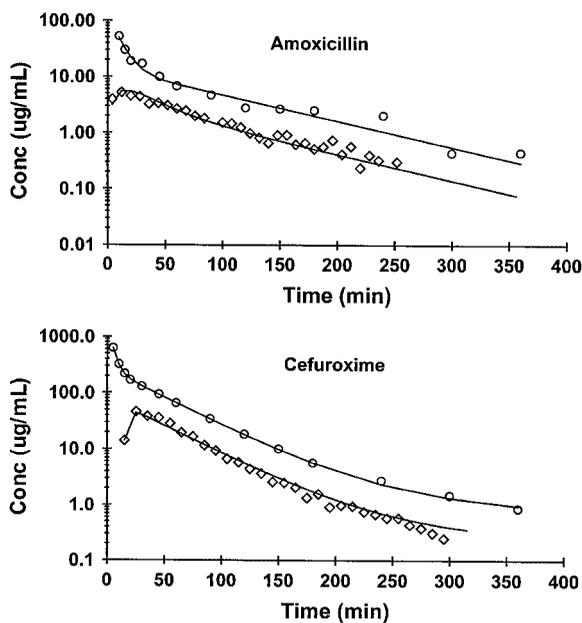


Figure 3. Examples of plasma (\circ) and skin (\diamond) simultaneous fitting in semi-log scale. Upper panel: AMX (25 mg/kg; i.v. bolus); Lower panel: CFX (50 mg/kg; i.v. bolus)

Prediction of pharmacological effect

It has been demonstrated that for β -lactam antibiotics the time spent above the MIC is directly correlated with the effect [2]. In an attempt to show a practical application of the proposed model, the plasma and the skin concentrations from clinically relevant doses were predicted and the time above the MICs against some bacteria were measured. The MICs used in this project were obtained *in vitro* and are defined as the lowest concentration of drug that inhibits more than 99% of the bacterial population. Therefore, MICs are bacterium and antibiotic dependent but not host dependent.

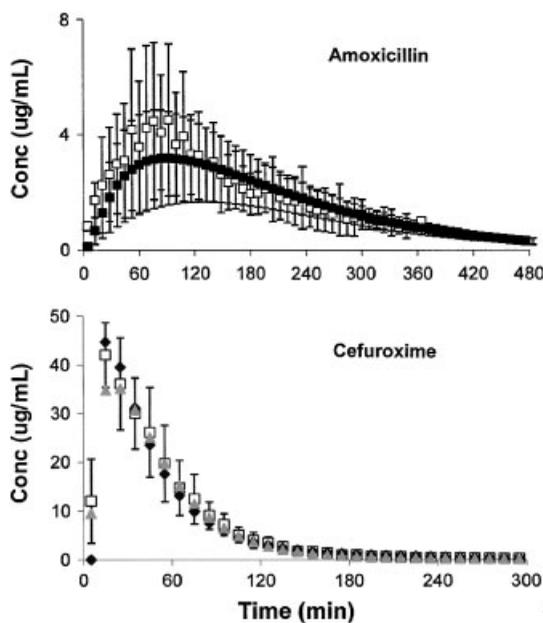


Figure 4. Upper panel: Comparison of measured (\square) (mean \pm SD) vs predicted (\blacksquare) (mean \pm SD) skin concentrations of AMX following oral administrations of 25 mg/kg ($n = 5$); Lower panel: Comparison of concentrations of CFX measured in skin following an i.v. bolus administrations of 50 mg/kg (\square) (mean \pm SD, $n = 3$) with the skin concentrations predicted from model Equation (15) using the transfer constants estimated from the 25 (\blacklozenge) and 100 (\blacktriangle) mg/kg administrations, respectively

Skin concentrations of AMX remained above the MIC against *Bacillus anthracis* [19] strain: ST-1 (MIC $0.06 \mu\text{g/ml}$) for 7.9 ± 0.4 h ($n = 5$); and against strain: Sterne (MIC $0.125 \mu\text{g/ml}$) for 7.2 ± 0.7 h ($n = 5$). While the corresponding plasma concentrations of AMX remained above the MIC against *Bacillus anthracis* strain: ST-1 (MIC $0.06 \mu\text{g/ml}$) for 7.8 ± 0.3 h ($n = 5$); and against strain: Sterne (MIC

0.125 µg/ml) for 7.6 ± 0.8 h ($n = 5$). Considering a typical 8 h dosing interval, both the skin and plasma concentrations remained above the MIC for almost the entire dosing interval in all rabbits except one, showing that AMX plasma concentrations are good predictors of drug activity in the skin for both strains of anthrax bacterium.

In the case of CFX, skin concentrations remained above the MIC against *Staphylococcus aureus* lower limit (MIC 0.5 µg/ml) for 2.3 ± 0.1 h ($n = 3$) and against the *Staphylococcus aureus* upper limit (MIC 2 µg/ml) for 1.4 ± 0.1 h ($n = 3$); while the corresponding plasma concentrations remained above MIC 0.5 µg/ml for 3.3 ± 0.3 h ($n = 3$) and above MIC 2 µg/ml for 1.9 ± 0.3 h ($n = 3$). On the other hand, skin concentrations of CFX remained above the MIC against *Streptococcus pyogenes* (MIC 0.5 µg/ml) for 2.3 ± 0.1 h ($n = 3$); while the corresponding plasma concentrations remained above the MIC for 3.3 ± 0.3 h ($n = 3$).

For CFX, the skin concentrations remained above the MIC against *Staphylococcus aureus* [20] for only 18–28% of the dosing interval while plasma concentrations remained above the MIC for 23–40% of the dosing interval. In the case of *Streptococcus pyogenes* [21] the skin concentrations of CFX remained above the MIC for 28% of the dosing interval while plasma concentrations remained above the MIC for 40% of the dosing interval. These findings show a possibly clinically relevant difference in time above MIC between plasma and skin that needs to be addressed in further studies.

Discussion

The objective of this study was to develop and validate a pharmacokinetic model to predict skin interstitial concentrations from plasma levels. The idea was to estimate the transfer rate constants plasma/skin and skin/plasma from an experiment in which both plasma and skin interstitial concentrations were measured and then to use the estimated parameters to predict the interstitial skin concentration from any plasma level as needed. The study was conducted in rabbits in order to demonstrate the feasibility of the approach.

Skin is a tissue to which hydrophilic molecules such as β-lactam antibiotics distribute by passive diffusion most likely via the gaps between cells at the capillary level. Compared with other barriers in the body that control the distribution of compounds to the site of action, e.g. the blood–brain barrier, this type of barrier is considered as not rate-limiting and distribution is mostly controlled by blood flow [22]. Ideally, at steady state, the concentration of unbound drug should be the same in the tissue interstitial fluid and in plasma water. Consequently, the use of plasma concentrations as a predictor of tissue concentration is justified. However, for single dose administrations or intermittent multiple doses the interstitial fluid concentration may differ between the two sites depending on how long it takes to reach distributional equilibrium. For drugs whose effect is strictly correlated to the length of time above a certain concentration, as for antibiotics, the determination of the concentration–time course at the site of action is essential for a successful therapy. Microdialysis permits the sampling of the tissue interstitial fluid concentration in a relatively safe and non-invasive way, but it is technically more demanding than blood sampling. Therefore the possibility to predict skin interstitial concentrations from plasma levels would be very helpful in pre-clinical and clinical drug development. This is the first time, to the best of our knowledge, that the simultaneous fitting of non-steady-state data has been attempted for plasma and microdialysis data in the skin.

In order to develop a model for prediction of skin concentrations, dialysate and plasma samples were collected serially and then several models were tested before opting for the model depicted in Figure 1. Specifically the following approaches were carefully investigated: (i) skin was considered as part of one of the peripheral tissue compartments; (ii) skin was modeled as independent compartment linked to plasma by bi-directional rate constants; (iii) skin was modeled as an independent, single or multiple compartments but not counted in the mass balance of plasma. Indeed for AMX, it was necessary to add a deeper compartment in skin in order to obtain an appropriate fitting. The third approach gave the best results based on

analysis of residuals, CV% of parameters and AIC [23]. Not counting the microdialysis sampling compartment as part of the plasma mass balance has been adopted in several modeling approaches proposed when linking plasma and microdialysis data [24]. The assumption that the skin compartment is negligible in the mass balance of plasma data helps in reducing the number of parameters and in the stabilization of the minimization process. This was a data-rich type of approach in which the plasma and skin concentrations time courses were well characterized in the same subject, and replicate and dose escalation could also be done in the same subject too. Therefore the use of a population approach was not necessary. The model developed to link skin to plasma concentration time course (Equations (8)–(10)) is an attempt to integrate physiologically relevant parameters in an otherwise empirical approach. It is an effort to explain the distribution to skin in terms of blood flow and partitioning between the two tissues, parameters that may be estimated independently and possibly can be used to predict how changes in these physiological variables can affect the distribution. The model was inspired by that developed for the distribution to brain of fluvoxamine [24], a drug whose distribution to brain is perfusion limited. The partition ratio becomes equal to unity when the concentration in the two tissues' extracellular fluid becomes equal. It is interesting to note that P is similar to the ratio of the $AUC_{\text{skin}}/AUC_{\text{plasma-u}}$ (Table 2). Probe recovery was calculated by *in vivo* retrodialysis. The method was tested previously *in vitro* by measuring both gain and loss [25] for escalating concentrations. In this way, it was demonstrated that recovery is independent of concentration and that gain and loss are similar, substantiating the hypothesis that retrodialysis is a suitable method for the estimation of recovery. *In vivo* and *in vitro* recoveries were also similar ($p > 0.05$) suggesting that AMX and CFX move freely in the skin interstitial space.

Table 2 shows that K_{in} and K_{out} were estimated with acceptable precision for both drugs with a CV% lower than 79 [23]. The external validation of the proposed model showed a good agreement between predicted and observed skin concentrations of AMX and CFX (Figure 4). The range of

doses used in this study was selected on the bases of the assay sensitivity rather than of clinical relevance. Since the linearity of distribution was demonstrated for both AMX and CFX, the model was then used to predict the time course of AMX and CFX in skin following lower doses, typically used to treat infections by *Bacillus anthracis* for AMX and by *Staphylococcus aureus* or *Streptococcus pyogenes* for CFX. AMX concentrations in skin remained above the MIC against the two different strains of *Bacillus anthracis*, ST-1 and Sterne throughout the entire dosing interval, conversely CFX concentrations remained above the MIC against *Staphylococcus aureus* and *Streptococcus pyogenes* for less than 50% of the dosing interval pointing to the possibility that skin suboptimal concentrations may lead to therapy failure or development of resistance. Therefore using plasma concentrations of CFX may lead to an overestimation of exposure time in skin. Naturally, these findings must be further verified in the presence of disease and in human subjects [26].

Inspection of the concentration profiles in plasma and skin (Figure 2) and of the pharmacokinetic parameters presented in Table 1 shows that AMX reached distributional equilibrium as the terminal half-lives are similar. For CFX instead the terminal half-lives appear different possibly because the equilibrium phase in skin occurred when the concentration were below the detection limit. Therefore the contribution of this terminal phase to the skin concentration would be negligible. In addition, it can be noted that both K_{in} and K_{out} are larger for CFX than AMX, suggesting that the exchange of drug between the two tissues occurs faster for CFX than AMX.

Microdialysis was used to study the pharmacokinetics of AMX in muscle interstitial fluid of rats [27] and in middle ear of chinchilla [28], while in the case of CFX microdialysis was applied to study the pharmacokinetics in the skeletal muscle of humans during cardiac surgery [29] and in the rat brain [12]. This is the first time microdialysis has been applied to study the dermal pharmacokinetics of AMX and CFX in rabbits. According to Marchand *et al.* [27] the exposure of AMX in rat muscle was similar to the unbound plasma concentration, conversely it was found in this study that skin exposure of

AMX is only 17% of the unbound plasma levels. While for CFX the skin exposure was 20% of the unbound plasma exposure. The observed low tissue exposure might be due to the fact that skin is a less perfused tissue compared with muscle. Indeed, blood flow to the skin is 300 ml/min; while blood flow to the inactive muscle is 750 ml/min [22].

In conclusion, a semi-physiological model was developed to predict skin interstitial concentrations from any plasma level once the transfer rate constants between the tissues are estimated. The underlying assumption is that the skin interstitial concentration is a linear, time independent function of plasma levels, as it was demonstrated by administering three increasing doses of drug. The experimental design and the model proposed could possibly be used in preclinical and clinical practice to predict the time course of antibiotics with linear distribution to skin. This approach may be extremely helpful in drug development and dose selection for investigational antibiotics.

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