Pharmacokinetic Modeling of Free Amoxicillin Concentrations in Rat Muscle Extracellular Fluids Determined by Microdialysis

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The aim of the present study was to investigate amoxicillin (AMX) distribution in muscle interstitial fluid by microdialysis in healthy, awake rats. Microdialysis probes were inserted into the jugular vein and hind leg muscle. Probe recoveries in each rat were determined by retrodialysis with cefadroxil. AMX was administered as a bolus dose of 50 mg · kg⁻¹, and microdialysis samples were collected during 180 min. Concentrations of unbound drug in blood and muscle were analyzed simultaneously by a population approach. Simulations were conducted using a hybrid, physiologically based pharmacokinetic model to investigate the potential impact of tissue blood flow on muscle AMX distribution. A two-compartment pharmacokinetic model described adequately the unbound amoxicillin concentration-time profiles in blood and muscle. Muscle AMX distribution equilibrium was rapidly achieved. Consequently, the best results were obtained by considering concentrations in muscle as part of the central compartment. The ratio of the concentration of unbound drug in muscle to that in blood (R_{model}) was estimated to 0.80 by the model, which is close to the mean value obtained by noncompartmental data analysis ($R_{\text{area}} = 0.86 \pm 0.29$). Simulations conducted with a hybrid, physiologically based pharmacokinetic model suggest that a muscle blood flow reduction of 30% to 50%, such as could be encountered in critical care patients, has virtually no effect on muscle AMX concentration profiles. In conclusion, this study has clearly demonstrated that AMX distributes rapidly and extensively within muscle interstitial fluid, consistent with theory, and that altered muscle blood flow seems unlikely to have a major effect on these distribution characteristics.

Microdialysis allows multiple determinations of free drug concentrations within tissue extracellular fluids over time (7). It has recently been increasingly used to investigate the distribution of antibiotics, in particular amino- β -lactam compounds, including cefaclor (8), ceftriaxone (12), cefpodoxime (13), cefpirome (11), piperacillin (3, 5, 15, 20), imipenem (19), and meropenem (21), in muscle interstitial fluid (MIF) both in laboratory animals (5, 8, 12, 13, 15) and in humans (3, 11, 13, 19, 20, 21). Muscle was chosen because it is easier to access than other target tissues or organs such as lung, where infection may occur, and because muscle interstitial fluid drug concentrations are likely to accurately reflect concentrations in interstitial fluid in other tissues without physiological barriers, such as lung (14). Clinical studies have reported that piperacillin (3) and imipenem (19) distributions are reduced in critical care patients, which was attributed in part to altered blood flow. The distribution of amino- β -lactam antibiotics in MIF is presumably rate limited by tissue blood flow (16). Therefore, decreased blood flow may actually have an effect at least on the rate of distribution. The objective of this study was to precisely characterize the MIF distribution of one of these antibiotics in a well-controlled microdialysis experiment in animals, with special reference to the potential effect of tissue blood flow reduction on the MIF distribution rate. Because in a previous study (14), the imipenem distribution in MIF in rats appeared

to be almost instantaneous, this particular compound did not seem to be a good candidate for this investigation. Amoxicillin (AMX) was a better candidate, not only because its distribution in MIF had never been investigated using microdialysis (although it is one of the most frequently used antibiotic), but more importantly because previous experiments in animals have demonstrated a multiexponential decay of plasma AMX concentrations with time (22, 23), suggesting slow and more complex equilibration processes that are more likely to be affected by changing blood flow. Therefore, the objective of the present study was to investigate the AMX distribution within MIF of awake rats.

MATERIALS AND METHODS

Chemicals. AMX and cefadroxil were purchased from Sigma (Saint-Quentin Fallavier, France). AMX sodium, used for intravenous (i.v.) administration, was obtained from GlaxoSmithKline (Clamoxyl, France). Solvents, including water, were of analytical grade.

Animals. Eleven male Sprague-Dawley rats from Janvier Laboratories (Le Genest-St-Isle, France), weighing 333 ± 49 g (mean $\pm$ standard deviation), were used and handled as previously described (14). This work was done in accordance with the Principles of Laboratory Animal Care (NIH publication no. 85-23, 1985).

Vein and muscle probe implantation. The day before the experiment, rats were anesthetized by isoflurane (Forene; Abbot, Rungis, France) inhalation (14). A polyethylene cannula was inserted into the left femoral vein for drug administration, and two CMA/20 probes (polycarbonate; membrane length, 10 mm) (CMA microdialysis; Phymep, Paris, France) were inserted into the right jugular vein and the right hind leg muscle as previously described (14). In brief, an incision was made in the skin to expose the right pectoral muscle. An introducer (corresponding to a needle inserted into a tubing) was inserted through the pectoral muscle into the right jugular vein. By removing the needle, the microdialysis CMA/20 probe, perfused with 0.1% low-weight heparin at a flow rate of 3.5 $\mu\text{l} \cdot \text{min}^{-1}$ (CMA 100 microdialysis pump; Phymep, Paris, France), was inserted through the tubing. The probe was then secured by suture on the

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pectoral muscle, and the tubing was removed. The right hind leg was then exposed, and the probe, perfused with Ringer solution (perfusion fluid T1 for peripheral tissues) (CMA microdialysis; Phymep), was inserted with the help of an introducer. The inlet and the outlet of probes as the femoral catheter were passed subcutaneously to exit at the nape. Rats were allowed to recover consciousness. During the entire night, the muscle probe was perfused with blank Ringer solution at a flow rate of $0.5 \mu\text{l} \cdot \text{min}^{-1}$. Food was withdrawn approximately 12 h before the experiment, but animals had free access to water.

Microdialysis experiment. The day of the experiment, the CMA/20 vein probe and the muscle probe were perfused at a flow rate of $0.5 \mu\text{l} \cdot \text{min}^{-1}$ with Ringer solution for 1.3 h to stabilize the system and to obtain a blank sample. A retrodialysis period was then started by changing the blank perfusion solution to Ringer solution containing cefadroxil ($8,000 \text{ nmol} \cdot \text{liter}^{-1}$) as a calibrator (10). Equilibration was performed for 2 h before the bolus administration of AMX ($50 \text{ mg} \cdot \text{kg}^{-1}$) via the left femoral vein. The AMX solution was prepared by dissolving an adequate amount of AMX sodium salt in 0.9% NaCl so that the final volume of administration was set to 1 ml. Microdialysate samples were collected automatically with a CMA/140 microfraction collector (CMA microdialysis; Phymep) for 180 min in fractions corresponding to 7.5 min intervals during the first 30 min, to 10-min intervals until the end of the first hour, and to 20-min intervals during the final 120 min. All dialysates were analyzed on the day of experiment.

To determine the *in vivo* recovery, the concentrations of cefadroxil in the perfusate (C_{in}) and in dialysates (C_{out}) were determined by high-performance liquid chromatography. This *in vivo* relative recovery by loss was expressed as a percentage ($\text{RL}_{\text{in vivo}}$) and calculated according to equation 1:

$$\text{RL}_{\text{in vivo}} = [(C_{\text{in}} - C_{\text{out}})/C_{\text{in}}] \times 100 \quad (1)$$

Measured AMX concentrations in each individual dialysate were corrected by the corresponding estimated $\text{RL}_{\text{in vivo}}$ value.

Microdialysis sample analysis. Microdialysis samples were directly injected onto a Kromasil C_{18} column ($5\text{-}\mu\text{m}$ particles, 250 by 3 mm [inside diameter]; Varian, Les Ulis, France). The chromatographic system consisted of a Shimadzu LC-10AS pump (Croissy Beaubourg, France) and a CMA 200 refrigerated micro-sampler (Phymep, Paris, France) connected to a UV detector (SPD 10A Shimadzu UV detector) at 225 nm . Data were recorded and analyzed on a Kromasystem integrator (Bio-Tek, St. Quentin en Yvelines, France). The mobile phase consisted of 95% monobasic potassium phosphate buffer (0.067 M) and 5% (vol/vol) of methanol at a flow rate of $0.5 \text{ ml} \cdot \text{min}^{-1}$. With an $8\text{-}\mu\text{l}$ injection volume, the limit of quantification of AMX in microdialysates was $250 \text{ nmol} \cdot \text{liter}^{-1}$. The within-day variability of the method was characterized for AMX and cefadroxil at one concentration ($4,000 \text{ nmol} \cdot \text{liter}^{-1}$ for AMX and $8,000 \text{ nmol} \cdot \text{liter}^{-1}$ for cefadroxil) but at three volumes of injection (2.5 , 5 , and $8 \mu\text{l}$) and was always below 6%. The between-day variabilities for AMX and cefadroxil were characterized at $4,000 \text{ nmol} \cdot \text{liter}^{-1}$ and $8,000 \text{ nmol} \cdot \text{liter}^{-1}$, respectively, and at three volumes of injection (2.5 , 5 , and $8 \mu\text{l}$) and were always below 13%.

Noncompartmental pharmacokinetic analysis. The total areas under the concentration-versus-time curves (AUCs) for muscle interstitial fluid (AUC_{MIF}) and blood ($\text{AUC}_{\text{blood}}$) were estimated using the trapezoidal method with extrapolation to infinity according to standard procedures (14), using the software WinNonLin (professional edition, version 1.5; Pharsight Corporation, Mountain View, CA). Corresponding $\text{AUC}_{\text{MIF}}/\text{AUC}_{\text{blood}}$ ratios were estimated individually and are referred to as R_{area} .

Simultaneous modeling of concentrations of unbound drug in blood and muscle interstitial fluid by a population approach. Data for unbound drug concentrations in blood and MIF were analyzed simultaneously using various multicompartment pharmacokinetic models, with concentrations in MIF being part of the central or peripheral compartments. A two-compartment model with MIF drug concentrations within the central compartment allowed the best fitting. It was parameterized for clearance (CL), steady-state volume of distribution (V_{ss}), central-to-total volume ratio (AA), intercompartmental clearance (Q), and unbound MIF-to-blood drug concentration ratio equal to R_{model} at any time. A population approach was used, and interanimal variability modeled exponentially was added on CL, V_{ss} , and R_{model} .

In the exponential variance model (equation 2), P_i and P_{pop} are the parameters for the i th ($i = 1, \dots, n$) subject and the mean population estimates, respectively. η_i is a zero-mean and normally distributed variable with standard deviation ω , which has been estimated according to equation 2:

$$P_i = P_{\text{pop}} \times \exp(\eta_i) \quad (2)$$

To characterize the residual variability, a combined random error model was used for concentrations of unbound drug in blood ($Y_{\text{obs, plasma}}$) as well as in MIF

($Y_{\text{obs, MIF}}$). For the j th site of sampling ($j = 1$ or 2 for plasma or MIF, respectively), the general model is described by equation 3, where $Y_{\text{pred},j}$ is the predicted concentration, $\epsilon_{1,j}$ is a zero-mean normally distributed variable with standard deviation $\sigma_{1,j}$ given for the additive random error, and $\epsilon_{2,j}$ is a zero-mean normally distributed variable with standard deviation $\sigma_{2,j}$ given for the proportional random error:

$$Y_{\text{obs},j} = Y_{\text{pred},j} + \epsilon_{1,j} + (Y_{\text{pred},j} \times \epsilon_{2,j}) \quad (3)$$

The population parameters were estimated by maximum likelihood with the software NONMEM (nonlinear mixed-effects model) version V and using the first-order conditional estimate interaction method (1). The adequacy of the model was checked by visual inspection of the residuals as well as the predictions (individual and population). Population pharmacokinetic parameter estimates are given, with the relative standard error (RSE) of estimation expressed as a percentage.

PB-PK model simulations. A previously described (17, 18) blood flow-limited, hybrid physiologically based pharmacokinetic (PB-PK) model was used to simulate AMX concentrations in MIF (C_{MIF}) by using equation 4

$$V_{\text{MIF}} \frac{dC_{\text{MIF}}}{dt} = Q_M \left(C_b - \frac{C_{\text{MIF}}}{R_{\text{area,av}}} \right) \quad (4)$$

where C_b correspond to experimentally determined concentrations of unbound AMX in blood and $R_{\text{area,av}}$ to the mean of the individual R_{area} values previously determined (equal to 0.86). V_{MIF} is muscle interstitial fluid volume, and Q_M is muscle blood flow. In these simulations, estimates of V_{MIF} and Q_M of rats were obtained from the literature (2, 4). V_{MIF} was fixed to 14.7 ml , corresponding to approximately 11% of muscle tissue (4), with the percentage of muscle tissue approximately equal to 40.4% of total body weight (2). Q_M was fixed to $30.7 \text{ ml} \cdot \text{min}^{-1}$, corresponding to approximately 28% of the total blood flow (2). C_b values were obtained from the population modeling approach described above but using blood data only. Interindividual variability was modeled exponentially and added on CL and V_{ss} , and a combined random error model was used to characterize the residual variability. Parameters were estimated using NONMEM with the first-order conditional estimate interaction method, and the population pharmacokinetic model was used as a forcing function in the hybrid PB-PK model. A total of 1,000 simulations were generated to take into account the variability in the population, using the MATLAB (version 6.1) software. The 95% prediction interval with the median was obtained from the 1,000 simulations and compared with the observed data for AMX concentrations in MIF estimated by microdialysis.

To assess the effect of muscle blood flow on MIF AMX distribution, a series of simulations was conducted, letting Q_M vary between 100% and 1% of its physiological value.

RESULTS

Probes recoveries varied between $48.1\% \pm 5.2\%$ and $87.2\% \pm 5.1\%$ in blood and between $56.2\% \pm 6.6\%$ and $83.9\% \pm 12.2\%$ in muscle but were stable over the full study duration (180 min). Mean concentrations of unbound AMX in blood and MIF were virtually identical at any time, except for the first dialysate fractions collected during the initial 7.5 min postdosing, in which concentrations were lower in MIF than in blood (Fig. 1). However, MIF concentrations were most often (8/11 instances) maximum in the first dialysate fraction (0 to 7.5 min), demonstrating that distribution equilibrium was achieved within this initial collection period. Accordingly, the best modeling results were obtained when MIF concentrations were considered as part of the central compartment of a two-compartment model. In particular, no trend was observed in checking residuals, and a good correlation was observed between predicted and observed concentrations when MIF was considered part of the central compartment (Fig. 2a) but not when it was considered part of the peripheral compartment (Fig. 2b). The corresponding R_{model} was estimated at 0.80 with 40% interanimal variability, which was close to the mean value obtained by individual noncompartmental data analysis

FIG. 1. Unbound AMX concentrations (means $\pm$ standard deviations) in blood (closed circles and solid line; $n = 11$) and in muscle (open circles and dashed line; $n = 11$) after i.v. bolus administration of AMX at a dose of $50 \text{ mg} \cdot \text{kg}^{-1}$ ($137 \text{ } \mu\text{mol} \cdot \text{kg}^{-1}$).

($R_{\text{area,av}} = 0.86 \pm 0.29$). Other pharmacokinetic population parameters are displayed in Table 1. RSEs of estimation obtained from NONMEM were satisfactory under the common rules ($\leq 20\%$ for mean population parameters and $\leq 50\%$ for

TABLE 1. Population pharmacokinetic parameter estimates of the mean population time course of amoxicillin after i.v. bolus administration of $50 \text{ mg} \cdot \text{kg}$ of body weight $^{-1}$ ($137 \text{ } \mu\text{M} \cdot \text{kg}^{-1}$) in rat, using a two-compartment model and considering concentrations in MIF as part of the central compartment, with a tissue penetration factor (R) relating free concentrations in muscle and plasma

Parameter (Unit)	Value (RSE, %)	
	Mean population	% Interanimal variability
CL ($\text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$)	30.4 (9.1)	27.0 (44.1)
V_{ss} ($\text{mL} \cdot \text{kg}^{-1}$)	828 (11.3)	12.6 (74.2)
AA	0.664 (22.3)	
Q ($\text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$)	5.6 (17.3)	
R (%)	80.0 (12.9)	40.4 (38.3)
Proportional residual variability (%) of blood data	21.3 (11)	
Proportional residual variability (%) of MIF data	26.1 (9.6)	
Additive residual variability (μM) of plasma data	0.7 (25.1)	
Additive residual variability (μM) of MIF data	1.2 (53.4)	

variability parameters), except for the interanimal variability on V_{ss} .

Hybrid PB-PK model simulations of MIF AMX concentrations showed remarkably good agreement between predicted and observed values (Fig. 3a and b). Simulations conducted by changing muscle blood flow suggest that this parameter must be tremendously altered to have an effect on MIF AMX distribution rate (Fig. 4a and b).

DISCUSSION

In previously published studies on distribution of antibiotics in tissue by using microdialysis in rats, total plasma drug concentrations were determined after traditional blood sampling (5, 8, 12, 13, 15). A limitation of this approach is that free concentrations are determined in tissues whereas total concentrations are measured in plasma, requiring further correction for protein binding, which had to be estimated in separate experiments. It may therefore be of benefit to introduce a microdialysis probe in a vein as well as in tissue to directly determine and compare multiple free antibiotic concentrations within the two media with time (25). This has been done by several authors to investigate the distribution of morphine in the central nervous system of rat (24) or that of AMX in the middle ear of chinchilla (8), but this experimental setting was recently used for the first time to study antibiotic distribution in rat muscle (14). Estimates of free concentrations by microdialysis require previous probe recovery estimates, which add complexity to the experiment, but cefadroxil had previously been carefully validated as an acceptable calibrator for probe recovery determinations during microdialysis studies with AMX (8). Thus, a unique advantage of this approach is that it does not require multiple blood sampling, which may have an effect on hematocrit, albuminemia, etc., and therefore drug distribution characteristics, especially when conducting experiments with small animals with limited blood volume such as rats.

FIG. 2. Individual predictions versus observed unbound AMX concentrations in MIF (open circles) and blood (closed circles) obtained by population simultaneous modeling using a two-compartment model and considering concentrations in MIF as part of the central (a) or the peripheral (b) compartment, after i.v. bolus administration of AMX at a dose of $50 \text{ mg} \cdot \text{kg}^{-1}$. Diagonal lines correspond to identity lines.

FIG. 3. Predicted 95% interval (dashed lines) with the median (solid line) of AMX concentration-time profiles in MIF, using PB-PK model simulations ($n = 1,000$) based on experimental blood and MIF AMX concentrations after an i.v. bolus administration of AMX in rat. Panel a highlights concentrations at early times on a decimal scale, and panel b shows concentrations as a semilog plot. Circles correspond to measured concentrations in MIF.

The pharmacokinetic parameters estimated in the present study have been derived from unbound concentrations and therefore may not be directly compared to previously published values obtained from total concentration measurements. However, to our knowledge only a few articles have been published on the pharmacokinetics of AMX administered i.v. to rats (22, 23). The AMX volume of distribution at steady state ($V_{ss} = 828 \text{ ml} \cdot \text{kg}^{-1}$) was slightly higher than the total body water volume ($668 \text{ ml} \cdot \text{kg}^{-1}$) (6), although this may not have any particular physiological meaning. More interestingly, as previously described, multiexponential decay of blood (or plasma) amoxicillin concentrations with time was observed, with a prolonged initial decay phase (22, 23), whereas with most other antibiotics of this family the initial decay phase was rapid, as, for example, with cefaclor (8), ceftriaxone (12), or piperacillin (5, 15), and sometimes even not observable, as was the case with imipenem (14). The experimental protocol was optimized in order to carefully characterize these two phases. Multiple microdialysis samples were collected at early times to characterize the initial decay phase, and a compartmental

FIG. 4. Medians of concentration-time profiles in MIF, using the PB-PK model, obtained after 1,000 simulations with different values of Q_M , i.e., the physiological value (dashed line) and -80% (thin solid line), 90% (medium solid line), and -99% (thick solid line) of the physiological value. Panel a shows concentrations at early times on a decimal scale, and panel b shows concentrations as a semilog plot.

pharmacokinetic analysis of the data was conducted in order to best define each of these two phases. Individual data analysis demonstrated that most profiles were best fitted using a two-compartment model, but a few exceptions were noticed, corresponding to a monoexponential decay in one rat and a triexponential decay in two rats. A population analysis was therefore conducted in order to fit a single two-compartment model to the data.

The MIF AMX distribution was characterized by R values estimated by individual noncompartmental ($R_{\text{area}} = 0.86 \pm 0.29$) and simultaneous population ($R_{\text{model}} = 0.80$) modeling, which were close to unity, meaning that at equilibrium unbound AMX concentrations in blood and muscle interstitial fluid are comparable, as could be expected with free concentrations of a compound at the two sides of a semipermeable membrane. The volume of the central compartment estimated by the population modeling approach was relatively large ($550 \text{ ml} \cdot \text{kg}^{-1}$) and, in particular, was larger than the rapidly accessible extracellular body water volume ($297 \text{ ml} \cdot \text{kg}^{-1}$) (6), in which AMX is thought to distribute almost instantaneously (16). Although inspection of mean concentration-versus-time profiles (Fig. 1) suggests that AMX distribution equilibrium may not have not been reached by the time of the first dialysate collection (0 to 7.5 min), concentrations in MIF were most often (8/11 instances) maximum in this first dialysate fraction

and then decayed in parallel with concentrations in blood (Fig. 1), leading to much better results when modeling was done with MIF considered part of the central rather than the peripheral compartment. Interestingly when multiexponential decays were observed with other amino- β -lactam antibiotics (5, 8, 12, 13, 15), MIF concentrations were always considered a priori to be part of the peripheral compartment for modeling, although this could have apparently resulted in major peak underestimation in tissue (5, 8, 15). The very rapid distribution of AMX in MIF is consistent with previous knowledge on amino- β -lactam antibiotic pharmacokinetics, suggesting that this process is rate limited not by permeability characteristics but rather by blood supply to the tissue (16). In agreement with that, the previously published blood flow-limited hybrid semi-physiological model (17, 18) was satisfactory for describing the observed MIF data. In particular, the presumably virtually instantaneous distribution of AMX in MIF was predicted by the model, indicating that maximum MIF AMX concentrations should be reached at about 4 min postdosing, corresponding approximately to the middle of the time of the first microdialysate fraction (Fig. 3a). Furthermore, the model allowed assessment of the potential effect of changing blood flow on MIF AMX concentration profiles. According to the model, only major reductions of tissue blood flow should have a detectable effect on both peak concentrations in MIF and time to peak (Fig. 4a). As an example, a reduction by about 25% of peak MIF drug concentration (from 200 μ M to 150 μ M), with a slight lengthening of time to peak, should require a 90% reduction of blood flow (Fig. 4a). However, the robustness of this hybrid PB-PK model in such an extreme range of blood flow values is questionable. More importantly, the model predicts that blood flow reduction of 30 to 50%, as can be expected in critical care patients (9), should have virtually no effect on MIF AMX concentrations (Fig. 4a and b). However, these are only simulation results, which should be confirmed experimentally. It should also be interesting to assess whether MIF AMX distribution characteristics such as those observed during this study may apply to other amino- β -lactam antibiotics, since reduced tissue blood flow, which is frequently proposed to explain their altered distribution in the MIF of critical care patients (3, 19), is not supported by this analysis.

In conclusion this study has clearly demonstrated that AMX distributes rapidly and extensively within MIF, consistent with theory, and that altered muscle blood flow seems unlikely to have a major effect on these distribution characteristics.

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