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Technical Note

Evaluation of Free Tissue Concentrations of Fleroxacin After Oral Administration

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INTRODUCTION

The activity of antibiotic drugs can be quantified *in vitro* by their minimum inhibition concentrations (MIC). These can be used as target concentrations, which should be at least equaled and then maintained as long as possible at the site of infection for the drug to be active. Also, this target concentration will be the free concentration in tissue since only the unbound drug can be active (1,2). However, tissue samples cannot be analyzed easily without disturbing their physiological properties. Hence, the unspecific binding and the free drug concentration in tissue are difficult to determine. Since most of the treated infections are located in tissues the problem of evaluating the free drug concentration at this site is of importance. Attempts to calculate free concentrations in tissue using a noncompartmental pharmacokinetic approach have been made (3–6). Previously a two-compartmental model was proposed to predict free tissue concentrations after intravenous bolus administration assuming passive diffusion of the drug (7). In this paper the model is expanded to calculate the free tissue concentrations after oral administration. The method was applied to the quinolone antibiotic drug fleroxacin and calculated results were compared with experimental data.

MATERIALS AND METHODS

Experimental Studies

Study Design. Twelve healthy young male volunteers (age between 20 and 27 years) who were free from liver and kidney diseases on the basis of history, physical examination, chemistry profile, blood analysis, and urine analysis participated in this study. None of them had a known allergy to 4-quinolones. All subjects were nonsmokers. No other medication was allowed during the time of study and alcoholic beverages and caffeine were withheld for the trial period. On the morning of the study and following an overnight fast, each subject received a single 400-mg oral dose of fleroxacin (8). Each dose was taken with 200 ml of water.

Sampling. Blood samples were drawn at 0, 10, 20, and 30 min and at 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hr after dosing; blister fluid samples were drawn at 0 and 30 min and at 1, 2, 3, 4, 6, 8, 12, and 24 hr after dosing.

Sample Analysis. An HPLC system was used with a C_{18} Nova Pak column. The mobile phase consisted of 77% phosphate buffer (pH = 3.25) containing 5 m*M* tetrabutylammonium hydrogen sulfate and 23% methanol as organic modifier. A fluorescence detector with 254-nm excitation and 425-nm emission wavelengths was used. The drug was extracted from the biological fluids with a mixture of methylene chloride and 2-propanol (95:5). The sensitivity of this assay was 0.01 μg/ml of drug in plasma; the coefficient of variation from day to day was less than 4.4% and the recovery after extraction was 83%. The correlation coefficient of the calibration curves was higher than 0.99 in the concentration range between 0.05 and 10 μg/ml.

Pharmacokinetic Model

To estimate the free tissue levels of fleroxacin, a two-compartment model with first-order absorption was used. For this model the equation for the plasma concentration $C_p(t)$ at time t is given by Eq. (1):

$$C_p(t) = A * e^{-\alpha t} + B * e^{-\beta t} - (A + B) * e^{-k_a t} \quad (1)$$

with the constant coefficients A and B, the first-order absorption rate constant k_a, and the two hybrid constants $\alpha > \beta$. By knowing the plasma concentration vs time curve of a drug, these parameters can be determined by nonlinear regression and the miocroconstants k_{10}, k_{12}, and k_{21} can be calculated (9). The differential equation for the rate of change of amount of drug in the peripheral tissue compartment X_T can be described by Eq. (2):

$$dX_T/dt = k_{12} * X_c - k_{21} * X_T \quad (2)$$

with the amount in the central compartment X_c and the two first-order intercompartmental transfer rate constants k_{12} (from plasma to tissue) and k_{21} (from tissue to plasma).

Solving this differential equation leads to the function

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$X_T(t)$, which describes the total amount of drug in tissue at different times t:

$$X_T(t) = Z * [(\alpha - \beta)e^{-k_a t} + (\beta - k_a)e^{-\alpha t} + (k_a - \alpha)e^{-\beta t}] \quad (3)$$

with $Z = k_a * F * D * k_{12}/[(\alpha - \beta) * (\alpha - k_a) * (\beta - k_a)]$, where D is the dose and F is the fraction of the dose absorbed. In all calculations complete absorption was assumed ($F = 1$).

To convert these amounts into the tissue concentrations, the total amount of drug X_T has to be divided by the volume of distribution in tissue V_T. Since this is not known, another approach can be used to calculate the free tissue concentrations. At the end of the equilibration phase in a two-compartment body model, the free concentration of the drug in the peripheral compartment reaches its maximum and is equal to the free plasma concentration (7):

$$C_T^{free}(t_{max}) = C_p^{free}(t_{max}) \quad (4)$$

in which t_{max} is the time of the maximum concentration of the drug in the peripheral compartment. At this time point the free plasma concentration $C_p^{free}(t_{max})$ can be calculated with the help of the protein-bound fraction fb_p:

$$C_p^{free}(t_{max}) = C_p(t_{max}) * (1 - fb_p) \quad (5)$$

Assuming constant tissue binding, the free concentration of drug in tissue is proportional to the total amount of drug in the tissues and both functions $C_T^{free}(t)$ and $X_T(t)$ reach their maximum at the time t_{max}. One way of etimating t_{max} is by iteration of $X_T(t)$ [Eq. (3)]. Another way of calculating t_{max} is by setting the first derivative of $X_T(t)$ equal to zero:

$$dX_T/dt(t_{max}) = 0 = Z * [-k_a(\alpha - \beta)e^{-k_a t_{max}} - \alpha(\beta - k_a)e^{-\alpha t_{max}} - \beta(k_a - \alpha)e^{-\beta t_{max}}] \quad (6)$$

In most cases the absorption phase is over at t_{max} with $k_a > \alpha > \beta$ and Eq. (6) can be simplified to calculate t_{max}:

$$0 = Z * [-\alpha(\beta - k_a)e^{-\alpha t_{max}} - \beta(k_a - \alpha)e^{-\beta t_{max}}] \quad (7)$$

in which Z cannot be equal to zero, following the assumption that α is not equal to k_a and to β. Solving Eq. (7) for t_{max} leads to Eq. (8):

$$t_{max} = \ln[\alpha(k_a - \beta)/\beta(k_a - \alpha)]/(\alpha - \beta) \quad (8)$$

Therefore $C_T^{free}(t_{max})$ can be calculated from Eqs. (1), (4), and (8). Since free concentrations and total amounts in tissue are directly proportional at all times, a factor K can be defined by dividing the total amount in tissue $X_T(t_{max})$ by $C_T^{free}(t_{max})$ in Eq. (9):

$$X_T(t_{max})/C_T^{free}(t_{max}) = K = V_T/(1 - fb_T) \quad (9)$$

This proportional factor K combines the two unknown parameters volume of distribution in tissue and the fraction bound to tissue (fb_T). With the help of this, an equation for predicting free tissue concentrations at all times can be derived.

$$C_T^{free}(t) = X_T(t)/K \quad (10)$$

or

$$C_T^{free}(t) = \frac{k_a * F * D * k_{12} * C_T^{free}(t_{max})}{X_T(t_{max}) * (\alpha - \beta) * (\alpha - k_a) * (\beta - k_a)} * [(\alpha - \beta)e^{-k_a t} + (\beta - k_a)e^{-\alpha t} + (k_a - \alpha)e^{-\beta t}]$$

RESULTS

Calculation of Free Tissue Concentrations from Experimental Plasma Data

The individual fleroxacin plasma and blister fluid concentrations were used to determine the pharmacokinetic parameters by nonlinear regression. The best data fit was obtained for a two-compartment body model with first-order absorption. The resulting parameters are listed in Table I. The plasma protein binding of fleroxacin (fb_p) was determined to be 0.48 and independent of concentration (10). The time of the maximum tissue concentration t_{max} was calculated with Eq. (7) and averaged 5 hr. Figure 1 shows total concentrations in plasma and blister fluid as well as the model-calculated free and total concentrations for one of the subjects. The free tissue levels and the free concentration in plasma cross over at t_{max}. During the elimination phase they have identical half-lives with higher free concentrations in the tissues than in plasma.

Comparison of Calculated Tissue Concentrations with Experimentally Determined Blister Fluid Concentrations

To validate the described pharmacokinetic model, total blister fluid concentrations were compared with the calculated free tissue levels. Assuming constant tissue binding, the free and the total concentrations in tissue are directly

Table I. Pharmacokinetic Parameters of the Subjects

	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
A (μg/ml)	3.94	1.00	2.57	1.00	12.71	4.73	1.63	4.08	2.08	46.30	7.33	2.23	7.47	12.13
α (hr^{-1})	0.58	0.96	0.49	0.85	0.97	0.98	0.90	0.69	0.49	1.15	0.86	0.85	0.81	0.20
B (μg/ml)	3.08	5.70	6.73	3.38	6.43	3.38	5.67	3.95	3.44	5.54	2.71	5.65	4.64	1.38
β (hr^{-1})	0.056	0.042	0.054	0.028	0.081	0.052	0.041	0.067	0.054	0.040	0.041	0.036	0.049	0.014
k_a (hr^{-1})	1.06	1.05	3.77	1.05	1.68	3.93	1.22	2.11	10.59	1.30	1.26	1.34	2.53	2.62
fb_T	0.35	0.30	0.18	0.52	0.73	0.60	0.59	0.53	0.59	0.63	0.57	0.57	0.51	0.15
t_{max} (hr)	5.9	6.0	5.3	6.1	3.7	3.5	5.1	4.3	5.2	4.9	5.1	5.1	5.0	0.8

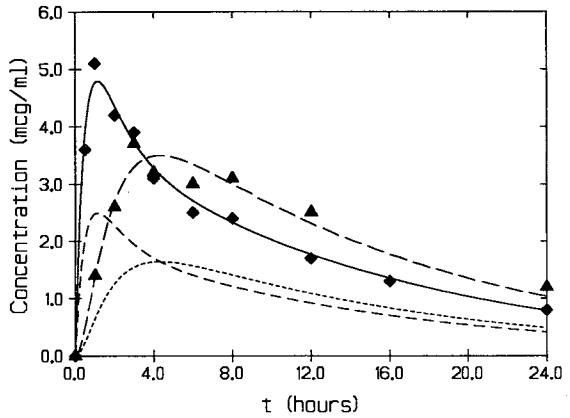

Fig. 1. Fleroxacin plasma concentration–time profile after a single oral dose of 400 mg to subject 8. Shown are the total (— —) and calculated free (---) concentration in plasma as well as the total (-- --) and calculated free (·····) concentration in blister fluid.

proportional. From that ratio it is possible to calculate the fraction bound in blister fluid (fb_T). The average value of fb_T was found to be 0.51 ± 0.15 and was not significantly different from the fraction bound to plasma proteins. Figure 2 shows a comparison of the calculated total blister fluid concentration with the experimentally determined data for all subjects at all times studied. The relationship is linear with no systematic deviation in the investigated concentration range.

DISCUSSION

Whenever the active site of a drug is located in tissue, such as receptors at cell membranes or bacteria in the extracellular fluid, the measured plasma concentrations do not represent the concentration at the site of action. It is rather the free, nonbound concentration at the active site that will determine the effect and can be related to pharmacodynamic *in vitro* data such as minimum inhibitory concentration or receptor affinity. The calculation of the free tissue concentrations is possible on the basis of plasma concentration–time profiles and knowledge of the degree of plasma protein binding. Concentration-dependent protein binding will complicate the equations, but it can be included in this model. One interesting result from a practical standpoint is the fact that the method allows predictions of free peak concentrations in tissue and the time at which they occur. In the case of fleroxacin peak tissue levels are reached after 5 hr, hence much later than the peak levels in plasma are observed (t_{max} = 1.3 hr).

However, there are limitations to this approach. If the active site is located in a very small compartment and the drug distribution into that compartment cannot be detected by the plasma concentration–time curve, the microconstants for the intercompartmental drug exchange cannot be determined and the drug concentrations at that site are not predictable. Also, the model is limited to drugs that are distributed by passive diffusion only.

The presented approach has been used to describe free tissue levels after intravenous administration of antibiotics (7). The use of the method after oral administration is more limited and depends on the relative magnitude of the pharmacokinetic parameters k_a, α, and β. A triexponential plasma concentration curve will be observed for $k_a > \alpha > \beta$ even if k_a approaches α (11). However, without intravenous data it is not possible to assign the calculated parameters unambiguously to k_a and α. Furthermore, it could be shown (11) that the triexponential curve collapses to a two-exponential curve when k_a approaches k_{21} and may result in erroneous determination of the absorption rate constant. Hence, prediction of free tissue levels from plasma concentrations is much more limited after oral than after intravenous administration.

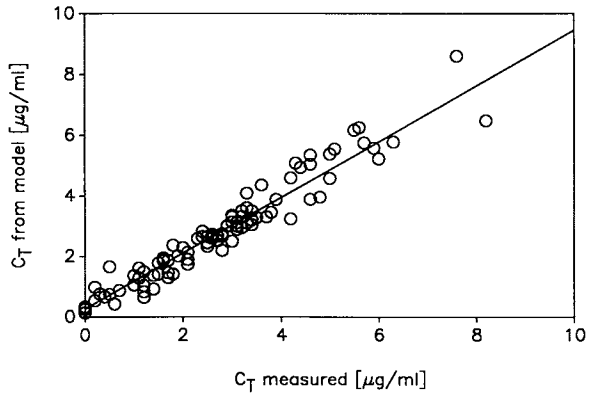

Fig. 2. Comparison of model-calculated and experimentally measured fleroxacin concentrations in blister fluid.

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