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A Comparative Study of Ofloxacin and Ciprofloxacin Erythrocyte Distribution

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ABSTRACT: The present work deals with the *in vitro* and *in vivo* distribution of ofloxacin and ciprofloxacin in erythrocytes. *In vitro* studies were carried out in standard solutions prepared using fresh blood for a concentration range between 100 and 0.25 μg mL^{-1}. A 5 mg kg^{-1} bolus dose was administered to rabbits and erythrocyte and plasma kinetics were determined over 8 h.

A linear model was used to establish the relationship between plasma and erythrocyte concentrations of both quinolones *in vitro*. The mean partition coefficient values obtained were 1.04 ± 0.02 and 1.32 ± 0.03 for ofloxacin and ciprofloxacin, respectively. A decrease in the ciprofloxacin partition coefficient was observed at higher concentrations. Values ranged between 2.54 ± 0.40 and 1.38 ± 0.15 as the concentrations increased.

The partition coefficients obtained from the linear relationship between plasma and erythrocyte concentrations established from the *in vivo* data were 0.80 ± 0.58 for ofloxacin and 0.61 ± 0.30 for ciprofloxacin. *In vivo* plasma and erythrocyte data analysis was performed by a deconvolution method and the theoretical transfer curves in erythrocytes were estimated. The distribution of both quinolones to erythrocytes is very rapid, probably due to a high permeability of erythrocyte membranes to these drugs. This was also confirmed by the parallelism between plasma and erythrocyte kinetics. © 1998 John Wiley & Sons, Ltd.

Key words: erythrocyte distribution; ofloxacin; ciprofloxacin; pharmacokinetics; rabbits

Introduction

The distribution of antimicrobial agents is of great interest since such drugs need to access the infected organ or tissue for the therapeutic response to be achieved. The intracellular distribution of drugs constitutes a specific phase included within the whole distribution process, and very often determines the drugs' pharmacological and/or toxicological effects. It may also be the slowest phase in the tissue distribution process.

It is therefore useful to find valid models of intracellular distribution which will allow one to establish the kinetic behaviour of drugs in such structures and hence the distribution kinetics in the corresponding organ or tissue.

Drug distribution studies are scarce and most of them have only examined the partition coefficients for different organs or tissues. The reasons for this are twofold: first, the difficulty in obtaining tissue or cellular samples, and second, the complexity of understanding the experimental results and defining robust mathematical methods or models which best fit the results. The aim of the present work was to study the distribution kinetics of two quinolones, ofloxacin and ciprofloxacin, in red blood cells from the experimental data obtained from *in vitro* and *in vivo* drug plasma and whole blood concentrations.

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Experimental Section

Chemicals

Ofloxacin and ciprofloxacin were supplied by Hoescht Iberica S.A. (Barcelona). The commercially available form Baycip® (Bayer) was used for ciprofloxacin. All other chemical and reagents were of analytical grade.

In Vitro Studies

Solutions of ciprofloxacin and ofloxacin were prepared using fresh blood as solvent with a concentration range of 100–25 μg mL^{-1}. These solutions were incubated at 37°C for a period of 3 h after which equilibrium was reached. After incubation, blood was centrifuged at about $2000 \times g$ for 10 min and the supernatant was separated and kept at −20°C until assay.

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In Vivo Studies

The *in vivo* study was carried out on 12 healthy male New Zealand white rabbits (mean weight 2.43 ± 0.45 kg) divided into two groups of six animals each. One group received ofloxacin and the other ciprofloxacin. All animals were fasted for 12 h prior to the start of the study, though water was provided *ad libitum*. The quinolone was administered at a dose of 5 mg kg^{-1} as a bolus injection through the marginal ear vein and blood samples were withdrawn by venipuncture to the contralateral ear at the following times from administration: 2, 5, 10, 15, 30, and 45 min and at 2, 4, 6, and 8 h. Each blood sample was divided in two aliquots. One of these was immediately centrifuged at about $2000 \times g$ for 10 min and the supernatant was separated and used to determine the quinolone plasma concentration. The other aliquot was used to determine quinolone concentration in whole blood.

In all cases (*in vitro* and *in vivo* experiments) quinolone levels in red blood cells were calculated from plasma and whole-blood concentration data and haematocrit values, which were experimentally determined with fresh blood from the corresponding animal or fresh blood used as solvent in the *in vitro* study [1] using the following expression [2]:

$$C_e = \frac{C_b - C_p(1-H)}{H} \quad (1)$$

where C_e is the erythrocyte concentration, C_b is the whole-blood concentration, C_p is the plasma concentration, and H is the haematocrit value.

The erythrocyte–plasma partition coefficient was calculated by the C_e/C_p ratio.

Analytical Assay

Blood and plasma ofloxacin and ciprofloxacin levels were measured by a reverse phase, ion-pair HPLC method with fluorescence detection. The HPLC system comprised a Varian 5000 pump and injector and a Kontron SFM variable-wavelength fluorescence detector, equipped with a 25.0 cm × 4.6 mm (i.d.) column packed with reverse phase 10 μm C_{18}.

The mobile phase was prepared with 10% acetonitrile and 90% of a mixture of 1 L 0.025 M phosphoric acid and 15 mL 40% tetrabutyl ammonium hydrogen sulphate, adjusted to pH 3 with 1/15 M phosphate buffer; excitation and emission wavelengths were 330–450 and 277–445 nm for ofloxacin and ciprofloxacin, respectively, and the flow rate was 2 mL min^{-1}.

Sample Treatment

1 mL of blood was vortexed for 30 s with 1 mL Triton, 0.25 mM; then 4 mL 6% trichloroacetic acid was added. The mixture was vortexed again for 30 s and centrifuged for 10 min at about $2000 \times g$ and 100 μL of the supernatant was injected.

Table 1. A statistical comparison of ofloxacin and ciprofloxacin partition coefficients

C^a	R^b_{oflo}	R^c_{cipro}	p^d
100	1.06 ± 0.10	1.38 ± 0.15	1.13×10^{-3}
50	1.08 ± 0.13	1.29 ± 0.37	0.23
10	1.66 ± 0.04	1.86 ± 0.32	0.17
5	1.05 ± 0.10	1.20 ± 0.36	0.34
2.5	1.01 ± 0.13	1.73 ± 0.15	4.52×10^{-6}
1	1.32 ± 0.23	2.30 ± 0.35	1.78×10^{-4}
0.5	1.18 ± 0.19	2.41 ± 0.33	1.19×10^{-5}
0.25	1.31 ± 0.31	2.54 ± 0.40	3.02×10^{-5}

[a] Quinolone blood concentration.
[b] Oxfloxacin partition coefficient.
[c] Ciprofloxacin partition coefficient.
[d] Statistical significance.

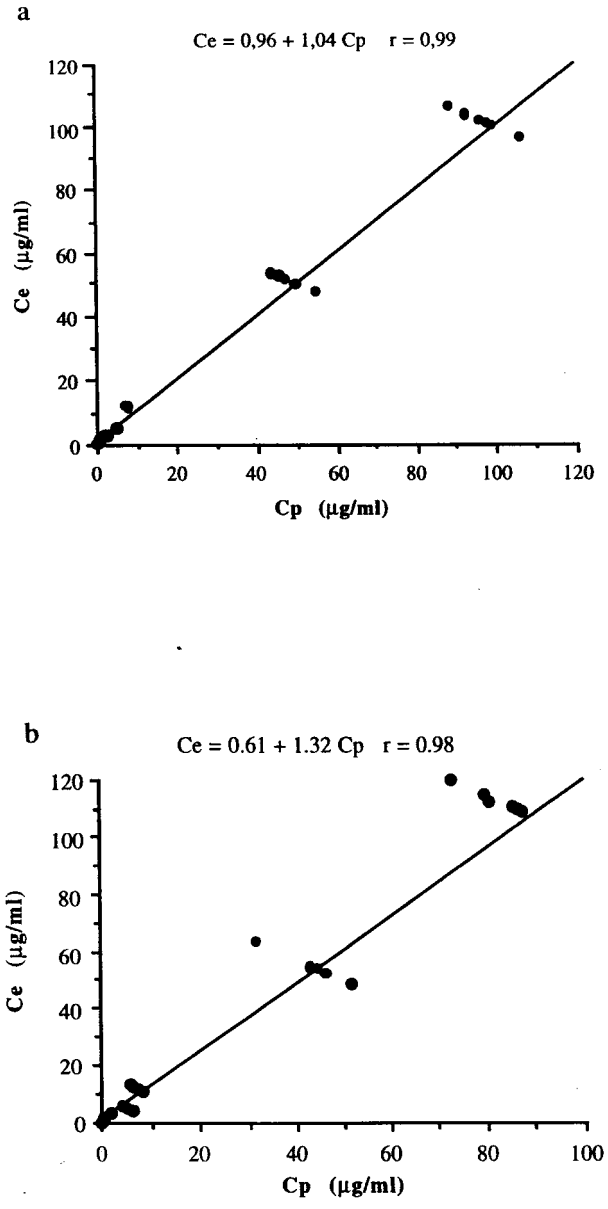


Figure 1. *In vitro* ofloxacin (a) and ciprofloxacin (b) erythrocyte and plasma concentration ratios

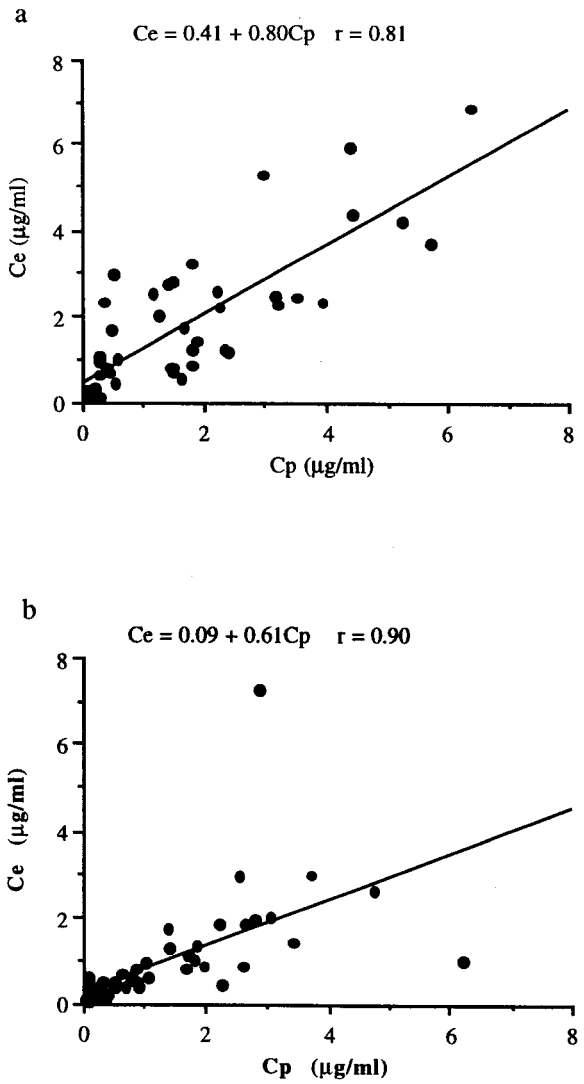


Figure 2. *In vivo* ofloxacin (a) and ciprofloxacin (b) erythrocyte and plasma concentration ratios

For plasma level determination the same procedure was used but starting from 0.5 mL sample and using the corresponding reactive proportions.

Blood and plasma standard solutions of concentrations between 100 and 0.4 µg mL^{-1} were subjected to the same treatment.

Under these chromatographic conditions, a complete separation of ofloxacin and ciprofloxacin peaks was obtained, with retention times of 5 and 9 min, respectively.

An external standard method was used for quantification. It was necessary to use three calibration curves. The detection limits were 0.03 and 0.05 µg mL^{-1} for plasma and blood, respectively.

Kinetic Analysis

Plasma and blood concentration–time curves were fitted to a biexponential equation corresponding to the classical two-compartment model with linear kinetic [3]. Optimization of the parameters defining this model was carried out using a non-linear regression computer program (MULTI) [4].

A deconvolution technique was used to evaluate the kinetics of the quinolone in the erythrocytes. Since drug transport across the erythrocyte membrane is a diffusion process, the factor controlling the transference rate would be the drug concentration gradient, according to the following differential equation [5]:

$$\frac{dX_e(t)}{dt} = Cl_t[f_1C_p(t) - f_2C_e(t)] \quad (2)$$

where C_p and C_e are the plasma and erythrocyte quinolone concentrations, respectively; f_1 and f_2 are unbound drug fractions on both sides of the cellular membrane; Cl_t is the tissue clearance; and X_e is the amount of quinolone inside erythrocytes.

If drug tissue amount (X_e) is substituted by the product of erythrocyte volume (V_e) and concentration (C_e) the following equation is obtained:

$$\frac{dC_e}{dt} = \frac{Cl_t f_2}{V_e}[FC_p(t) - C_e(t)] \quad (3)$$

or

$$\frac{dC_e}{dt} = K[FC_p(t) - C_e(t)] \quad (4)$$

where $F = f_1/f_2$ represents the quinolone plasma and erythrocyte unbound fraction ratio.

According to the corresponding mass balance equation in a non-eliminating tissue and considering a flow-limited physiological model the following relationship is established:

$$K = \frac{Cl_t}{V_eR} = \frac{k_{e1}}{R} \quad (5)$$

where k_{el} is the first-order erythrocyte output constant and R is the partition coefficient between erythrocytes and plasma.

Assuming a linear kinetic behaviour, the erythrocyte drug concentrations as a function of time can be expressed as a convolution integral which takes the expression

$$C_e(t) = FK\ e^{-Kt} * C_p(t) \quad (6)$$

where $*$ represents the convolution operation. Algebraic deconvolution of the erythrocyte and plasma functions allows one to determine the impulse response in erythrocytes, $G(t)$,which is defined by

$$G(t) = FK\ e^{-Kt} \quad (7)$$

The exponential term (e^{-Kt}) is representative of the unit dose impulse response function of the drug in the erythrocyte.

On integrating Equation (7), the following expression is obtained:

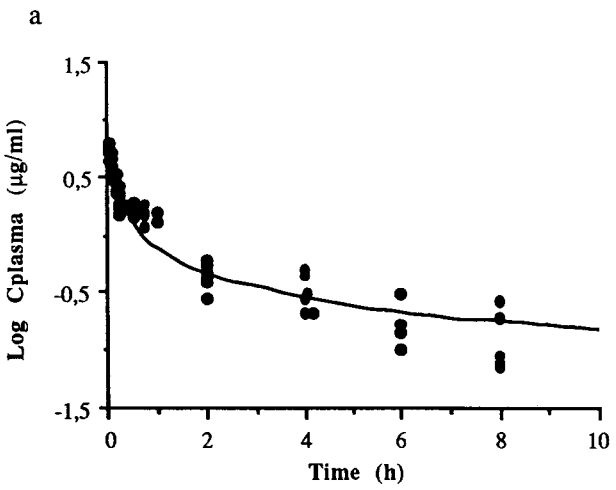
$$A(t) = F(1 - e^{-Kt}) \quad (8)$$

The deconvolution approach applied in this study requires previous fitting of erythrocyte and plasma levels curves to a biexponential equation using non-

linear regression methods, as indicated previously. Optimized coefficients and exponent values are used to carry out the deconvolution technique according to the Langenbucher method [6] and the approach of Lanao *et al*. [7].

Statistical Analysis

Normal distribution of the data was tested by the Saphiro–Wilks test [8]. The homocedasticity and heteroscedasticity of the samples were tested using the Cochran test [9]. According to the results of the above-mentioned test, parametric and non-parametric tests were used for comparison of the experimental data. Only for the comparison of *in vitro* and *in vivo* results was the non-parametric test of Wilcoxon [10] used as a result of the heteroscedasticity of compared samples. For all other statistical comparisons one-way analysis of variance [11] was used.



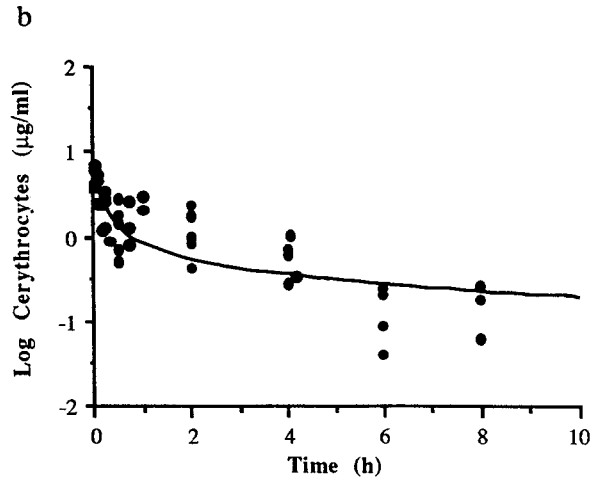
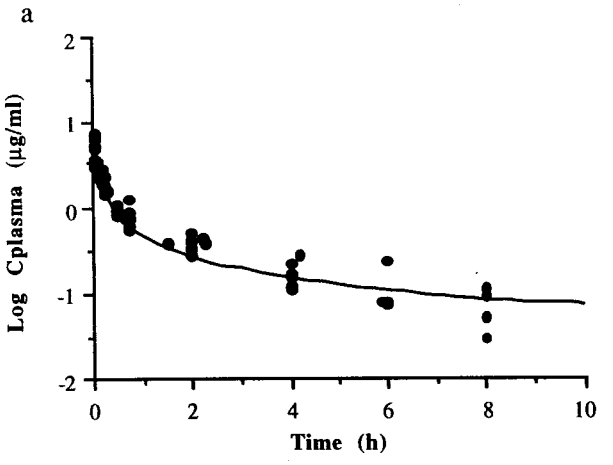


Figure 3. Plasma (a) and erythrocyte (b) ofloxacin kinetics ($D = 5$ mg kg^{-1}) obtained form the whole data set



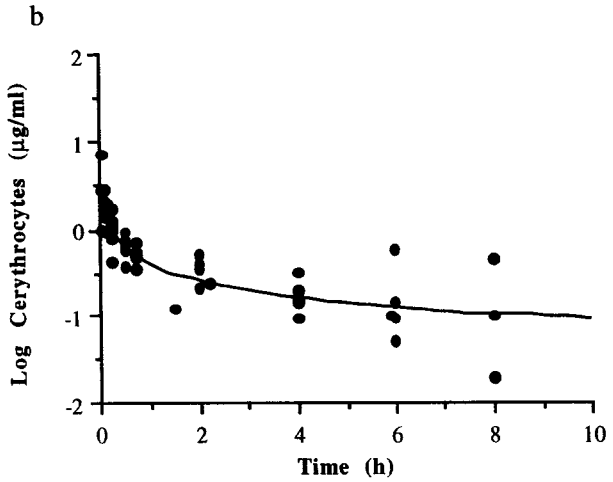


Figure 4. Plasma (a) and erythrocyte (b) ciprofloxacin kinetics ($D = 5$ mg kg^{-1}) obtained from the whole data set

Results and Discussion

In Vitro Studies

A linear model was used to establish the relationship between plasma and erythrocyte concentrations of both quinolones *in vitro*. Partition coefficient values were calculated from the slope of the curve.

Table 1 shows the mean partition coefficient values of ofloxacin and ciprofloxacin as well as the results obtained in the statistical comparison of the two quinolones. Ciprofloxacin shows higher *in vitro* partition values than ofloxacin. The differences had statistical significance for some concentration values, especially when concentrations were less than 5 µg mL^{-1}.

Regarding ciprofloxacin, the partition coefficient values decrease as the blood concentration increases. For a blood concentration value of 0.25 µg mL^{-1}, this coefficient has a value of 2.54 ± 0.40; this decreases to 1.38 ± 0.15 when the blood concentration is 100 µg mL^{-1}. Statistical comparison revealed significant differences ($p < 0.001$).

Figure 1 shows the linear relationship and the corresponding linear equation whose slope values are $1.04 \pm 1.80 \times 10^{-2}$ and $1.32 \pm 3.26 \times 10^{-2}$ for oxfloxacin and ciprofloxacin, respectively.

The fact that a linear model was used to establish the relationship between plasma and erythrocytes for ciprofloxacin and at the same time a decrease in the partition coefficient was observed at higher concentrations could be explained by the existence of non-linear processes that saturate at low concentrations, as happens in methotrexate kinetics [12]. To confirm and characterize this possible non-linear process for ciprofloxacin, experimental studies at concentration values lower than 0.25 should be carried out.

In Vivo Studies

Using the same linear model as indicated for the *in vitro* studies, the plasma and erythrocyte concentration relationship was established and the corresponding partition coefficients were determined from the curve slope values. Figure 2 shows the relationship for ofloxacin and ciprofloxacin, whose slope values are 0.80 ± 0.58 and 0.61 ± 0.30, respectively.

This linear model points to low precision in estimating the partition coefficients, probably due to the high variability of the *in vivo* data.

Differences are observed on comparing the *in vitro* and *in vivo* results. First, the *in vivo* mean partition coefficient value is lower than the *in vitro* value for both quinolones. The Wilcoxon test shows statistical differences for ciprofloxacin ($p = 3.15 \times 10^{-2}$) and non-statistical differences for ofloxacin ($p = 0.07$) for the same concentration range (0.25–10 μg mL^{-1}). These results confirm the difficulty in extrapolating *in vitro* results to *in vivo* conditions for some drugs.

Table 2. Mean pharmacokinetic ofloxacin and ciprofloxacin parameters ($\pm$S.D.) in rabbits

Parameter	Ofloxacin	Ciprofloxacin
α $(h^{-1})^a$	2.39 ± 0.72	5.52 ± 2.18
β $(h^{-1})^b$	0.23 ± 0.09	0.35 ± 0.10
k_{12} $(h^{-1})^c$	1.14 ± 0.48	3.05 ± 1.47
k_{21} $(h^{-1})^d$	0.65 ± 0.23	1.24 ± 0.59
k_{10} $(h^{-1})^e$	0.82 ± 0.29	1.58 ± 2.64
V_c $(L)^f$	2.95 ± 0.71	3.06 ± 0.67
$V_p(L)^g$	5.49 ± 3.45	7.53 ± 1.98
V_{dss} $(L)^h$	8.44 ± 3.45	10.58 ± 1.97
Δ_{dss} $(L\ kg^{-1})^i$	3.87 ± 1.57	4.01 ± 1.06
$t_{1/2\beta}$ $(h)^j$	3.61 ± 1.68	2.16 ± 0.83
Cl_p $(L\ h^{-1})^k$	2.29 ± 0.58	4.70 ± 1.08

[a] Rapid disposition constant.
[b] Slow disposition constant.
[c] Distribution microconstant from central to peripheric compartment.
[d] Distribution microconstant from peripheric to central compartment.
[e] Elimination rate constant.
[f] Central compartment distribution volume.
[g] Peripheric compartment distribution volume.
[h] Steady-state distribution volume.
[i] Steady-state distribution coefficient.
[j] Slow-phase elimination half-life.
[k] Plasma clearance.

Plasma and erythrocyte levels, corresponding to the administration of 5 mg kg^{-1} of ofloxacin and ciprofloxacin by intravenous injection to rabbits, are shown in Figures 3 and 4. Both exhibit a biexponential profile. Table 2 shows the mean plasma pharmacokinetic parameter values corresponding to a two-compartment model. The differences observed between both quinolones are similar to those observed in healthy human volunteers [13,14]: namely a larger distribution volume for ciprofloxacin and a longer elimination half-life value for ofloxacin.

Figure 5 shows the individual asynthotic curves of ofloxacin and ciprofloxacin to erythrocytes obtained from individual *in vivo* plasma and whole-blood experimental data using the numerical deconvolution approach.

Figure 6 shows the mean theoretical curves in erythrocytes of ciprofloxacin and ofloxacin obtained from all the plasma and whole-blood experimental data. When working with the whole set of experimental data instead of individual curves, erythrocyte kinetic parameters were estimated with a more acceptable level of accuracy. The estimated mean values of these parameters are $F = 0.93 \pm 8.13 \times 10^{-3}$ and $K = 4.11 \pm 0.32\ h^{-1}$ for ofloxacin and $F = 0.63 \pm 4.82 \times 10^{-3}$ and $K = 2.49 \pm 0.13\ h^{-1}$ for ciprofloxacin. Using the above mentioned K values and the mean erythrocyte partition coefficient values calculated from the experimental *in vivo* data ($R_{oflo} = 1.28$; $R_{cipro} = 0.79$), the corresponding output rate constants were estimated; the values for these constants are 5.26 and 1.97 h^{-1} for ofloxacin and ciprofloxacin, respectively.

Summarizing the results of the present study it can be concluded that both quinolone access to red blood cells and erythrocyte kinetics are governed by plasma kinetics. The erythrocyte partition coefficient values corresponding to a wide concentration range suggest a mainly linear kinetic behaviour together with a diffusion process as the main transport mechanism. Nevertheless, the differences between the partition coefficient values corresponding to whole-blood concentration values reveals the possibility of a non-linear kinetic process which saturates at low concentration. This may be a consequence of binding to intracellular structures. Other authors [15] have already suggested this possibility but have reported that it is not possible to draw conclusions about specific intracellular structures involved in the binding process because of the lack of information regarding this topic.

According to the *in vivo* results, the erythrocyte partition coefficient of ofloxacin is higher than that of ciprofloxacin in contrast to the *in vitro* results. As

previously commented, the extrapolation of *in vitro* results to *in vivo* situations is complicated and requires a deep knowledge of the physiological and functional differences between *in vitro* and *in vivo* conditions. Anatomical and structural erythrocyte characteristics remain in *in vitro* conditions but energy-consuming processes do not. It would be easier to understand an increase in the partition coefficient under *in vivo* conditions as a consequence of the existence of active transport out of the cells and tissue metabolism explain why in the case of several drugs *in vivo* partition coefficients in specific tissues never reach the predicted values [16]. Taking into account that ciprofloxacin is strongly metabolized [17], intraerythrocyte metabolism and/or active transport out of the cells could be suggested as an explanation for the *in vitro–in vivo* differences observed. Considering that erythrocytes comprise about 40–50% of the total volume of blood it is interesting to clarify the differences, if they exist, between plasma and red blood cell kinetics. The result obtained in this study point to a strong parallelism in the erythrocyte and plasma curve profiles for both quinolones. This means that the *in vivo* distribution of ofloxacin and ciprofloxacin in erythrocytes fits a flow-limited kinetic model, probably due to the high permeability of erythrocyte membranes to these quinolones. The high permeability is additionally supported by the results from the deconvolution approach, which point to rapid erythrocyte transfer kinetics with high output constant values.

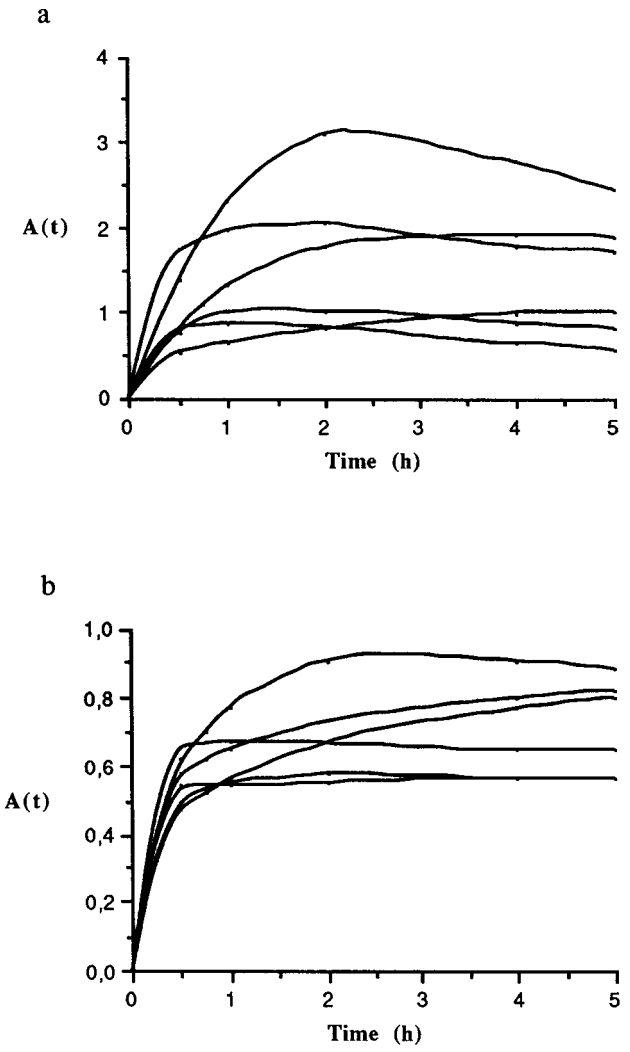


Figure 5. Theoretical individual asynthotic curves of ofloxacin (a) and ciprofloxacin (b) in erythrocytes obtained by deconvolution

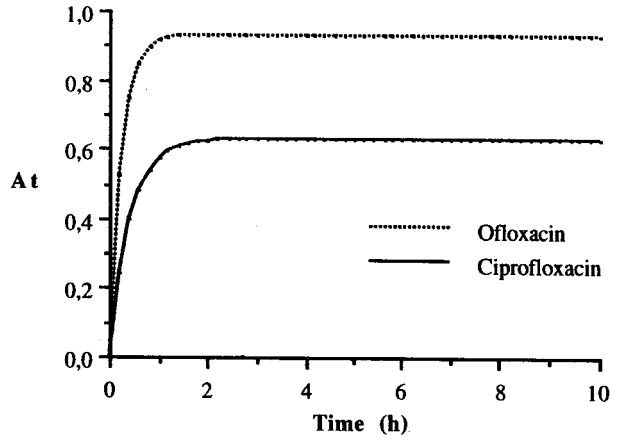


Figure 6. Theoretical asynthotic curves of ofloxacin and ciprofloxacin obtained by deconvolution from the whole data set

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