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In Vitro and In Vivo Ciprofloxacin Pharmacokinetics in Human Neutrophils

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Early in vitro investigations have shown that ciprofloxacin is concentrated within human neutrophils (polymorphonuclear leukocytes [PMNs]) at between 3 and 11 times the extracellular concentration. The elution of ciprofloxacin from cells is relatively rapid when the extracellular concentration is reduced. In order to estimate the in vivo intracellular penetration of ciprofloxacin and to determine its intracellular pharmacokinetics, PMNs were recovered from blood samples drawn from healthy volunteers at different times during a 24-h period after they were given a 750-mg oral dose. High-performance liquid chromatographic determination of ciprofloxacin in serum and cells showed that the intracellular/serum ratio was 3.7 at 1.5 h (maximum concentration of drug in serum), 5.7 at 12 h, and 20 at 24 h. The area under the curve ratio was 3.73. The mean elimination half-lives of ciprofloxacin were 3.7 and 6.2 h in serum and PMNs, respectively. These data show that in vivo findings are in agreement with in vitro findings. The large uptake of ciprofloxacin by PMNs combined with a prolonged intracellular half-life described under the conditions of human therapy should provide the basis for the use of ciprofloxacin in infections caused by susceptible intracellular bacteria.

Fluoroquinolones represent a new group of compounds that exhibit very high activity against a broad spectrum of bacteria, including those that develop intracellularly and that have important extravascular diffusion. Moreover, among the currently marketed compounds, ciprofloxacin has the lowest MICs and the greatest apparent volume of distribution. Thus, it should be one of the most promising agents for the treatment of intracellular infections. Because the ability of an antimicrobial agent to penetrate into phagocytic cells is the first essential step for activity against facultative intracellular organisms (7), in this study we examined the ability of ciprofloxacin to enter human polymorphonuclear leukocytes (PMNs). The uptake of ciprofloxacin by several cell types has been studied previously. These were in vitro experiments only (2, 3), i.e., they were done under experimental conditions, which is very different from the in vivo interactions between the antibiotic and cells. However, these data can be considered very informative, and they demonstrate that ciprofloxacin has a high degree of intracellular penetration followed by a rapid release into the extracellular medium. At present, no information regarding the intracellular behavior of ciprofloxacin in patients receiving the drug has been published.

The purpose of this study was to determine whether the pattern of ciprofloxacin penetration into cells observed in vivo corroborates the in vitro data and which findings might be made regarding the clinical use of ciprofloxacin.

MATERIALS AND METHODS

The first part of this study was devoted to characterization of the principal parameters of ciprofloxacin penetration into human PMNs in vitro. Following this, the drug was administered to humans and the intracellular penetration was examined by sequentially obtaining blood samples after ciprofloxacin administration.

In vitro experiments. (i) Preparation of human PMNs. Approximately 25 ml of venous blood was collected from healthy volunteers by using an heparinized syringe. Granulocytes (largely PMNs) were isolated by dextran sedimentation and Ficoll-Hypaque density gradient centrifugation. At least 97% of the granulocytic cells were neutrophils, and more than 90% of these were PMNs. More than 95% of these phagocytes were viable, as judged by trypan blue exclusion. Finally, the PMNs were suspended in 10 ml of RPMI 1640 tissue culture medium, so that the cell concentration was about 2.0×10^6 to 4.0×10^6 cells per ml.

(ii) Determination of antibiotic uptake. Uptake of ciprofloxacin by human PMNs was estimated by a velocity gradient centrifugation technique. PMNs were incubated with 10 mg of ciprofloxacin per liter, and at intervals, phagocytes were separated from the extracellular antibiotic by velocity gradient centrifugation. This was accomplished by centrifugation of PMNs through a water-impermeable barrier of silicone oil into formic acid, which dissolved the cells. The tubes were then frozen, and the layers were separated by using a razor. The experiment was performed in triplicate. The intracellular water space was measured by using tritiated water, and the extracellular marker was ^{14}C-labeled inulin. Ciprofloxacin was measured by high-performance liquid chromatography, and the results are expressed as the ratio of the cellular to the extracellular antibiotic concentration.

(iii) Determination of ciprofloxacin in plasma and cells. The ciprofloxacin concentrations were determined by high-performance liquid chromatography by using a RP18 5 μm (4.6 by 25 mm) reversed-phase column (Licrospher; Merck, Darmstadt, Federal Republic of Germany), and the detection was performed by fluorimetry, with excitation λ (λ_{ex}) = 400 nm and emission λ (λ_{em}) = 462 nm. The mobile phase was a mixture of acetonitrile and phosphate buffer (20/80 [vol/vol]) containing 0.005 M tetrapentyl ammonium bromide adjusted to pH 2.5.

Briefly, sample preparation was performed as follows. A

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total of 0.5 ml of plasma was added to 0.5 ml of pH 7 buffer and extracted with 8 ml of dichloromethane. Then, 5 ml of the organic phase was reextracted with 200 μl of 0.1 M hydrochloric acid. Finally, 10 μl of the acid phase was used for chromatographic analysis. The cell pellets were sonicated for 1 h, and after centrifugation a 10-μl portion of the supernatants was used for analysis.

Quantitative standards were run for blood samples from each subject and for plasma and cellular determinations. In both cases, a standard curve was determined by using the total area under the peak as determined by electronic integration. The detection limit in serum and intracellular water was 0.01 mg/liter. Two separate but overlapping standard curves for serum were prepared (10 to 0.5 and 1.0 to 0.02 mg/liter). Least-squares regression analysis gave coefficients of correlation of >0.998. Within-day reproducibility was assessed by using spiked serum (n = 10) at 5 and 0.1 mg/liter. Coefficients of variation were 3 and 4.5%, respectively. Between-day reproducibility was assessed with pooled serum samples, with assay values of 5 and 0.1 mg/liter for the high (n = 10) and low (n = 10) range, respectively; coefficients of variation were 4 and 5%, respectively.

In vivo experiments. (i) Donor blood. Blood samples were obtained from eight healthy volunteers who received no other medication except the 750-mg dose of ciprofloxacin. The eight healthy subjects participated in the study after written informed consent was obtained. The subjects were four males and four females with an average age of 28 years (range, 23 to 37 years) and an average weight of 65 kg (range, 46 to 82 kg). All subjects received a complete medical history, physical examination, and laboratory profile, including a complete blood count. The experiment started after approval of the protocol by the Ethical Committee of the Nice University Hospital.

(ii) Drug administration. After fasting for at least 12 h, each subject received a single 750-mg dose of ciprofloxacin by the oral route with a 100-ml volume of water.

(iii) Sample collection and pharmacokinetics study. Blood samples were collected (15 ml) from an arm vein at the following times: 0 h (before ciprofloxacin administration) and 0.5, 0.75, 1, 1.5, 2, 3, 5, 9, 12, and 24 h (after ciprofloxacin administration). Each sample was immediately treated to obtain viable PMNs as described above. Ciprofloxacin was then determined simultaneously in plasma and cells. Then, pharmacokinetic analysis was performed by using the SIPHAR computer resource, which uses a nonlinear least-squares regression and a weighted least-squares algorithm that uses the weighting factor 1/y (calc) ^2. The area under the curve (AUC) of ciprofloxacin concentrations in serum or intracellular water versus time from time zero to infinity ($AUC_{0-\infty}$) and the mean residence time (MRT) were estimated in serum and cells with noncompartmental method. The AUCs were calculated by the log trapezoidal rule method and were extrapolated to infinity by dividing the last value for the concentration in serum or intracellular water by the elimination rate constant (derived from at least the last three values for concentration in serum or intracellular water). The MRT was obtained by calculating the ratio between the area under the first moment versus time curve to the AUC (obtained by the trapezoidal rule method and extrapolated to infinity). The maximum ciprofloxacin concentration in serum (C_{max}) was graphically determined in plasma and cells, and the time to C_{max} (T_{max}) was determined as the time to achieve the highest measured concentrations. The elimination half-life was calculated by dividing the natural logarithm of 2 by the elimination rate constant.

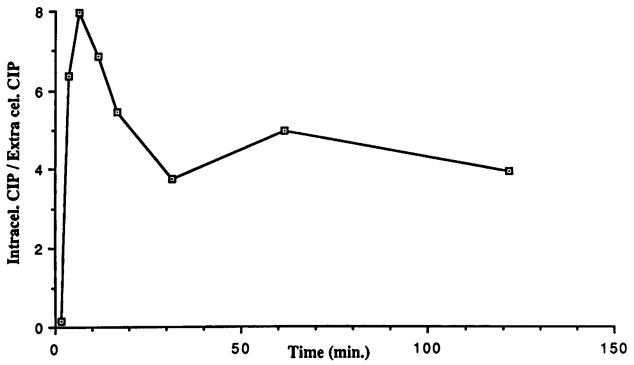

FIG. 1. Kinetics of intracellular penetration of ciprofloxacin (CIP) in human PMNs in vitro.

In order to be compared with the concentrations in plasma, the intracellular ciprofloxacin concentrations were expressed as milligrams per liter of intracellular water.

(iv) Statistical analysis. The means and standard deviations of each of these pharmacokinetic parameters were determined, and the differences between them for serum and intracellular water were determined by using the two-tailed Student's t test. A probability of less than 5% was taken as statistically significant.

RESULTS

Intracellular volume of water in PMNs. The intracellular volume of water in 10^7 PMNs was 2.18 ± 0.07 μl, which was calculated by subtracting the extracellular water volume from the total volume of water measured in the PMN samples. This result was in fairly good agreement with the results of another study (7).

In vitro behavior of water. Analysis of the intracellular penetration (Fig. 1) of ciprofloxacin showed a rapid uptake of the antibiotic by the cells, because T_{max} was obtained within 5 min of incubation at 37°C. Ciprofloxacin was concentrated within the neutrophils at between four and eight times the extracellular concentration, with an extracellular antibiotic concentration of 10 mg/liter. Then, after a relatively rapid decrease in the intracellular concentration, a plateau was reached at about 30 min and was maintained until the end of the experiment, i.e., 2 h.

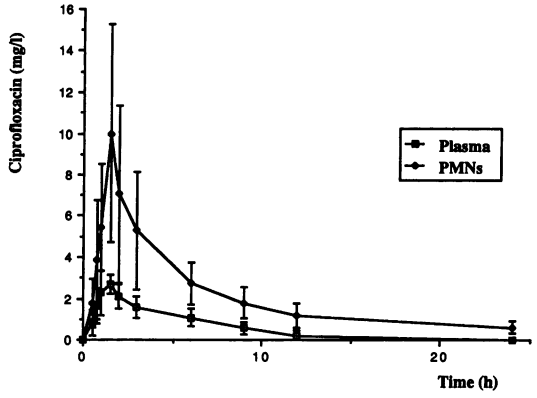

FIG. 2. Mean pharmacokinetic profile of ciprofloxacin in plasma and PMNs of volunteers receiving 750 mg orally (n = 8).

TABLE 1. Pharmacokinetic parameters of ciprofloxacin[a]

	C_{max} (mg/liter)	Concn (mg/liter) at:		T_{max} (h)	$AUC_{0-\infty}$ (mg · h · liter^{-1})	MRT (h)	$t_{1/2}$ (h)
		12 h	24 h				
Serum	2.7 ± 0.46	0.21 ± 0.2	0.03 ± 0.07	1.5	15 ± 6.3	5.74 ± 1.6	3.7 ± 1.105
PMNs	10.0 ± 5.2	1.19 ± 0.58	0.61 ± 0.31	1.5	56 ± 19.8	10 ± 3	6.2 ± 1.8
P[b]	0.003	0.001	0.001		0.001	0.02	0.0025
R[c]	3.70	5.66	20.3	1	3.7	1.7	1.6

[a] Values are means for eight volunteers who received 750 mg of ciprofloxacin orally. $t_{1/2}$, elimination half-life; abbreviations of the other pharmacokinetic parameters are defined in the text.
[b] Statistical significance for $P < 0.05$.
[c] R, PMN/plasma ratio.

In vivo behavior of ciprofloxacin. The pharmacokinetic behavior of ciprofloxacin in plasma and human PMNs after a single 750-mg oral dose is shown in Fig. 2. The C_{max} values of ciprofloxacin in plasma and PMNs were obtained 1.5 h after administration and were 2.7 ± 0.46 and 10.0 ± 5.25 mg/liter, respectively. At 12 h after administration, the antibiotic concentrations in plasma and PMNs were 0.21 ± 0.22 and 1.19 ± 0.58 mg/liter, respectively, showing that intracellular ciprofloxacin levels constantly remain above the levels in plasma. The calculated pharmacokinetic parameters and the corresponding ratio of PMNs/plasma are presented in Table 1. It is important to point out that the intracellular half-life of ciprofloxacin in vivo appeared to be about twofold higher than that in plasma, i.e., 6.2 versus 3.7 h. The main pharmacokinetic parameters and the related PMNs versus plasma ratios are presented in Table 1. It is clear that the $AUC_{0-\infty}$ and the MRT of ciprofloxacin in PMNs are greater than those observed in serum. Moreover, the ratio of the ciprofloxacin concentrations is increasingly more favorable for the intracellular medium from 1.5 (C_{max}) to 24 h, showing a fivefold increase during that period of time.

DISCUSSION

When clinicians attempt to treat infections caused by bacteria capable of remaining alive inside cells, it is essential that an antimicrobial agent that can be transferred into phagocytes be used. Fluoroquinolones (particularly ciprofloxacin) are antibiotics with spectra of activity against a number of intracellular pathogens (mycobacteria, legionellae, chlamydiae). A previous in vitro study has shown that ciprofloxacin is taken up rapidly by both human neutrophils and mouse peritoneal macrophages (4). It does not appear to be bound firmly within the cell, and it can be eluted readily if the extracellular concentration is lowered. Indeed, analysis of the subcellular distribution of the fluoroquinolones has demonstrated that these antibiotics are soluble in the intracellular medium, with a small proportion associated with the azurophil granules of PMNs (1). Our in vitro experiments were performed with human neutrophils and without withdrawing the extracellular antibiotic in order to simulate better the in vivo findings. Some of the results are in agreement with the data reported previously by Easmon and Crane (3), i.e., rapid and important intracellular penetration of ciprofloxacin, because the C_{max} was reached within a few minutes and the intracellular/extracellular ratio varied from about 5 to 8 during the 2-h period of observation. We also observed a rapid elution of ciprofloxacin from the intracellular medium, but a plateau was obtained in about 20 min and the intracellular/extracellular ratio stabilized at about 4. This may have been due to the occurrence of an equilibrium between intracellular and extracellular ciprofloxacin concentrations. This suggests that, in vivo, because ciprofloxacin remains present in the blood several hours after dosing, a relatively high intracellular concentration should be maintained during the entire dosing interval (8 or 12 h). The results correlate with previous experiments which demonstrated that the good intracellular penetration of fluoroquinolones, particularly ciprofloxacin, in vitro results in a significant reduction in the number of intracellular bacteria; this differs from the results obtained with antibiotics such as clindamycin, which accumulate largely in PMNs but which are much less effective (4, 7).

The pharmacokinetic study performed in vivo confirmed these findings. Indeed, the parameters that we evaluated showed that intracellular ciprofloxacin concentrations are largely higher than the concentrations in serum, particularly 12 and 24 h postdosing. They remained above the MICs for the majority of susceptible bacteria, e.g., *Staphylococcus aureus*, *Legionella pneumophila*, *Mycobacterium hominis*, and *Mycobacterium pneumoniae*, for at least 12 h and at least 8 h for less susceptible species, i.e., *Ureaplasma urealyticum*, *Chlamydia trachomatis*. Because this experiment was performed after administration of a single dose, one can speculate that at steady-state after a dosing regimen of two or three times daily, the intracellular ciprofloxacin concentration will be above the MICs for these bacteria. Indeed, intracellular ciprofloxacin penetration does not seem to be saturable within the therapeutic range of concentrations (4, 5, 9). Moreover, the longer elimination half-life of ciprofloxacin in PMNs compared with those in serum (6.2 versus 3.7 h) might support the potential efficacy of a dosing regimen of two or three times daily for infections caused by intracellular bacteria. Lastly, the intracellular ciprofloxacin AUC and MRT, which were 3.9 and 1.6 times the values in plasma, respectively, emphasize the favorable intracellular disposition of this antibiotic.

In conclusion, the results of this study demonstrate that the good intracellular penetration of ciprofloxacin previously described in vitro is also observed in vivo under conditions of therapeutic use in humans. The high intracellular concentrations and the prolonged half-life of 6 h suggest that ciprofloxacin could be an antibiotic of choice for the treatment of infectious diseases caused by susceptible intracellular bacteria. In this case, the dosing regimen should be 750 mg orally two or three times daily, depending on the susceptibility of the strain.

REFERENCES

1. **Carlier, M. B., B. Scorneaux, A. Zenebergh, and P. M. Tulkens.** 1987. Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 622.
2. **Easmon, C. S. F., and J. P. Crane.** 1985. Uptake of ciprofloxacin

by macrophages. J. Clin. Pathol. **38**:442–444.
3. **Easmon, C. S. F., and J. P. Crane.** 1985. Uptake of ciprofloxacin by human neutrophils. J. Antimicrob. Chemother. **16**:67–73.
4. **Easmon, C. S. F., J. P. Crane, and A. Blowers.** 1986. Effect of ciprofloxacin on intracellular organisms: in vitro and in vivo. J. Antimicrob. Chemother. **18**(Suppl. D):43–48.
5. **Garraffo, R.** 1989. Intracellular pharmacokinetics of ciprofloxacin. Association des Pharmacologistes, Réunion d'Automne, Besançon, France.
6. **Koga, H.** 1987. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leucocytes. Antimicrob. Agents Chemother. **31**:1904–1908.
7. **Mandel, G. L.** 1973. Interaction of intra leukocytic bacteria and antibiotics. J. Clin. Invest. **52**:1673–1679.
8. **Fraub, W. H.** 1984. Intraphagocytic bactericidal activity of bacterial DNA gyrase inhibitors against *Serratia marcescens*. Chemotherapy **30**:379–386.
9. **Van der Auwera, P., T. Martsumoto, and M. Husson.** 1988. Intraphagocytic penetration of antibiotics. J. Antimicrob. Chemother. **22**:1185–1192.