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Inter- and Intrasubject Variabilities in the Pharmacokinetics of Rufloxacin after Single Oral Administration to Healthy Volunteers

BRUNO P. IMBIMBO,[1]* GIAMPIETRO BROCCALI,[2] MARINA CESANA,[1] FRANCESCA CREMA,[3] AND GIUSEPPE ATTARDO-PARRINELLO[3]

Medical Department, Mediolanum Farmaceutici, Milan,[1] B.T. Biotecnica, Saronno,[2] and Institute of Medical Pharmacology, University of Pavia, Pavia,[3] Italy

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Rufloxacin is a new long-acting, once-daily quinolone antibacterial agent. We evaluated inter- and intrasubject variations in pharmacokinetics of rufloxacin following oral administration of 400 mg (two capsules) under controlled conditions, at an interval of 2 weeks (periods I and II), to 12 healthy male subjects. Plasma and urine samples were collected up to 48 h after drug administration. Plasma drug levels determined by bioassay were higher than those measured by high-performance liquid chromatography, indicating that one or more active metabolites were formed. Individual high-performance liquid chromatography plasma rufloxacin concentrations were fitted with a one-compartment open model with first-order input. There were considerable variations in the plasma concentration-time profiles among subjects; for example, the elimination half-life in plasma varied from 14.6 to 95.5 h. However, pharmacokinetic parameters calculated for the two periods did not differ significantly. These results suggest that the intrasubject variation in the pharmacokinetics of rufloxacin is usually small in spite of the considerable intersubject variation.

Rufloxacin (MF 934; Fig. 1) is a new quinolone carboxylic acid derivative (3) that is highly active in vitro against a broad spectrum of gram-negative and gram-positive organisms, including those resistant to β-lactam antibiotics (9). In animals (rats, dogs, and monkeys), the drug is absorbed well after oral administration, with an absolute bioavailability of about 60%; is distributed extensively in tissues, with high tissue-plasma ratios; and has a long half-life of 12 to 24 h, and about 30 to 40% is excreted in the urine (12).

The aim of this study was to evaluate the inter- and intrasubject variations in pharmacokinetics of rufloxacin after a single oral administration to humans.

Twelve healthy male volunteers, ranging in age from 23 to 30 years (mean, 25.1), in weight from 61 to 88 kg (mean, 73.6), and in height from 168 to 184 cm (mean, 179.1), participated in this study. Written informed consent was obtained. All subjects had normal histories, physical examinations, and laboratory tests (complete blood count, serum urea, creatinine, total protein, alkaline phosphatase, glucose, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and complete urine analysis). The study was approved by the Ethical Committee of the University of Pavia. Rufloxacin hydrochloride was given as two capsules with 200 ml of water; each capsule was equivalent to 200 mg of rufloxacin base. Subjects fasted from 9 p.m. after a standard meal on the night before the experiment to avoid any possible effects of food. The drug was administered between 8 and 9 a.m. on the next day. No food or drink other than water was permitted until 4 h after the dose. Bread (200 g), roast beef (200 g), milk (180 ml), and water (ad libitum) were allowed 4 h after drug administration. Venous blood samples (7 ml) were drawn from a forearm into heparinized tubes (25-U vacuum tube) through an indwelling butterfly needle, immediately before and at 1, 2, 4, 6, 8, 10, 12, 24, and 48 h after dose. After centrifugation, the plasma samples were transferred to sterilized vials and frozen at −20°C until analysis. Urine was collected for intervals of 0 to 6, 6 to 12, 12 to 24, and 24 to 48 h after administration. Each sample was shaken, and after measurement of the volume, a 10-ml aliquot was removed and frozen for later analysis. All subjects were given the same dose again 2 weeks later, and all samples were obtained in the same manner.

The isocratic high-performance liquid chromatographic (HPLC) method described by Lombardi (8) was adopted. The method employs a 10-μm PRP1 column and a UV detector operating at 300 nm. The mobile phase was a ternary mixture of 0.017 M phosphoric acid, acetonitrile, and tetrahydrofuran (880/120/5, vol/vol/vol) adjusted to pH 5.0 with triethylamine. Plasma samples, after addition of 25 μl of pipemidic acid solution (300 μg/ml) as an internal standard, were deproteinized with 70% perchloric acid and centrifuged, and 10 μl of the supernatant was injected. For analysis of the urine, after addition of internal standard the sample was salified with sodium phosphate and extracted with dichloromethane. The analytical method is linear over the range 0.4 to 20 μg/ml for plasma and 10 to 80 μg/ml for urine. The detection limit is 0.25 μg/ml for plasma, whereas the precision is better than 3.6% for plasma and 9.5% for urine.

The microbiological assay method described by Fonio (4) was adopted for detecting rufloxacin in plasma and urine. Agar (Antibiotic Medium No. 1; Difco Laboratories, Detroit, Mich.) inoculated with *Escherichia coli* ISF 432 was employed. The agar well method was utilized to measure concentrations of rufloxacin by plotting diameters of inhibition zones on a calibration curve (2). To determine rufloxacin levels in plasma, pooled human plasma was used to

* Corresponding author.

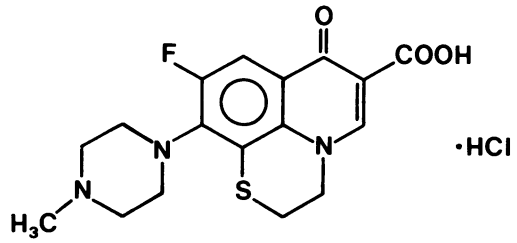

FIG. 1. Structural formula of rufloxacin.

dilute samples and for standard solutions. To determine rufloxacin levels in urine, 0.2 M phosphate buffer (pH 7.0) was used to dilute samples and for standard solutions. The method is linear over the ranges 0.62 to 10 μg/ml in plasma and 0.31 to 5 μg/ml in phosphate buffer. The limits of detection are 0.45 μg/ml in plasma and 0.25 μg/ml in phosphate buffer. The precisions are better than 4.44% for plasma and 2.23% for urine, and the accuracies are better than 8.00% for plasma and 7.60% for urine.

Pharmacokinetic parameters were calculated from the HPLC data by fitting individual plasma rufloxacin concentration data points of each subject according to a one-compartment open model, with first-order input (14). The one-compartment model was selected by using Akaike's information criterion (1), the Schwartz test (10), and the Ip index (7). Nonlinear regression analysis was performed with the program TOPFIT. The apparent volume of distribution (V/F), absorption rate constant (k_a), and elimination rate constant (k_{el}) were obtained by extended least-squares nonlinear regression. All other pharmacokinetic parameters were derived by standard methods (6). The area under the serum concentration-time curve from zero to infinity ($AUC_{0-\infty}$) was calculated as $C(0)/k_{el} - C(0)/k_a$, in which $C(0)$ is the coefficient and k_a and k_{el} are the exponents of the fitted biexponential equation. The absorption half-life ($t_{1/2\alpha}$) was calculated as $\ln(2)/k_a$, and the elimination half-life ($t_{1/2}$) was calculated as $\ln(2)/k_{el}$. The mean residence time (MRT) was calculated as $[C(0)/k_{el}^2 - C(0)/k_a^2]/[C(0)/k_{el} - C(0)/k_a]$. V/F was calculated as dose/k_{el} · $AUC_{0-\infty}$, and the apparent total body clearance (CL/F) was calculated as dose/$AUC_{0-\infty}$, assuming complete bioavailability. The maximum drug concentration (C_{max}) and the time to reach it (T_{max}) were obtained from the individual data. The area under the serum concentration-time curve from zero to 48 hours (AUC_{0-48}) was calculated by the linear trapezoidal rule. The renal clearance (CL_R) was calculated by dividing the amount of drug excreted in the urine in the 48 h by the AUC_{0-48}. The percent of the drug excreted in the urine (f_e) was calculated by dividing the total amount excreted in the urine up to 48 h by the dose.

The significance of differences between the pharmacokinetic parameters of periods I and II was examined by the Student t test for paired data. The 95% confidence intervals for the differences between two means were calculated. Statistical power ($1 - \beta$) to detect a 20% difference between two means was also calculated at the $\alpha = 0.05$ level. Results are expressed as means ± standard deviation.

C_{max}s (2.74 ± 0.61 μg/ml in period I and 2.56 ± 0.47 μg/ml in period II) were generally reached after 2 or 4 h (T_{max}, 3.8 ± 2.6 h and 4.0 ± 2.4 h, respectively), although principal or secondary peaks were also observed up to 10 h after administration. AUC_{0-48}s were 81.0 ± 14.6 μg · h/ml for period I and 71.6 ± 15.4 μg · h/ml for period II. The pharmacokinetic parameters derived from one-compartment model fitting of plasma rufloxacin concentrations for period I and period II are shown in Table 1. The pharmacokinetic analysis shows that the drug was absorbed very fast, with $t_{1/2\alpha}$s of 29 ± 54 min and 20 ± 21 min for the two periods. V/Fs were 155 ± 33 and 163 ± 32 liters for periods I and II, respectively. The drug remained in the body for a long time (MRT, 57 ± 29 and 45 ± 15 h, respectively) and was cleared slowly from plasma (CL/F, 53 ± 18 ml/min and 69 ± 31 ml/min, respectively). The $t_{1/2}$s were 38.9 ± 20.5 h for period I and 30.5 ± 10.1 h for period II. Because of the long half-life, the $AUC_{0-\infty}$s (142.9 ± 56.7 μg · h/ml and 110.8 ± 40.1 μg · h/ml) were considerably higher than the AUC_{0-48}s.

Mean concentrations of rufloxacin in the 0- to 6-h urine fractions were 22.0 ± 5.2 μg/ml in period I and 28.0 ± 28.2 μg/ml in period II; the corresponding concentrations for 6 to 12 h were 23.4 ± 5.3 and 19.8 ± 6.3 μg/ml and were still 26.7 ± 8.9 and 27.7 ± 11.2 μg/ml in the 12 to 24 h after the administration. Two days after the administration, the concentrations of rufloxacin in urine were even higher (47.1 ± 15.2 μg/ml in period I and 53.6 ± 12.9 μg/ml in period II). CL_Rs were 18 ± 7 ml/min for period I and 23 ± 9 ml/min for period II, these values being 34 and 33% of the apparent total body clearances. The f_es were 21.4 ± 7.8% for period I and 24.2 ± 10.0% for period II.

There was considerable variation in the plasma concentration-time profiles for the different subjects. However, the

TABLE 1. Pharmacokinetic parameters (means ± standard deviation) for rufloxacin in 12 subjects in periods I and II, determined by HPLC

Pharmacokinetic parameter	Measurement for period:		95% confidence interval	Power
	I	II		
$t_{1/2\alpha}$ (min)	29.4 ± 54.4	19.8 ± 20.9	−28.8 to 48.2	0.92
T_{max} (h)	3.83 ± 2.62	4.00 ± 2.41	−2.36 to 2.03	0.81
C_{max} (μg/ml)	2.74 ± 0.61	2.56 ± 0.47	−0.24 to 0.59	0.92
$t_{1/2}$ (h)	38.9 ± 20.5	30.5 ± 10.1	−6.2 to 23.0	0.65
V/F (liters)	154.8 ± 33.5	163.2 ± 32.1	−32.9 to 16.2	0.92
AUC_{0-48} (μg · h/ml)	81.0 ± 14.6	71.6 ± 15.4	−1.1 to 19.9	0.93
$AUC_{0-\infty}$ (μg · h/ml)	142.9 ± 56.7	110.8 ± 40.1	−7.9 to 72.2	0.55
MRT (h)	56.9 ± 29.2	44.5 ± 14.7	−8.5 to 33.3	0.64
CL/F (ml/min)	52.7 ± 18.0	68.6 ± 30.6	−36.7 to 4.9	0.59
CL_R (ml/min)	17.9 ± 6.8	22.8 ± 9.3	−48.6 to 38.2	0.58
f_e (%)	21.4 ± 7.8	24.2 ± 10.0	−7.7 to 2.3	0.57

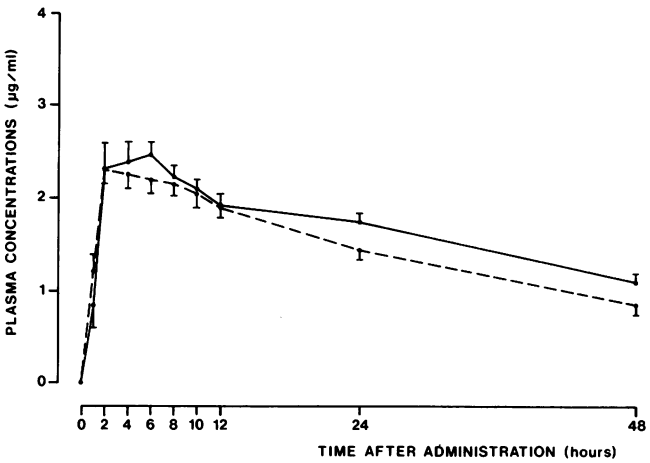

FIG. 2. Average HPLC plasma concentration-time profiles of rufloxacin for 12 subjects in periods I (——) and II (– – –). Each point is the mean (± standard error of the mean) plasma concentration for 12 subjects.

plasma concentration-time curves for period I were similar to those for period II in most of the subjects. As a consequence, the average plasma concentration-time curve for period I was very similar to that for period II (Fig. 2). Table 1 lists the pharmacokinetic parameters for rufloxacin and 95% confidence intervals for their differences between the two periods, together with statistical powers ($1 - \beta$) to detect 20% differences at the $\alpha = 0.05$ level. Both confidence intervals and the t test show that there were no significant differences for any of the parameters between the two periods. There was a significant within-subject linear correlation for most of the pharmacokinetic parameters between the two periods ($r = 0.792$ for C_{max}; $r = 0.742$ for $t_{1/2}$; $r = 0.882$ for AUC_{0-48}).

Plasma rufloxacin levels determined by the microbiological assay were higher than those determined by HPLC. Also in this case, there was a considerable intersubject variation in the plasma concentration-time profiles. However, the plasma concentration-time curves for period I were comparable to those for period II for most of the subjects, and the average plasma concentration-time curve for period I was very close to that for period II.

The most important aspect of the pharmacokinetic profile of rufloxacin after single oral administration is its long half-life in plasma (31 to 39 h). Since plasma samples were collected only up to 48 h from drug administration, the estimation of the $t_{1/2}$ could not be accurate. Also, the very low CL/F (60 ml/min) and the high V/F could be affected by the imprecision in estimating the $AUC_{0-\infty}$ and the assumption that bioavailability remains constant for each study day. However, a quite lengthy plasma $t_{1/2}$ (30 to 36 h), as well as a low CL/F (26 to 68 ml/min) and a high V/F (70 to 280 liters), was also previously reported by Cocuzza et al. (3a). Another unusual pharmacokinetic parameter observed in the present study is the extremely low CL_R (20 ml/min). The low CL/F may be due to the high protein binding of the drug in plasma (about 80% [11]). Similarly, the long plasma $t_{1/2}$ could be linked to the low free fraction of the drug in tissue (5). In addition, the probable enterohepatic recycling of rufloxacin indicated by late slight peaks in plasma might contribute to the long stay of the drug in plasma. Indeed, rufloxacin was found to be excreted with bile in patients with T-tube drainage although the percentage of dose excreted is quite low (about 1% [13]). Finally, the low CL_R of rufloxacin (about 20 ml/min) seems to be due to the high plasma protein binding of the drug (80%) and corresponds to the value of the glomerular filtration rate corrected for the free fraction of the drug in plasma.

The concentrations of rufloxacin in plasma determined by bioassay were higher than those determined by HPLC, indicating that one or more active metabolites were formed in humans. Microbiological concentrations greater than 2 μg/ml, a value equal to the MICs for most of the susceptible bacteria (7), were detected in serum for at least 12 h after the drug administration.

The percentage of the drug excreted in urine was about 20 to 25% of the dose in the 0- to 48-h period. However, a considerable portion of the drug (about 7%) was found in the urine 24 to 48 h after the dose, indicating that significant amounts of drug might be eliminated later by the kidney. The CL_R of about 20 ml/min represents about 33% of the CL/F of rufloxacin in both periods, indicating that a considerable amount of the drug is cleared via hepatic metabolism. Rufloxacin concentrations in urine (20 to 50 μg/ml) were much higher than the MICs for most organisms responsible for urinary tract infections even up to 48 h after the dose.

Although there were no significant differences in any parameters between the two periods, the low power values for $t_{1/2}$, $AUC_{0-\infty}$, MRT, CL/F, CL_R, and f_e imply that more data are needed to test whether these parameters in the two periods are statistically equivalent in the range of 20% difference at $\alpha = 0.05$.

In conclusion, pharmacokinetic parameters of oral rufloxacin appeared to vary only slightly within individuals over a short period (2 weeks) under the controlled conditions. This suggests that the physiology responsible for rufloxacin disposition in the body (such as gastric emptying, gastrointestinal absorption, or glomerular filtration rate) might be stable over at least 2 weeks in a given subject, although it might vary greatly in different subjects.

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